出國報告
（類別：其他）

參加第三屆抗生素替代物質國際研討會：動物保健產品之挑戰與解決方案

服務機關：行政院農業委員會動植物防疫檢疫局
姓名職稱：黃怡銘技士
派赴國家：泰國曼谷
報告日期：109 年 2 月 27 日
出國期間：108 年 12 月 15 日至 12 月 19 日

目次

一、緣起及目的

世界動物衛生組織（World Organisation for Animal Health, OIE）與美國農業部農業研究局 （United States Department of Agriculture, Agricultural Research Service, USDA-ARS）於 2019 年 12 月 16~18 日於泰國召開第 3 屆抗生素替代物質（Alternatives to antibiotics, ATA）國際研討會。該研討會的重點在於預防及治療動物疾病方面最新科學上的突破及技術，提供替代抗生素策略，以減少農業方面醫療重要抗生素之使用。雖然其中有些新技術用於防疫一體策略 （One Health approach） 及直接應用於人類醫療健康，但研討會的重點在於動物健康與生產，以及食品安全，並進行下面 6 大領域專題簡報及討論：（1）可用於減少醫療重要抗生素使用之疫苗、（2）微生物衍生產物，例如益生菌及嗜菌體基因產物、（3）非營養性的植物性化學成分，包含益菌生（Prebiotics）、（4）免疫相關產物，例如抗體、微生物胜肽及細胞素（Cytokines）、（5）核發替代抗生素物質使用許可證之法規途徑。此研討會目標著重抗生素替代物質之應用及進展，用以不具有產生微生物抗藥性(Antimicrobial resistance)篩選壓力(Selection pressure ）的物質預防及治療疾病，強化農業生產措施。

二、行程及會議議程

- 2019 年 12 月 16 日（日）：臺北往曼谷。
- 2019 年 12 月 17 日（一）：

時間	議程	主持人/主講人
8：00 ~ 8：10	開幕式及引言	USDA-ARS的Dr. Cyril Gay
8：10 ~ 8：30	開幕式摘要報告： 動物 AMR 及替代物質之全球目標	OIE的Elisabeth Erlacher-Vindel
8：30 ~ 9：00	開幕式摘要報告： 研發人體保健用之抗生素替代物質全球策略	美國國家衛生研究院（National Institutes of Health, NIH）國家過敏和傳染病研究所（National Institute of Allergy and Infectious Disease, NIAID）的Dennis Dixon
9：00 ~ 9：30	開幕式摘要報告： 動物之抗生素、細菌及 ATA	USDA-ARS的Hyun Lillehoj
9：30 ~ 10：00	茶敘、壁報論文展覽	
第一節：疫苗		
10：00 ~ 10：25	肉雞免疫接種抵抗壞死性腸炎之進展及可行性	加拿大貴湖大學 （University of Guelph）的John Prescott
10：25 ~ 10：50	*Salmonella* 疫苗的地理多樣性及流行病學環境變化之未來展望	比利時根特大學（Ghent University）的Filip Van Immerseel
10：50 ~ 11：05	以雞隻抗頂複門之單株抗體辨識新型疫苗抗原	日本大阪府立大學（Osaka Prefecture University）的Kazumi Sasai
11：05 ~ 11：20	以 *Clostridium perfringens* 酵素為標的，研發次單位疫苗，管控肉雞壞死性	美國俄亥俄州立大學 （The Ohio State University）的Lisa Bielke

時間	議程	主持人/主講人
	腸炎	
11：20 ~ 11：35	以具毒力的 *Clostridium perfringens* 野外菌株製作新型候選基因重組抗原疫苗與接種，探討肉雞壞死性腸炎之部分保護力	USDA-ARS動物生物科學及生物科技實驗室的Charles Li
11：35 ~ 12：00	第一節講師組成專家小組並開放提問	加拿大的John Prescott及美國的Cyril Gay
12：00 ~ 13：30	午餐、壁報論文展覽	
第二節　微生物來源產品（Microbial-derived products）		
13：30 ~ 13：55	腸道菌叢保健—對抗AMR之現代工具及其努力目標	香港大學的Tun, Hein Min
13：55 ~ 14：20	以非抗生素方式調整乳牛微生物菌叢—對產乳量、動物健康及食品安全之影響	美國喬治亞大學的Todd Callaway
14：20 ~ 14：45	益生菌代謝產物作為ATA—小型分子量代謝物調節生長及免疫	USDA-ARS的Inkyung Park
14：45 ~ 15：00	畜禽及水生動物商品化益生菌產品—微生物品質及其可能為AMR基因傳播來源	泰國朱拉隆功大學（Chulalongkorn University）的Rungtip Chuanchuen
15：00 ~ 15：15	豬源性益生菌*Lactobacillus plantarum*調節豬腸道內源性防禦胜肽的合成	中國北京市農林科學院的Jing Wang
15：15 ~ 15：45	茶敘、壁報論文展覽	
15：45 ~ 16：10	腸道菌叢的管理—從科學至實務面	美國切遲杜威公司 （Church & Dwight Co., Inc.）的Tom Rehberger
16：10 ~	離乳豬營養補充 *Bacillus*	泰國 Evonik 公司的 Thammakit

時間	議程	主持人/主講人
16：25	*amyloliquefaciens* CECT 5940之反應	Thammathipborworn
16：25 ~ 16：40	發酵生物活性物質對亞急性瘤胃酸中毒及*Stretoccocus uberis*乳房炎的影響，以減少牛使用抗感染藥	美國Inc Cedar Rapids的Leon Samuel Barringer
16：40 ~ 17：00	第二節講師組成專家小組並開放提問	香港的Tun, Hein Min及美國的Toddy Callaway
18：00 ~ 20：00	晚宴	

■ 2019 年 12 月 17 日（二）：

時間	議程	主持人/主講人
第三節　新型之藥物、化學物質及酵素（Innovative drugs, chemicals and enzymes）		
8：30 ~ 8：55	中國減少豬隻使用抗生素策略	中國農業大學的 Junjun Wang
8：55 ~ 9：20	於分子生物時代，以非抗生素物質治療蜜蜂疾病	USDA-ARS的Judy Chen
9：20 ~ 9：35	Gly-substituted DLP4陽離子胜肽抵抗 *Staphylococcus aureus* CVCC 546的體內及體外試驗	中國農業科學院的 Bing Li
9：35 ~ 9：50	細菌死亡後的細胞碎片－胃腸道中的peptidoglycan	丹麥Novozymes生物科技公司的 Christian Nyffenegger
9：50 ~ 10：20	茶敘、壁報論文展覽	
10：20 ~ 10：45	優化胃腸道功能以對抗AMR－全面性療法	瑞士DSM製藥公司的Pietro Celi
10：45 ~ 11：10	單胃動物使用有機酸作為ATA	加拿大曼尼托巴大學（University of Manitoba）的Chengbo Yang
11：10 ~ 11：25	無抗生素飼料管理計畫之商用肉雞必不可缺的工具－25-OH-D_3	新加坡亞太區DSM製藥公司的 Thau Kiong Chung
11：25 ~ 11：40	動物用抗微生物藥品替代物質－聚焦豬下痢之新歐盟計畫	丹麥農業知識研究院（SEGES）的 Poul Jesper Baekbo
11：40 ~ 12：00	第三節講師組成專家小組並開放提問	美國的 Lyun Lillehoj 及瑞士的 Pietro Celi
12：00 ~ 13：30	午餐、壁報論文展覽	
第四節　植物化學成分（Phytochemicals）		
13：30 ~	腸道健康對微量營養及植物化學成分	澳大利亞墨爾本大學（University

時間	議程	主持人/主講人
13：55	的偵測及反應	of Melbourne）的 John Furness
13：55 ~ 14：20	白頭草湯（Pulsatilla Decoction, PD）治療*Escherichia coli*性下痢的作用機制	中國北京農業大學的 Hong Dong
14：20 ~ 14：35	富含花青素的紫薯萃取物可減少高脂肪飲食及Lipopolysaccharide（LPS）引起的肥胖與輕微的腸炎（Low-grade gut inflammation）	中國江西中醫藥大學的 Hua Zhang
14：35 ~ 14：50	食用馬鈴薯抗性澱粉改變豬隻免疫狀態及微生物菌叢以限制*Salmonella*	USDA-ARS 的 Crystal L. Loving
14：50 ~ 15：20	茶敘、壁報論文展覽	
15：20 ~ 15：45	邁向下一個世代之植物營養素	美國 Full Circle Science 的 Emma Wall
15：45 ~ 16：10	於後抗生素時代，以科學為基礎，使用植物萃取物改善動物健康之進展	墨西哥 AVT Natural 公司的 Prashant Mishra
16：10 ~ 16：25	植物萃取物（Phytobiotic）於體外抗*Eschericia coli* 及 *Mycoplasma gallisepticum*之活性	印尼 PT Medion Farma Jaya 的 Elvina Jonas Jahja
16：25 ~ 16：40	Neutrapath ™ 體內及體外抗*Salmonella Typhimurium*之療效評估	美國 Amlan International 的 Hongyu Xue
16：40 ~ 17：00	第四節講師組成專家小組並開放提問	墨西哥的Prashant Mishra及美國的Emma Wall

■ 2019 年 12 月 18 日（三）：

時間	議程	主持人/主講人
第五節　免疫來源產物（Immune-derived products）		
8：30 ~ 8：55	以被動免疫及類 IgG 抗體（IgG-like antibody）作為 ATA	丹麥技術大學 （Technical University of Denmark）的 Peter Heegaard
8：55 ~ 9：20	以具抗微生物及免疫調節活性之宿主防禦胜肽作為ATA	美國農業部國家農業研究中心（Beltsville Agricultural Research Center, United States Department of Agriculture）的Woohyum Kim
9：20 ~ 9：35	以新型腸菌素專一性之卵黃抗體控制革蘭氏陰性細菌	美國田納西大學 （University of Tennessee）的 Jun Lin
9：35 ~ 9：50	高量篩選宿主自然防禦胜肽化合物作為ATA	美國奧克拉荷馬州立大學（Oklahoma State University）的 Glenn Ahang
9：50 ~ 10：20	茶敘、壁報論文展覽	
10：20 ~ 10：45	將研究試驗轉變至現場應用	加拿大阿爾伯塔大學（University of Alberta）的Douglas Korver
10：45 ~ 11：10	重新編製（Reprogramming）先天性免疫反應作為ATA	荷蘭烏特勒支大學（Utrecht University）的Henk Haagsman
11：10 ~ 11：25	雄性肉雞受到實驗室感染引起壞死性腸炎期間，投予乾燥蛋品（Dried egg product, DEP）之效力	美國禮來動物保健（Elanco Animl Health）公司
11：25 ~ 11：40	肉雞Salmonella Enteritidis感染症─酵母細胞壁（Yeast cell wall, YCW）之免疫調節效果及對腸道完整性之療效	巴西ICC的Ekachai Jenwitheesuk
11：40 ~ 12：00	第五節講師組成專家小組並開放提問	加拿大的Douglas Korver及荷蘭的Henk Haagsman

時間	議程	主持人/主講人
12：00 ~ 13：30	午餐、壁報論文展覽	
第六節　ATA 登記上市之法規及鼓勵利害關係者研發（Regulatory pathways to enable the licensing of alternatives to antibiotics and incentives from stakeholders to support their development）		
13：30 ~ 13：55	美國FDA動物ATA法規	美國食品藥物管理署動物藥品中心（Food and Drug Administration, Center for Veterinary Medicine, FDA-CVM）的 Joshua Hayes
13：55 ~ 14：20	歐盟促進動物用ATA之核准	歐洲藥品管理局（EMA）的 Javier Pozo
14：20 ~ 14：45	ATA的核准/訂定之法規架構	日本農林水產省（Ministry of Agriculture, Forestry and Fisheries, MAFF）的 Takashi Kozasa
14：45 ~ 15：10	以業界角度看ATA登記上市	全球動物醫療健康協會（health for animals global animal medicines association）的 Erik De Ridder
15：10 ~ 15：40	茶敘、壁報論文展覽	
15：40 ~ 16：30	第六節講師及其他相關學術界、政府機關代表組成專家小組，開放提問。	美國FDA的Joshua Hayes及英國獸醫局的Peter Borriello
16：30 ~ 17：00	研討會總結及後續行動	USDA-ARS 的 Dr. Cyril Gay

- 2019 年 12 月 19 日（四）：曼谷返回臺北。

三、過程及會議內容

（一）開幕式及引言

鑒於法規限制及微生物抗藥性(Antimicrobial resistance, AMR)的興起，造成醫療重要性抗生素（Medically important antibiotics）的選擇持續減少，並受到全球關注，故舉辦科學研討會，以評估抗生素替代物質（Alternatives to antibiotics, ATA）之研究及研發程度。本次研討會主要聚焦於具前景之動物用 ATA 研究結果及新技術，同時評估其商品化及其使用所面臨挑戰，另提供可行策略以支持其發展。本次研討會著重於 5 類產品，以減少醫療重要抗生素的使用：（1）疫苗、（2）微生物來源產品(Microbial-derived products)、（3）植物化學成分（Phytochemicals）、（4）免疫來源產物（Immune-derived products）、（5）新型之藥物、化學物質及酵素。AMR 為防疫一體（One Health）優先重要議題，其於公共衛生及農業方面具重大影響力。

目前受到注意的是，21 世紀面臨的挑戰為需增加農業產量以符合不斷增加的世界人口，農業產量可否增加完全依賴是否具有預防動物及植物疾病措施。最重要的是，全球農業預防及控制疾病之成功與否將直接影響全球糧食安全（Global food security）及全球衛生安全綱領（Global Health Security Agenda, GHSA），此重要計畫已受到世界動物衛生組織（World Organisation for Animal Health, OIE）、聯合國糧食及農業組織 （Food and Agriculture Organization, FAO）及世界衛生組織（World Health Organization, WHO）的認可。有一點必須強調，本次研討會並非要刪除動物用抗生素，因為特定動物疾病仍需抗生素治療。本研討會亦非要提倡未受到科學實證且無法對付抗藥性病原的措施。本研討會挑選的議題為預防及治療動物疾病的新產品研究並強化之，同時該產品對微生物不具有抗藥性篩選壓力。

因此，新藥及 ATA 的研究及發展已被納入美國國家型行動方案對抗細菌抗藥性計畫(United States National Action Plan for Combating Antimicrobial Resistant Bacteria, CARB）之重要策略目標。全球細菌性病原抗生素抗藥性的增加被認為是因人類及動物健康方面過度及濫用抗生素所致。其中一項

公共衛生考量為在農業方面，存在於產食動物設備的菌株與食媒性細菌可能產生抗藥性，此可能嚴重減少醫療用藥的選擇。因此，於農業需要發展管理計畫（Stewardship programs）及替代抗生素物質。此外，不斷有科學證據顯示，某些抗生素會破壞腸道正常菌叢、且對免疫系統、抗病力（Disease tolerance）及健康產生負面影響。21 世紀後，世界人口不斷增加，隨之增加糧食及營養需求，尋求 ATA 以改善動物健康及其產品已成為全球性的議題，此亦有助於減少世界之貧窮及飢餓問題。

（二）開幕式摘要報告

1. OIE 的 Elisabeth Erlacher-Vindel 簡報「動物 AMR 及替代物質之全球目標」，AMR 為全球人類及動物健康（Animal health）的關注議題，其與人類及動物醫學抗微生物藥品的使用有關。2013 年 3 月 13 日至 3 月 15 日 OIE 於法國巴黎召開的「動物抗微生物藥品謹慎及負責使用之 OIE 全球研討會－對抗 AMR 之國際共識」，給會員國的第 13 項建議（如附件 1）提及：「以延長抗微生物藥品的使用及降低抗藥性的產生為目標，支持相關研究以增加對現有抗微生物藥品效力的瞭解，研發新型分子及尋找產食動物 ATA。」2015 年 AMR 全球行動計畫五大策略目標為：第一、改善對 AMR 之警覺性及瞭解。第二、透過監控（Surveillance）及研究，強化專業知識。第三、減少感染的發生。第四、優化抗微生物藥品之使用。第五、考量所有國家的需求，於新藥、診斷工具、疫苗及其他措施研發方面，增加及發展永續投資。為對抗 AMR，OIE 以科學為基礎制定政府之間的標準及指引，包含陸生及水生動物。OIE 參與 WHO 之 AMR 全球行動方案的工作，而此參與係由 OIE 的 180 個會員國於 2016 年 11 月一致決議通過。在之後的常任代表全球會員大會（World Assembly of Delegates）要求下，OIE 將其 AMR 措施彙整成一項策略計畫。OIE 的 AMR 策略計畫，包含謹慎使用抗微生物藥品，摘述目標及措施以支持會員國對抗 AMR，並鼓勵會員國實施國際標準。OIE 策略計劃的架構係為了支持全球行動方案的目標所建立，四大目標

分別為（1）增加對 AMR 之警覺認知及瞭解、（2）透過監測及研究，強化專業知識，包含蒐集產食動物及伴侶動物之抗微生物藥品使用量。（3）建立良好的管理體系及量能，以及（4）鼓勵實施國際標準。OIE 遵循這些目標，提供指引及支持 ATA 的研究（包含疫苗）並與夥伴組織共同鼓勵 ATA 的研發、登記上市、接受度；及鼓勵受到科學實證的產品可替代抗生素使用，進而減少 AMR 的產生及散布。在此體系規則下，OIE 協助美國農業部在 OIE 總部舉辦前兩次的「抗生素替代物質國際研討會－動物保健產品之挑戰與解決方案」（分別 2012 年 9 月及 2016 年 12 月在法國巴黎召開）。此外，OIE 支持動物與人畜共同主要傳染病研究統籌全球策略（The Global Strategic Alliances for the Co-ordination of Research on the Major Infectious Diseases of Animals and Zoonoses, STAR-IDAZ）國際研究聯盟，促進國際間研究計畫的調和，參與研發新型及改善動物保健策略，包含 AMR 議題及 ATA 研發。

2. 美國國家衛生研究院（National Institutes of Health, NIH）國家過敏和傳染病研究所(National Institute of Allergy and Infectious Diseases, NIAID）的 Dennis Dixon 簡報「研發人體保健用之抗生素替代物質全球策略」，AMR 為世界公共衛生重要議題之一。特別受到關注的是，原本對人類細菌感染具強效的抗生素卻失去效果。病原持續接觸現有抗生素、新種類抗生素數量少與製藥公司研發的投資報酬率低等因素，造成 AMR 的挑戰更複雜。NIAID 致力於處理美國國內及國際間的這些挑戰，處理方式包含提升診斷、預防及治療疾病的基礎研究、轉譯醫學（Translational research）及臨床研究，以及提供臨床前與臨床資源，以減少民間公司的投資風險。NIH 抗生素抗藥性研究計畫包含強化研發例行性使用之 ATA。另獎勵該領域研究，包含公告專案基金。

3. 美國農業部農業研究局（Agricultural Research Service, United States Department of Agriculture, USDA-ARS）的 Hyun Lillehoj 簡報「動物之抗生素、細菌及 ATA」，食用動物產業（Food animal industry）於飼料中添加抗生素的方式已超過 60 年以上，此方式不僅為了控制傳染病，亦為了增加飼料效率及促進生長。對雞隻而言，相較於使用無抗生素飼

料，使用未達治療劑量之抗生素飼料，可增加 8%的體重並減少 5%飼料轉換率 （飼料轉換率 = 飼料攝取量/體重增加量, feed conversion ratio = feed intake/body weight gain） 。全球濫用抗生素已造成食用動物及人體產生多重抗藥性的「超級細菌」（Multi-drug resistant “superbugs”）。美國食品藥物管理署（The United States Food and Drug Administration, FDA）已要求農業生產者停止飼料添加未達治療劑量的抗生素。因此，迫切需要尋找取代生長促進藥物的新型 ATA，以使畜牧產業的永續發展。本研討會針對畜牧業，將討論研發新型 ATA 的各種策略，我們將學到更多新型 ATA 的作用機制。在本次討論，USDA-ARS 的 Hyun Lillehoj 檢視促進生長藥物的可能機制、抗生素對腸道微生物菌叢的作用及 ATA。抗生素過去被認為透過減少腸道正常菌叢的數量及多樣性而促進動物生長，因其增加宿主對營養物質的生體可用率（Bioavailability）及／或減少微生物代謝產生傷害動物生長的有害物質。另一個可能機制是，抗生素過去被認為其直接作用於腸道上皮細胞，產生抗發炎作用，以促進動物生長。新興的分子生物學及生物資訊學（Bioinformatics）讓我們更加瞭解動物食入抗生素後發生的體內變化，包含腸道炎症反應的變化、腸道微生物菌叢結構及多樣性的變化。由於腸道微生物、免疫系統及腦部功能之間具交互相作用，因此瞭解這些交互作用有助於研發新型 ATA。現今科技的進步，得以將分子生物學技術應用於食用動物的基因表現及代謝研究，更加了解生物化學的過程，以研發替代促進生長及免疫的新型 ATA。應該要有制度規則，以判定在無抗生素的使用下，天然化學性化合物可改善動物生長效能。

（三）第一節 疫苗

此節共有 5 篇口頭報告論文及 8 篇壁報論文展示，口頭報告重要內容摘述如下：

1. 加拿大貴湖大學（University of Guelph）的 John Prescott 簡報「肉雞免疫接種抵抗壞死性腸炎之進展及可行性」，免疫接種可能有幫助，但在

輔助控制肉雞壞死性腸炎之效果未臻至善。

2. 比利時根特大學（Ghent University）的 Filip Van Immerseel 簡報「*Salmonella* 疫苗的地理多樣性及流行病學環境變化之未來展望」，常見人類食物中毒的 *Salmonella* 病原型別為 *Typhimurium*，其源自於豬及家禽。其他血清型別之重要性可能有地區性差異。蛋雞使用 *Salmonella* 疫苗可有效減少雞蛋被 *Salmonella* 汙染，肉雞及豬的免疫效果具挑戰性。肉雞需在短暫的生命週期內，建立主動性免疫，但疫苗難以達成此目標。對豬而言，疫苗接種難以控制 *Salmonella* 於淋巴結的定殖（Lymph node colonization）。由於不同血清型別可能不具交叉保護力，故法規面的重要性在於，加速新興血清型別減毒活毒菌株疫苗上市。

3. 日本大阪府立大學（Osaka Prefecture University）的 Kazumi Sasai 簡報「以雞隻抗頂複門之單株抗體辨識新型疫苗抗原」，頂複門（Apicomplexans）是原生真核生物之下一個寄生囊泡蟲的門，為感染脊椎動物之細胞內寄生蟲。相對於哺乳動物而言，雞之體細胞機制產生的抗體具多樣性，可能有些抗體容易於雞產生，但難以在哺乳動物產生。此研究結果顯示頂複門寄生蟲媒介進入宿主細胞之關鍵抗原為 EF-1α，此抗原可作為候選疫苗抗原，以對抗頂複門寄生蟲。

4. 美國俄亥俄州立大學 （The Ohio State University）的 Lisa Bielke 簡報「以 *Clostridium perfringens* 酵素為標的，研發次單位疫苗，管控肉雞壞死性腸炎」，研究結果顯示，5 種胜肽混合物（MC）最能有效減少肉雞感染壞死性腸炎。

5. USDA-ARS 動物生物科學及生物科技實驗室的 Charles Li 簡報「以具毒力的 *Clostridium perfringens* 野外菌株製作新型候選基因重組抗原疫苗與接種，探討肉雞壞死性腸炎之部分保護力」，雞壞死性腸炎目前在市面上無有效疫苗。研究結果顯示，*Clostridium perfringens* 蛋白質混合 MONTANIDE™ ISA 71 VG 肌肉注射 2 次，可提供最佳保護力。

6. 其後第一節的講師組成專家小組並開放提問：

（1） USDA-ARS 的 Cyril Gay 表示，有專家學者曾提問：「我們在哪使用的抗生素量最多？或是哪個疾病使用的抗生素量最多？它的疫

苗又在哪？」我們仍使用很多抗生素，因為有些疫苗效果並不佳。因此，Cyril Gay 的提問為，在研發疫苗方面，尚有什麼問題需要突破？

- 比利時根特大學的 Filip 回應，第 1 個問題係可能需要良好的載體系統（Vector system），第 2 問題係為了補強免疫，可能需免疫接種很多次。第 3 個問題為成本問題，若抗生素的使用成本較疫苗低，且疫苗不會於使用後 1 個星期即有效果，因而產生成本問題。成本問題相當重要，若疫苗具有減少更多損失的效果，則更能說服大家使用疫苗。
- 加拿大貴湖大學的 John Prescott 認為「它的疫苗在哪？」是一個很好的問題，我們已經近在咫尺。我們需要有人跨出第一步，於田野試驗效果達 60~70%以上，因為美國有大量無抗生素飼養雞隻的需求。John Prescott 認為在法規及成本方面，證實其效力、安全性及核准登記上市方面具法規障礙。

（2） USDA-ARS 的 Cyril Gay 續提問：「Charles Li 簡報提到『需免疫注射 2 次』，這在現場實務面難以做到，特別是養禽業。因此，即使解決效力問題，尚須面臨使用方面是否務實的問題。所以我的問題是，如何做到一次免疫接種即產生免疫保護力？您的下一步為何？」

- Charles Li 回應，下一步將以飲水或飲食方式，給予黏膜免疫接種。目前研究係使用 2 次接種，但之後將嘗試 1 次免疫接種，因為食用家禽生命週期很短，僅有 6 週齡。

（四）第二節　微生物來源產品（Microbial-derived products）

此節有 8 篇口頭報告論文及 42 篇壁報論文展示，口頭報告重要內容摘述如下：

1. 香港大學的 Tun, Hein Min 簡報「腸道菌叢保健—對抗 AMR 之現代工具及其努力目標」，近年來使用微生物產品調節腸道菌叢（包含糞便微生物移植, Fecal microbiota transplant, FMT）以改善腸道健康之技術，於

畜牧產業已成為具前景之 ATA。FMT 在治療某些人類疾病（例如 *Clostridium difficile* 感染症）具療效，但 FMT 基礎科學仍未完全知曉，因此 FMT 於畜牧產業之應用需要更謹慎及更多的研究投入。

2. 美國喬治亞大學的 Todd Callaway 簡報「以非抗生素方式調整乳牛微生物菌叢—對產乳量、動物健康及食品安全之影響」，乳牛胃腸道微生物密集及具多樣性，這些微生物可將飼料及穀物轉換為高品質的肌肉及牛乳，故調整乳牛胃腸道微生物菌叢可改善其生產效率、動物健康及食品安全。調整乳牛胃腸道之非抗生素方法包含現場管理、飲食調整、有機酸、益生菌、益生菌飼料添加物及免疫接種，這些方法已廣泛用於全世界的乳牛產業，以提高產乳效率及飼料利用效率，同時亦改善動物健康。這些方法主要係以競爭性微生物排斥病原性微生物，進而提升動物產能、健康及食品安全。
3. USDA-ARS 的 Inkyung Park 簡報「益生菌代謝產物作為 ATA—小型分子量代謝物調節生長及免疫」，從 14 至 21 日齡，餵飼「Bacillus subtilis 1781 及 747」雞隻相較於無餵飼者，有餵飼之雞隻體重顯著增加。
4. 泰國朱拉隆功大學（Chulalongkorn University）的 Rungtip Chuanchuen 簡報「畜禽及水生動物商品化益生菌產品—微生物品質及其可能為 AMR 基因傳播來源」，本研究目標為針對泰國 9 項已上市的益生菌產品，確認其微生物品質及益生菌是否存在抗藥性基因。研究結果顯示，此 9 項益生菌產品均無被 *E. coli* 及 *Salmonella* 汙染。有些益生菌產品未含有其標示宣稱之微生物品種。有 3 項益生菌產品顯示具有抗 Sulphonamides、Streptomycin 及 Tetracycline 抗藥性基因。將此微生物產品使用於食用動物可能具效益，但也可能存在抗藥性基因傳播的風險。最後仍需要有法規規範益生菌產品及其使用，確保其品質及減少 AMR 基因的傳播。
5. 中國北京市農林科學院的 Jing Wang 簡報「豬源性益生菌 *Lactobacillus plantarum* 調節豬腸道內源性防禦胜肽的合成」，仔豬離乳後緊迫使其對病原的感受性增加，進而導致離乳後下痢，其特徵為仔豬嚴重下痢及高死亡率。宿主防禦性胜肽（Host defense peptides, HDPs）具有抗微生

物及免疫調節活性，並參與上皮細胞先天性免疫。飲食調整內源性 HDP 的合成可有效提升宿主的先天性免疫。以 *Lactobacillus plantarum* ZLP001 調整飼主的內源性 HDPs，可改善宿主腸道健康及增強離乳豬抵抗力，減少下痢。

6. 美國切遲杜威公司 （Church & Dwight Co., Inc.）的 Tom Rehberger 簡報「腸道菌叢的管理—從科學至實務面」，在沒有使用抗生素的狀況下，為了促進益生菌定殖（Colonization）及減少禽類病原性大腸桿菌（Avian pathogenic *Escherichia coli*, APEC）濃度，已有研發出 2 種乳酸菌，具有調節免疫能力，另 2 種 Bacillus 品種產生的代謝物可抑制 APEC 品種。這些益生菌係在孵化場階段一次性投予至雞隻。研究結果顯示，相較無治療雞隻，益生菌及 Gentamycin 兩者治療組均可減少兩週齡肉雞的 APEC 濃度。因此在孵化場階段，益生菌為有效的 ATA，可建立腸道健康、微生物菌叢及控制 APEC。
7. 泰國 Evonik 公司的 Thammakit Thammathipborworn 簡報「離乳豬營養補充 *Bacillus amyloliquefaciens* CECT 5940 之反應」，研究結果顯示，離乳豬補充 *Bacillus amyloliquefaciens* CECT 5940 之生長效能較使用生長促進用之 Colistin 者佳，此表示益生菌可改善無 AGP（抗生素類生長促進劑，antibiotic growth promoter）飼料餵飼豬隻之生長效能。
8. 美國 Inc Cedar Rapids 的 Leon Samuel Barringer 簡報「發酵生物活性物質對亞急性瘤胃酸中毒及 *Stretoccocus uberis* 乳房炎的影響，以減少牛使用抗感染藥」，研究結果顯示，餵飼這些新型生物活性物質可改變微生物菌叢及促進先天性免疫，減少牛隻使用抗感染藥。
9. 第二節的講師組成專家小組並開放提問：

   (1) USDA-ARS 的 Cyril Gay 提問：「我們是否知道腸道益生菌產生的代謝產物，其中有多少種類的代謝物會影響健康及免疫反應？」

   - Tun, Hein Min 回應：「在我們人類的研究，確實有許多腸道益生菌的代謝產物資料，但至今我們仍無代謝產物與健康、免疫反應產生關聯的資料庫，所以我認為在資料庫方面有很大的進步空間。」

- Todd Callaway 回應：「這問題很廣，我們並沒有這些資料，益生菌是否會產生一樣的代謝產物，改變宿主的生長速率及代謝？益生菌產生什麼代謝產物？我們仍嘗試瞭解中。」

(2) 一名菲律賓的獸醫師提問：「Tun, Hein Min 提到腸－腦軸線（Gut-brain axis），可否說明讓我們更加瞭解？因為關於腸道健康的研究資料太多了，而身為一個現場獸醫師尚須面臨呼吸系統問題」。

- Tun, Hein Min 回應：「我們最近進行一項研究，從健康人類腸道分離出細菌，再將其投予至新生無菌小鼠（Germ-free newborn mice），接著以流感病毒攻毒，結果發現，與對照組相比，投予健康類腸道菌的小鼠竟然存活下來了，此一定有我們未知的機制，腸道菌如何調解免疫系統及呼吸系統等」。

*（五）第三節　新型之藥物、化學物質及酵素 （**Innovative drugs, chemicals and enzymes**）*

此節有 8 篇口頭報告論文及 14 篇壁報論文展示，口頭報告重要內容摘述如下：

1. 中國農業大學的 Junjun Wang 簡報「中國減少豬隻使用抗生素策略」，抗生素已廣泛用於離乳豬，促進其生長效能及減少下痢。然而，由於抗生素抗藥性問題及動物來源食品抗生素殘留問題，迫使發展 ATA。中國 2019 年公告抗生素類生長促進劑（Antibiotic growth promoter, AGP）將於 2020 年全部退出，自 2020 年 1 月 1 日起，除了中草藥萃取物（Chinese herbal extracts）之外，停止核發 AGP 許可證，自 2020 年 7 月 1 日起禁止使用 AGP。由於 AGP 主要用於減緩離乳豬緊迫及促進其生長，為了因應此新法規，科學界及產業界被迫發展 ATA，低蛋白質飲食、嚴選飼料成分、環境管理措施等，以改善豬隻健康及生長。一般而言，ATA 為首選方法，包含酸化劑（Acidifier）、精油（Essential oil）、中鏈脂肪酸（Medium-chain fatty acids）、氧化鋅（Zinc oxide）、益生菌（Probiotics）、益菌生（Prebiotics）、寡醣（Oligosaccharide）、植物萃取物（Plant extracts）及新型已研發抗微生物胜肽（Newly developed

antimicrobial peptides），主要透過調整腸道微環境，包含上皮細胞屏障及共生菌而產生效能。另考量離乳豬腸道發育及酵素分泌功能尚未完全成熟，飼料成分應篩選易消化，低抗營養因子、低蛋白飲食但可均衡提供胺基酸需求；及補充有益纖維等方法已被廣泛測試及應用於離乳豬飲食。為了減少腸道消化的負擔，個別飼料成分使用前處理、酵素、發酵及熱處理等方式愈來愈流行，其可提供更多營養、更小的分子量、增加消化能力、促進吸收及減少飼料中抗營養因子及減少病原。最後，提供溫暖及乾淨環境，可有效控制離乳豬下痢，及時與合理之糞肥管理提供乾淨的空間，促進豬隻生長。總而言之，前述方法為中國目前努力減少飼料抗生素的措施，並促進畜牧產業的綠色發展（Green development）。

2. USDA-ARS 的 Judy Chen 簡報「於分子生物時代，以非抗生素物質治療蜜蜂疾病」，依據研究結果，RNA 干擾療法（RNA interference therapeutics）可有效治療蜜蜂疾病。
3. 中國農業科學院的 Bing Li 簡報「Gly-substituted DLP4 陽離子胜肽抵抗 *Staphylococcus aureus* CVCC 546 的體內及體外試驗」，研究結果顯示，抗 *S. aureus* 的 D13 多重機制作用可大大降低目標微生物產生抗藥性，D13 具有 ATA 潛力，可作為家畜 *S. aureus* 感染症的新型 ATA。
4. 丹麥 Novozymes 生物科技公司的 Christian Nyffenegger 簡報「細菌死亡後的細胞碎片－胃腸道中的 peptidoglycan」，Peptidoglycan 為細菌細胞壁的主要成分，細菌死亡後，這些大型、不可溶的細菌碎片會存留在腸道，阻礙動物攝取營養，所以你需要新型微生物溶菌酶（Novel microbial muramidase）將之分解及清除，則會改善腸道功能。
5. 瑞士 DSM 製藥公司的 Pietro Celi 簡報「優化胃腸道功能以對抗 AMR－全面性療法」，優化腸道功能最重要的方法為促使營養物質易消化、維持宿主生理功能（如先天及後天免疫反應）及維持對益生菌所需的微環境。為確保動物健康及福祉，必須謹慎使用抗生素、使用替代性物質及新技術，並結合飼養場衛生管理，以取代抗生素類生長促進劑（AGP）及減少預防性抗生素的使用，進而減少 AMR。由於法規不斷限制抗生

素的使用，促使益生菌及腸道保健產業迅速發展。各國/地區抗生素使用的法規環境摘陳如下：

(1) 美國：

- 2017 年 1 月刪除醫療重要抗生素（Medically important antibiotics）生長促進或改善生長效能之用途。
- 醫療重要抗生素自非處方箋取得管道（Over-the-counter [OTC] availability）轉變為獸醫師監管下始取得使用。
- 離子型抗球蟲藥（Ionophores）、Bacitracins、Bambermycins 不被認為是醫療重要抗生素。

(2) 拉丁美洲：

- 核准使用 AGP（除了巴西 2016 年 11 月禁止 Colistin 作為 AGP）。
- 准用所有抗球蟲藥（離子型抗球蟲藥及化學物質），未來並無規劃限制其使用。
- 不斷發展無抗生素飼養。

(3) 歐盟：

- 2006 年禁用抗生素類生長促進劑（AGP），不含家禽抗球蟲藥（例如離子型抗球蟲藥）。
- 2010 年針對動物性產品，透過監測抗生素的使用，進行抗生素減量計畫（Antibiotic reduction plan）。
- 准許在獸醫師監管下使用抗生素治療。
- 大多數鄰近歐盟的國家採用類似的法律。
- 出口至歐盟的國家具有類似的法律。
- 未來將有低鋅、低銅飲食的新法規。

(4) 中國：

- 2016 年 11 月禁止 Colistin 作為 AGP。
- 開始國家型 AMR 行動方案。
- 計畫禁止其他 AGP 及抗球蟲藥。
- 無 AGP 飼養受到重視。

(5) 亞洲及太平洋地區（APAC）

- 印尼：2018 年 1 月禁用 AGP。
- 孟加拉國禁用 AGP，但未確實執行。
- 其他國家：持續發展無 AGP 飼養 （泰國、南韓、台灣）。

6. 加拿大曼尼托巴大學（University of Manitoba）的 Chengbo Yang 簡報「單胃動物使用有機酸作為 ATA」，有機酸主要被用來防止食品腐敗及延長保存期限，進而被關注其是否可用於對抗細菌性疾病。目前已證實有機酸可增加單胃動物生產效能，具 ATA 潛力。不同種類有機酸與其他物質（例如精油）混合，可使有機酸的效果產生加乘作用。
7. 新加坡亞太區 DSM 製藥公司的 Thau Kiong Chung 簡報「無抗生素飼料管理計畫之商用肉雞必不可缺的工具－25-OH-D3」，根據 2017 年 31 個歐盟會員國，產食動物之抗微生物藥品銷售量占比分別如下，Tetracyclines 為 30.4%、Pencillines 為 26.9%、Sulfonamides 為 9.2%、Macrolides 為 7.4%、Lincosamides 為 7.3%、Aminoglycoside 為 4.6%、Polymyxins 為 3.4%、Pleuromutilins 為 3.3%、Fluoroquinolones 為 2.2%、Trimethoprim 為 1.4%、其他類抗微生物藥品為 3.8%。無抗生素飼養計畫造成商用肉雞腸炎的發生率增加。腸炎常為有臨床症狀/無臨床症狀之球菌感染症；或梭菌感染症併發球菌感染症，進而造成壞死性腸炎。25-OH-D3（一種維生素 D3 代謝物）已被證實參與抗發炎反應。在實驗室感染的情況下，25-OH-D3 可作為肉雞無抗生素飼養計畫的例行性使用工具，減緩腸炎之炎症反應。
8. 丹麥農業知識研究院（SEGES）的 Poul Jesper Baekbo 簡報「動物用抗微生物藥品替代物質（Alternatives to Veterinary Antimicrobials, AVANT）－聚焦豬下痢之新歐盟計畫」，AVANT 為歐盟 5 年期的創新行動計畫（Innovation action project），始於 2020 年 1 月。目標是研發及測試新型 ATA 於離乳豬下痢之效果。離乳豬下痢為全世界家畜使用最多抗微生物藥品的疾病之一，而治療毒素型大腸桿菌（Enterotoxigenic Escherichia coli, ETEC）感染症的 Colistin 及氧化鋅（Zinc oxide）缺乏有效的替代物質。AVANT 策略目標為聚焦於佔歐盟抗微生物使用量最大的目標動物別及其疾病，證明透過田野措施（Field interventions）可

顯著降低抗微生物藥品使用量（Antimicrobial consumption）。預期創新措施可減少抗微生物藥品使用量，減少疾病發生及減少生產損失，此將帶給動物保健產業革命性觀念及使 AMR 傳遞風險最小化，對商業界及社會均帶來重大影響，另對動物福利、公共衛生及經濟帶來正面影響。AVANT 臨床前研究包含 7 項措施組合，本研究將執行安全性試驗及效力試驗，同時優化產品劑型及投予方式。這些措施包含共生菌產品（Symbiotic product）、牧場端之糞便微生物移植（In-farm fecal transplantation）、抗腸出血型大腸桿菌嗜菌體及聚合體產品（Anti-EHEC phage- and polymer-based products）、免疫刺激劑（Immunostimulants）；及懷孕母豬或離乳豬的飼養策略。最具前景的方法將被選用於牧場試驗，以評估其臨床效力。此外，於試驗牧場端計算這些方法促使抗微生物藥品使用之減少量，再以歐盟為模型，推測這些方法可使歐盟抗微生物藥品減少之數量。AVANT 聯合集團是由 4 個產業龍頭共同組成，包含動物健康領域、4 家專門領域之小型及中型公司、5 家具聲望的大學及歐洲獸醫聯合會（Federation of Veterinarians of Europe）。這些領域共同合作可確保取得專業知識、工具及基礎建設之資源，促使研究方法迅速進展及執行。

9. 第三節的講師組成專家小組並開放提問：

(1) 英國獸醫局的 Peter Borriello 提問，Christian Nyffenegger「細菌死亡後的細胞碎片－胃腸道中的 peptidoglycan」簡報提到不需要著重於抗細菌活性，但酵素如何區分是要作用到死亡細菌，而不是作用到活細菌，雖然它可能作用到活的細菌，但不需要將活菌殺滅。

- Christian Nyffenegger 回應：「它確實沒有抗細菌活性，我們一開始從超過 10 萬種分子進行篩選，若其中一個酵素具有抗細菌活性，我們就很幸運，但最後並沒有找到。我們認為新型微生物溶菌酶（Novel microbial muramidase）可以區分活細胞及死亡細胞，因為活細胞具有內部結構支撐，使其產生內部壓力，處於張力狀態，而死亡細胞因為缺乏內部壓力，而處於鬆弛狀態。若細胞處於張力狀態，該細胞則無法有效與受質（Substrate）結合」

(2) 香港大學的 Tun, Hein Min 提問：「若使用 ATA 改善腸道健康，有時候我們無法管控碳足跡（Carbon footprints），所以如何能兼顧兩者，取得最佳的平衡？」

- 新加坡亞太區 DSM 製藥公司的 Thau Kiong Chung 回應：「此平衡難以做到。在研討會討論到使用有機酸減少疾病的發生，但同時你很難兼顧碳足跡的環境問題。此外，不是一個產品可解決所有的問題，有時你可能需要併用多個產品，並結合管理措施，以達效果。」

*（六）第四節　植物化學成分（**Phytochemicals**）*

此節有 8 篇口頭報告論文及 29 篇壁報論文展示，口頭報告重要內容摘述如下：

1. 澳大利亞墨爾本大學 （University of Melbourne）的 John Furness 簡報「腸道健康對微量營養及植物化學成分的偵測及反應」，胃腸道需要面對各式各樣的化學物質、病原及生化物質，因此胃腸道必須對這些物質進行分析及做出反應，包含優化營養吸收或對抗該物質。植物化學成分為食品之特殊成分，於食品含量低。植物營養素可減少食品腐敗、具抗微生物作用、改善嗜口性及促進腸道健康（包含免疫防禦及促進黏膜生長），然而目前對於植物化學成分的腸道受器並不完全瞭解。主要面臨的挑戰為找出植物化學物質的作用機制、效果量化評估、找出植物化學成分與微生物菌叢之交互作用及調查其對於特定生命階段（懷孕期、生長期）之效益。
2. 中國北京農業大學的 Hong Dong 簡報「白頭草湯（Pulsatilla Decoction, PD）治療 *Escherichia coli* 性下痢的作用機制」，PD 及其有效成分具有不活化細菌毒素的功能，進而保護微血管上皮細胞。Hong Dong 指出，中國 2019 年公告 2020 年禁止生長促進用之藥物飼料添加物，但不包含中獸醫藥品（Traditional Chinese Veterinary Medicine, TCVM）。習近平於 2019 年 10 月 25 日強調，中國應實踐中醫並使之創新，推動中藥走向世界，充分發揮中藥預防及治療疾病的獨特優勢和作用。

3. 中國江西中醫藥大學的 Hua Zhang 簡報「富含花青素的紫薯萃取物可減少高脂肪飲食及 Lipopolysaccharide（LPS）引起的肥胖與輕微的腸炎（Low-grade gut Inflammation）」，研究結果顯示，紫薯的 Phenolics 成分具抗炎症潛力，因此攝食深色根莖類植物具預防慢性病效及促進腸道健康。
4. USDA-ARS 的 Crystal L. Loving 簡報「食用馬鈴薯抗性澱粉改變豬隻免疫狀態及微生物菌叢以限制 *Salmonella*」，益菌生（Prebiotics）飼料添加物（例如抗性澱粉）已知可維持腸道微生物的生長及功能，並促進微生物產生 Butyrate，Butyrate 可維護菌叢恆定及調節宿主健康。研究結果顯示，馬鈴薯抗性澱粉有益於腸道微生物、宿主免疫反應及菌叢恆定，並減少人類食媒性病原於腸道定殖及排菌。
5. 美國 Full Circle Science 的 Emma Wall 簡報「邁向下一個世代之植物營養素」，植物營養素的重要性不應僅聚焦於其抗微生物活性，其對動物體的生理效果亦受到關注，例如其可改善消化、降低炎症反應及改善動物健康狀況。植物營養素及其他成分（例如益菌生）因對腸道環境產生減害反應（特別是其免疫效果），進而改善牧場動物生長效能。Emma Wall 強調，一個技術工具不應該因為法規缺乏適當定位及法規限制而阻礙其發展。
6. 墨西哥 AVT Natural 公司的 Prashant Mishra 簡報「於後抗生素時代，以科學為基礎，使用植物萃取物改善動物健康之進展」，最近的科學研究顯示，少量的植物萃取物，可直接作用於動物體，調節動物的生理反應。因現今已有各種分子生物學及基因營養學工具，故植物萃取物對基因的作用、細胞受器及發訊傳遞路徑已更加瞭解，此將有助於後抗生素時代，研發新技術，改善動物健康及生產效能。
7. 印尼 PT Medion Farma Jaya 的 Elvina Jonas Jahja 簡報「植物萃取物（Phytobiotic）於體外抗 *Eschericia coli* 及 *Mycoplasma gallisepticum* 之活性」，使用乙醇自蘇木（Sappan wood）、番石榴葉（Guava leaves）及紅薑（Red ginger rhizome）萃取之物質，具有抗 *E. coli* 及 *M. gallisepticum* 的潛力，可作為 ATA。

8. 美國 Amlan International 的 Hongyu Xue 簡報「neutrapath ™ 體內及體外抗 *Salmonella Typhimurium* 之療效評估」，體內及體外試驗的研究數據顯示，Neutrapath ™ 療法於肉雞具有減少 *S.Typhimurium* 定殖（Colonization）的潛力，且於 *S.Typhimurium* 感染期間，可保護雞腸道屏障的完整性。
9. 第四節的講師組成專家小組並開放提問：
   (1) 一名觀眾提問抗性澱粉與赤痢的關係為何？
   - Crystal L. Loving 回應：「並不是所有抗性澱粉都有一樣的效果，我們將它與其他纖維混合，用此產品做了試驗，它與大腸的黏液變化有關，我們還不完全瞭解它的機制，但確實看到效果。」

   (2) 一名觀眾提問針對 Hua Zhang 的「富含花青素的紫薯萃取物可減少高脂肪飲食及 Lipopolysaccharide（LPS）引起的肥胖與輕微的腸炎（Low-grade gut inflammation）」簡報提問：「我們每天要吃多少馬鈴薯才夠？就像是『每日一蘋果，醫生遠離我」（An apple a day keeps the doctor away.）』」。
   - Hua Zhang 表示無法回答此問題。

（七）第五節　免疫相關產物（Immune-related products）

此節有 8 篇口頭報告論文及 4 篇壁報論文展示，口頭報告重要內容摘述如下：

1. 丹麥技術大學 （Technical University of Denmark）的 Peter Heegaard 簡報「以被動免疫及類 IgG 抗體（IgG-like antibody）作為 ATA」，被動免疫方法（例如口服 IgG 及 IgY、專一性及非專一性免疫球蛋白、修飾型免疫球蛋白單方使用或搭配其他生物活性成分使用）將與免疫接種（即主動免疫）、母體免疫接種（Maternal vaccination）、品系育種、益菌生及嗜菌體方法進行比較。為達永續、使用簡易性、低成本及高效率目標，作為動物用 ATA，則需考慮其原料來源及生產方式。
2. 美國農業部國家農業研究中心（Beltsville Agricultural Research Center, United States Department of Agriculture）的 Woohyum Kim 簡報「以具抗

微生物及免疫調節活性之宿主防禦胜肽作為 ATA」，因病原抗藥性不斷增加，迫使發展抗微生物胜肽以替代抗生素。研究結果顯示，雞 NK-2 為宿主防禦胜肽，具有調節免疫的功能，包含誘發炎症反應之 Chemokines/cytokines 訊號傳遞。

3. 美國田納西大學 （University of Tennessee）的 Jun Lin 簡報「以新型腸菌素專一性之卵黃抗體控制革蘭氏陰性細菌」，專一性卵黃抗體的被動免疫為新興具前景之 ATA，可用於食用動物之各種黏膜性傳染病的預防及治療。研究人員優化蛋雞免疫接種方式，產生高免疫專一性卵黃 IgY，此抗體對於食用動物的革蘭氏陰性細菌具顯著預防及控制效果。
4. 美國奧克拉荷馬州立大學 （Oklahoma State University）的 Glenn Ahang 簡報「高量篩選宿主自然防禦胜肽化合物作為 ATA」，研究結果顯示，天然的宿主防禦胜肽誘發產生的化合物，個別或混合用於畜禽疾病的控制及預防，具有取代抗生素的潛力。
5. 加拿大阿爾伯塔大學 （University of Alberta）的 Douglas Korver 簡報「將研究試驗轉變至現場應用」，從使用抗生素作為生長促進或預防傳染病的階段，轉變使用 ATA 的過程，仍需要高標準的牧場、生物安全、管理及營養。ATA 要商品化常遇到許多障礙。第一、許多在管控下的研究缺乏適當的陰性對照組，難以證實其試驗產品與抗生素具同等效力。另外，該研究缺乏微生物感染亦無法確定其效力是否等同抗生素類生長促進劑（AGP），具有減少感染的效果。第二、在管控下的實驗常利用一個特定疾病作為模型。其抗微生物感染方面可能顯示具有效果，但在現場微生物感染的情況下，可能因不同區域、不同牧場及不同時間而有效果差異性。第三、即使研究採用自然感染模型，仍受到特定環境的限制。最後，在現場可能遭遇不同病原的感染，故以單一作用機制的 ATA 產品可能無法有效對抗各種病原。總之，無 AGP 飼養模式可能需要使用不同的 ATA 產品，互補彼此作用機制的不足，因此需要客製化的 ATA 產品。
6. 荷蘭烏特勒支大學（Utrecht University）的 Henk Haagsman 簡報「重新編製（Reprogramming）先天性免疫反應作為 ATA」，免疫接種的目標

係要誘發專一性免疫記憶，產生足夠的後天性免疫以對抗特定病原。然而，愈來愈多的證據顯示，有些廣泛使用的疫苗以特定的方式在對抗無相關的病原。此基因保護作用，可能可用先天性免疫記憶（或稱為受訓免疫反應 [Trained immunity]）來解釋。近年研究發現先天性免疫反應細胞，例如巨噬細胞及自然殺手細胞顯現具持續性記憶，此持續記憶可幫助這些細胞對抗再感染。各種不同的微生物產物顯示具有「訓練（“Train”）」先天性免疫反應的效果，而這些效果似乎係因表觀遺傳學（Epigenetics）的變化發揮作用所致。宿主防禦胜肽（Host defense peptides, HDPs）為存在所有脊椎動物的小型分子，其具抗微生物及免疫調節活性。Cathelicidins 為帶正電的 HDPs，其參與宿主初期對抗入侵病原的免疫反應。這些 HDPs 係白血球及上皮細胞在與病原微生物產生交互作用後，於黏膜表面分泌產生。若將 Cathelicidins 或 Cathelicidins 來源胜肽投予至實驗動物，可見這些胜肽對動物對後續的感染產生保護力。這些保護作用，部分可以用先天性免疫系統的訓練來解釋。因此，結論為透過微生物產物重新編製牧場動物的先天免疫系統，HDPs 具有減少抗生素使用量的潛力。

7. 美國禮來動物保健（Elanco Animl Health）公司的 Jeffery Escobar 簡報「雄性肉雞受到實驗室感染引起壞死性腸炎期間，投予乾燥蛋品（Dried egg product, DEP）之效力」，於相同環境下，同時有球蟲免疫接種的情況下，相較於未補充 DEP 的雞隻，補充 DEP 的雞隻生長效能明顯改善。
8. 巴西 ICC 的 Ekachai Jenwitheesuk 簡報「肉雞 *Salmonella Enteritidis* 感染症—酵母細胞壁（Yeast cell wall, YCW）之免疫調節效果及對腸道完整性之療效」，本研究的目標為肉雞受到 *S. Enteritidis* 感染後，評估使用 YCW 之療效。YCW 的治療可改善盲腸淋巴球性炎症反應，但無法改善肝臟的炎症反應。受 *S. Enteritidis* 感染的肉雞攝取 YCW 可改善腸道完整性。
9. 第五節的講師組成專家小組並開放提問：

   (1) 一名觀眾提問：「是否其實已存在抗病基因的動物並可做基因篩選

育種，所以我們不需要去找具潛在抗病基因的動物？我們可否就基因多樣性，找尋某些具抗病基因而不需要抗生素的動物（Some resistant animal that they do not need antibiotics）？」

- 一名講師回應：「基因學在動物免疫調節效果方面相當重要，但在此研究沒納入此變因，所以我沒有答案，我想在未來新的試驗，我們可以將之納入參數。」

(2) 香港大學的 Tun, Hein Min 向 Douglas Korver 提問：「簡報提到要改善實驗設計，許多營養試驗，特別是 ATA 顯示具生長促進效果，他們使用非常乾淨的環境進行試驗，一組使用抗生素、另一組使用試驗產品，而最終產生的結果可能係因乾淨的環境所致，而在骯髒的環境，特別是開發中的國家，就不會有那樣的效果，所以您的簡報對於產業界及學術界的實驗設計有什麼建議？」

- Douglas Korver 回應：「你需要將攻毒（Challenge）引入，在大學內，我們可用很糟的衛生環境以增加攻毒，我們看到的某些效果，歸功於益生菌，但其實並不是益生菌的效果。因此，當你在做實驗時，若你看到實驗組及對照組無顯著差異時，你要去確認攻毒是否成功。」

*（八）第六節　ATA 登記上市之法規及鼓勵利害關係者研發（**Regulatory pathways to enable the licensing of alternatives to antibiotics and incentives from stakeholders to support their development**）*

此節有 4 篇口頭報告，口頭報告重要內容摘述如下：

1. 美國食品藥物管理署動物藥品中心（Food and Drug Administration, Center for Veterinary Medicine, FDA-CVM）的 Joshua Hayes 簡報「美國 FDA 動物抗微生物藥品替代物質的法規」，由於 AMR 受到的關注增加，促使 ATA 產品的研發、認定及上市需求增加。美國依據多種因素以決定哪個單位為評估產品的主管機關，決定因素包含產品、主要作用機制及標示宣稱。FDA-CVM 業管法規為「聯邦食品、藥品和化妝品法案（FFDCA）」，管理範圍包含食品添加物、飼料添加物、動物用藥及公認安全物質（Generally recognized as safe, GRAS）。GRAS 為美國 FDA

針對化學物質或是食品添加物的分類，GRAS 指專家認為此種化學物質或食品添加物係安全的，因此可以不受「美國聯邦食品、藥品及化妝品法規（FFDCA）」中食品殘留容許量的限制。若該物質的主作用為非直接刺激免疫系統，並擬用於診斷、治療、減緩傷害或預防疾病者；或影響人體或其他動物結構或功能者，則被判定為新型動物用藥品。新型動物用藥品係依據效力試驗，核准特定適應症 （Specific indications）及使用情形 （例如：療程、動物別、品種、停藥期及處方箋等級等），同時須兼顧目標動物的風險、人類食品安全、人類使用者的安全及環境影響。美國國會過去已建立法定標準，以評估這些成分。過去的法規標準可能面臨有些物質係「沒有標準」方法的問題，包含這些物質可能係已發表的研究、國外研究或模型驗證的研究。FDA-CVM 已制定流程，協助具有創新科技（包含 ATA）的贊助商，並與贊助商討論研發初期階段的計畫核准程序。FDA-CVM 亦與國際法規機構合作，以減少全球登記上市的差異性，促使藥品核准上市。FDA-CVM 亦以免申請費用的方式作為藥品研發的誘因，使申請者有意願投入 ATA 的研發。

- **FDA-CVM 核准動物用藥程序**：第（1）項為調查新型動物用藥（Investigational New Animal Drug, INAD）文件：此程序分兩階段，分別為（A）送件前的討論會（Presubmission conference, PSC），同意此研發計畫。（B）送件資料包含作業程序、研究、數據資料、文獻報告及其他資訊。第（2）項為申請新型動物用藥。FDA-CVM 為了鼓勵新型動物用藥審查，故於第（1）項程序之前，再新增 1 項前置作業，包含研發早期階段的諮詢（Early information）、技術團隊（Tech Teams）及專案工作小組（Focus Groups）。研發早期階段的諮詢可使 FDA-CVM 及藥品研發贊助商及早進行資訊交流及討論。目標是於送件前的討論會（PSC）階段，使研發贊助商有效率符合調查所需要件，過程可能經歷政府與研發贊助商不斷來回的科學資訊交流及討論，並相互學習。
- **研發早期階段的諮詢（Early information）**：所有贊助商都可向 FDA-CVM 諮詢，並聚焦於研發贊助商所提出的單一產品。FDA-CVM 提

供贊助商早期研發諮詢，使贊助商所提出的研發計畫得以符合 FDA-CVM 的要求，此諮詢可在調查新型動物用藥（INAD）期間或之前。

- **技術團隊（Tech Teams）**：建立技術團隊使 FDA-CVM 能夠與贊助商共同學習新型科技，此有助於啟動調查新型動物用藥（INAD）後，減少 FDA-CVM 學習該技術所需的時間，並進行資訊交流。
- **專案工作小組（Focus Groups）**：專案工作小組為內部團隊，這些工作小組可能係與一個贊助商交流後而組成，負責處理廣泛性的議題，可能聚焦於技術方面，例如生物標記（Biomarkers）或流程改善，並提供審核意見。
- **ATA 需考量的問題**：（1）預期效果為何？（2）如何使用？（3）是否可符合 GMPs？（4）是否預期要改變產品製造方式？（5）是全身性作用或局部性作用？（6）此產品目標對象為小眾族群嗎？（7）是否有食安殘留問題？
- **FDA-CVM 公共衛生之目標**：目標是針對使用者，核准安全、有效、良好製造品質、適當標示的產品，新型動物用藥須達到治療效果及符合動物需求。
- **國際合作**：（1）法規合作委員會（Regulatory Cooperation Council）同時審視美國 FDA 及加拿大衛生部動物用藥處（Veterinary Drug Directorate, VDD）法規。（2）美國 FDA 每季與歐洲藥品管理局（European Medicines Agency, EMA）進行科學家之間的會談，並提供「同步科學諮詢」（Parallel scientific advice），所謂「同步科學諮詢」係指藥品研發贊助商的疑問可同時收到美國 FDA 及歐洲藥品管理局的諮詢回應，此使得新藥在核准之前的階段，美國 FDA 與歐洲藥品管理局之間即可進行調和及合作。透過減少分歧的研究，促進全球核准藥品登記上市。（3）參與國際動物用藥品檢驗登記技術資料一致化（Veterinary International Conference on Harmonization, VICH）。（4）法規管理者（Regulator）之間合作的其他機會（MOU 或與其他國家的機密協議）。
- **推動核准登記上市的全球計畫**：（1）不同國家的法規機關共享科學

資訊（2）單一套研究由多國核准（3）使已有的國內、國外科學資料物盡其用（4）使各國產品標示趨向一致化

- 2019 年 7 月美國 FDA 會議將新型動物用藥臨床調查（INAD）納入 ATA，而在 2020 年 7 月預計將有產業界指引（Guidance for Industry, GFI）。
- **鼓勵研發**：美國聯邦食品、藥品及化妝品法規（FFDCA）針對下列對象提供申請費用的減價或免申請費（1）符合特定條件的創新技術面臨重大障礙 （Significant barrier to innovation）。（2）審查過程之相關費用超過預期成本。（3）添加於任食飼料（Free choice feeds）的特定動物藥品。（4）供小眾動物別使用或小量使用。（5）小型公司。可參考「產業界指引（Guidance for Industry, GFI）」第 170 條（如附件 2）。
- **結論**：美國 FDA 認為該國有核准產品的法規途徑，必須符合其國會訂定的法規條件，並有流程指引，鼓勵業者早期階段溝通交流。業界負責產品研發，FDA 確保產品符合條件。

2. 歐洲藥品管理局（EMA）的 Javier Pozo 簡報「歐盟促進動物用抗微生物藥品替代物之核准」，全世界認為 AMR 對人類及動物健康造成重大威脅。對抗 AMR 其中一項重要基礎為鼓勵研發新型替代藥品以預防或治療抗藥性微生物感染及減少傳統抗微生物藥品（Conventional antimicrobials）的使用，此亦列為 EMA 及歐洲藥品法規之高度優先工作項目。EMA 的動物藥品委員會（Committee of the Veterinary Medicinal Products, CVMP）2016~2020 年抗微生物藥品策略包含研析促進 ATA 研發及上市策略。歐盟 2019/6 新法規（New Regulation （EU） 2019/6）強化因應 AMR 措施及鼓勵創新技術。CVMP 已草擬「歐盟促進抗微生物藥品替代物質核准登記上市」之意見反映報告（如附件 3），目標為進行現行措施之缺口分析及促進可替代傳統抗微生物藥品之動物藥品研發、核准及使用，並特別聚焦於動物用 ATA 領域。缺口的認定係依據類似產品之前在 EMA 的經驗、其他地區法規管理者（Regulator）的討論、利害關係者的回饋及檢視之前研討會的結果。已認定的缺口主要

為：

(1) **法規架構缺口**：現行歐盟法規架構（例如缺乏一致性的專有名詞、產品分類不明確、缺乏特定指引）顯示於法律上需建立適當條件、進行調和及制定技術要求的特定指引，以核准此類產品。根據其外觀（Presentation）、使用目的、標示宣稱，可能被分類為動物用藥品（VMP）、飼料添加物（Feed additive）或殺蟲劑（Biocide）。不同類別產品適用於不同的法規及主管機關，動物用藥品（VMP）的主管機關為歐洲藥品管理局（EMA），適用法規為 Directive 2001/82/EC;飼料添加物的主管機關為歐洲食品安全局（European Food Safety Authority, EFSA），適用法規為 Regulation（EC）1831/2003;殺蟲劑（Biocide）的主管機關為歐洲化學品管理局（European Chemical Authority, ECHA），適用法規為 Regulation（EC）528/2012。新方法及技術無法適用於現行歐盟法規，需考量如何設計試驗以支持 ATA 的安全性及臨床療效。

(2) **支持 ATA 研發及申請者的缺口**：創新工作小組（Innovation task force, ITF）協助研發者及申請者於產品研發及申請初期階段，取得科學上、法規上、程序上的諮詢，增加對小型、中型公司的誘因，幫助最具前景的 ATA 核准上市。研發後期階段、送件前或評估期間亦給予科學意見。針對研發後期及特定產品進行送件前討論會議（Pre-submission meetings）。創新動物醫療任務小組（Ad Hoc Group on Veterinary Novel Therapies, ADVENT）（新型治療方式）提供一般性指引，例如針對特定議題編撰 Q&A 文件。誘因鼓勵方面，以小型及中型公司計畫方案（Small and medium-sized enterprise scheme, SEM）提供行政、法規及財務方面的支援。以小眾動物別使用且用量少（Minor use minor species, MUMS）、市場需求有限之計畫方案，針對產食動物，調整資料條件要求及提供財務誘因。

(3) **與利害關係者合作與溝通交流方面的缺口**：於 ATA 研發方面，建立與利害關係者交流溝通平台，草擬藍圖建立工作項目的優先順序，並設定目標，追蹤所提出可行性建議措施之執行成效。此議題納入

EMA 的 2025 年法規科學計畫之優先議題，另進行國際交流。

結論：分析結果已確認出許多缺口，並提出對應措施，促進歐盟動物用 ATA 的研發、核准及使用。此仍需要長程計畫措施及一套與利害關係者於法規面及產業面相互協調與合作。歐盟的動物藥品委員會（CVMP）之意見反映報告已公開於 EMA 網站（https://www.ema.europa.eu/en/cvmp-reflection-paper-promoting-authorisation-alternatives-antimicrobials-eu，如附件 3）。

3. 日本農林水產省（Ministry of Agriculture, Forestry and Fisheries, MAFF）的 Takashi Kozasa 簡報「ATA 的核准/訂定之法規架構」，日本刻正推廣 ATA 發展以對抗動物病原微生物之抗藥性。因應 AMR 全球性威脅，日本首相安倍晋三召開部長級委員會（Ministerial Council），並有下列重要決定：（1）日本政府 2016 年 4 月 5 日採納 AMR 國家行動方案。（2）日本將針對人類及動物發展有效措施。（3）日本將領導國際措施的進展，例如支持其他國家的行動方案。（4）日本首相要求所有相關部會首長共同密切合作，使相關措施穩定發展。日本 AMR 國家行動方案 6 大目標之動物領域執行重點：（1）提高警覺性及教育：提高利害關係者的警覺性，包含畜禽飼養業者。（2）調查及監測：進一步促進人類及動物衛生之間的合作。擴大調查及監測範圍至水產養殖業。針對伴侶動物（Companion animals）建立監測及調查系統。（3）預防及控制感染：確保牧場符合衛生管理標準（Standards of Rearing Hygiene Management）。（4）適當使用抗微生物藥品：根據風險評估，徹底實施風險管理措施，另進一步推廣謹慎使用抗微生物藥品。（5）研究及發展：促進 ATA 研究及發展，包含疫苗。（6）國際合作：參與亞洲區域合作。

ATA 包含疫苗、細胞素（Cytokines）、酵素、免疫調解劑（Immunomodulators）、免疫刺激物（Immunostimulants）、有機酸（Organic acids）、益生菌（Probiotics）、草藥（Herbal medicines）及嗜菌體（Bacteriophages）等等。日本依據產品的活性成分、標示宣稱及使用目的，將之分類為動物用藥品（VMPs）及飼料添加物（Feed

additives）。VMPs 法規為醫藥品及醫療機器等產品的品質、有效性及安全性確保法（The Act on Securing Quality, Efficacy and Safety of Products including Pharmaceuticals and Medical Devices），簡稱藥物及醫療器材法（Pharmaceutical and Medical Device Act, PMD Act），規範的產品包含人類及動物醫療產品 （VMPs）於研發、製造、進口、行銷（Marketing）、零售（Retailing）及使用的每個階段。依據日本藥物及醫療器材法（PMD Act）第 2 條，藥物及醫療器材法所稱一般藥品（Pharmaceutical）定義係指下列項目：（1）日本藥典（Japanese Pharmacopoeia）所列品項。（2）用於診斷、治療或預防人類或動物疾病的品項。（3）影響人類或動物體的結構及功能的品項，且非為醫療用品或器材等（Medical appliances or instruments, etc.）。依據此法規，若業者擬上市 VMP，則需取得日本農林水產省的上市核准。若 VMP 的文件資料顯示該產品不具療效、有害副作用效果比治療效果強或品質不良，則不予核准。

飼料添加物法規為確保飼料安全及品質改善法規（The Act on Safety Assurance and Quality Improvement of Feeds），簡稱飼料安全法（Feed Safety Act）。此法規目標係透過規範飼料及飼料添加物，制定飼料規範、檢驗飼料是否符合主管機關規範，以確保飼料安全及品質，進而確保畜禽產品具公共安全性。該法規對於飼料添加物定義為透過添加、混合、浸潤的方式，預防飼料品質的惡化、提供營養成分及其他飼料的有效成分，促進飼料營養有效率被使用。此法規係由日本農林水產省諮詢農業材料委員會（Agricultural Materials Council）後制定。農業材料委員會負責審業者飼料添加物申請資料之查效力（Efficacy）、殘留（Residue）及安全性（日本政府核准/訂定動物用藥品及飼料添加物之程序如**第 36 頁**）。

日本促進 ATA 研究及發展重點摘陳如下：

(1) 動物用藥品（VMPs）為控制動物疾病的必要工具。飼料添加物對於生產之健康動物很重要。日本產業積極參與世界動物衛生之議題。

(2) 日本主管機關的任務為儘早提供安全、有效、高品質之產品供獸醫

師、農戶及寵物飼主使用。為達此目標，日本主管機關努力改良動物用藥品及飼料添加物之核准/訂定程序。

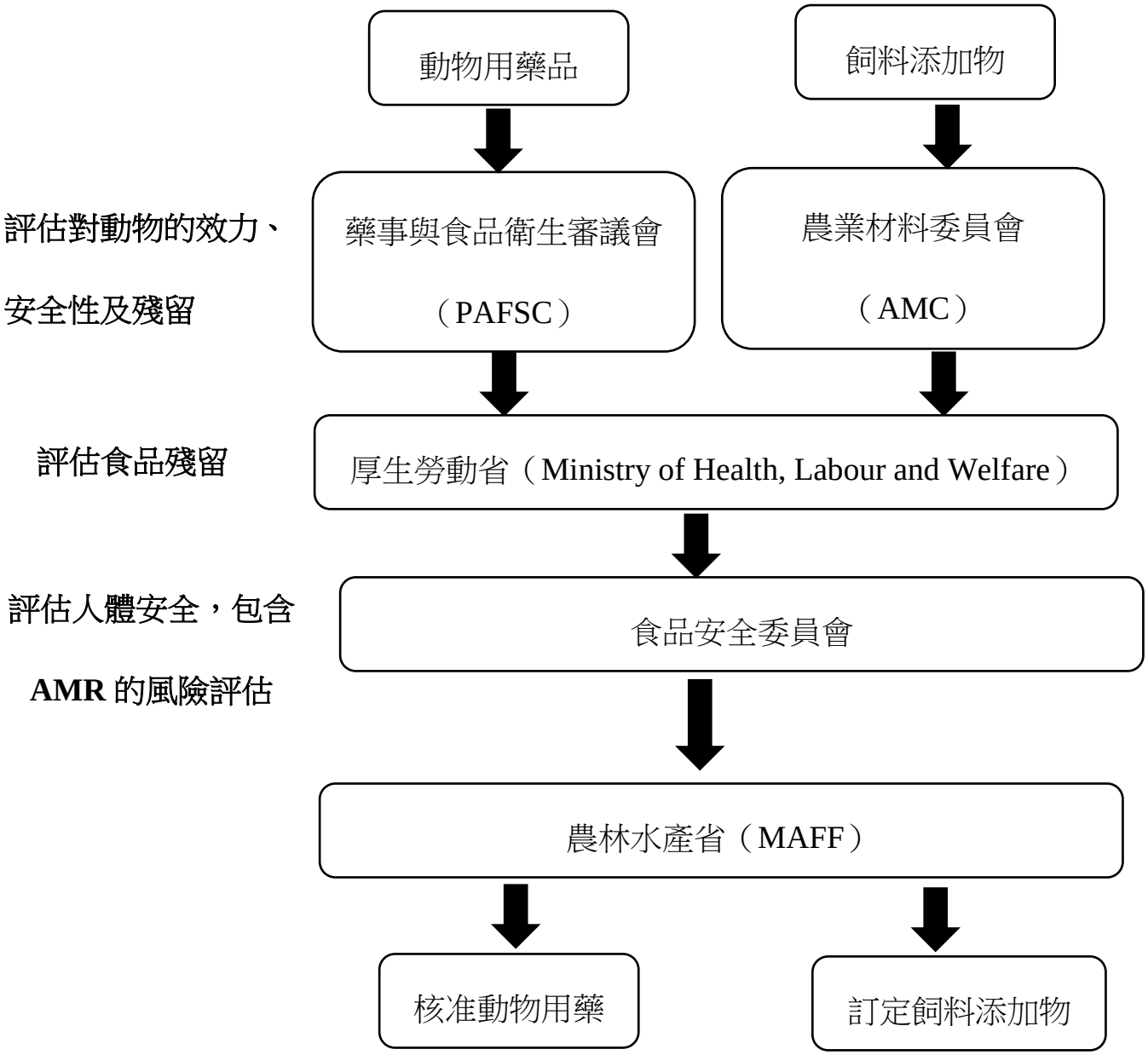
(3) 日本透過於 ATA 研發最後階段提供財務支援，以促進 ATA 發展。

(4) 技術指引為申請者研究及發展 ATA 的基礎，且由主管機關進行審查。日本促進動物用藥品發展新型國家及國際指引，包含產業界及學術界。

(5) 就對抗 AMR 的動物用藥品方面，考慮加速其核准速度。

(6) 維持主管機關及產業界之間的溝通交流係加速及核准 ATA 重要之措施，亦有助於未來之動物健康及公共衛生。

圖 1：日本政府核准/訂定動物用藥品及飼料添加物之程序：



4. 全球動物醫療健康協會（Health for animals global animal medicines association）的 Erik De Ridder 簡報「以業界角度看 ATA 登記上市」，基於動物及公共衛生的立場，對抗 AMR 的需求無庸置疑。鑒於過去幾十年，新型動物用抗生素治療選擇逐漸減少，故迫切需要有替代物質核准上市，以替代現有的抗生素。ATA 種類繁多，相較於以功能定義的抗生素，ATA 的定義並沒有很明確。此外，動物用藥品（Veterinary medicinal product, VMP）的定義可能因地區性而有差異。目前雖有典型 VMPs （疫苗、植物化學成分 [Phytochemicals] 等其他物質）及添加物（有機酸、草藥 [Herbal]、植物性化合物 [Botanical compounds]、礦物性化合物 [Mineral compounds]等其他物質）分類定義，但有一些物質（嗜菌體、飼料中的抗體、特定免疫調節劑、基因編碼技術來源產物）不易依據前述方式分類（歐盟動物藥物委員會 [Committee for Medicinal Products for Veterinary Use, CVMP]於 2019 年列出的 ATA 案例如**表 1**）。

表 1：歐盟動物藥物委員會於 2019 年列出的 ATA 案例

歐盟飼料添加物 （**Feed additives**）	模稜兩可產品 （**Borderline**）	歐盟動物用藥品 （**Veterinary drugs**）
有機酸 （Organic acids）	競爭排斥性產品 （Competitive exclusion product）	疫苗
草本/植物成分 （Herbals/botanicals）	嗜菌體 （Bacteriophages）	抗微生物胜肽 （antimicrobial peptides）
益生菌（Prebiotics）	益生菌與益生質的混合物（Symbiotics）	植物化學成分 （Phytochemicals）
礦物質（例如 ZnO）	飼料添加用之抗體（In-feed antibodies）	具物理功能之材料（Physical devices），例如「乳頭封膠（Teat sealant）」阻斷細菌入侵。
吸附毒素的產品（Toxin binding products）	基因編輯來源產品 （Gene editing derived products e.g. CRISPR-Cas9）	-
-	非專一性免疫刺激劑 （Non-specific immunostimulators）	-

ATA 法規面的障礙很多，包含產業及主管機關層面的障礙。第一項障礙為前述所提 ATA 缺乏定義及明確的分類，以核准新技術，進而產生第二個障礙。第二項障礙為 ATA 定義不明確，造成適用的法規及主管機關不明確。現行法規根據產品及標示宣稱雖給予明確定義，但無法處理新技術的模稜兩可及其特殊性質。主管機關目前對於是否同意 ATA 產品的標示宣稱有疑慮，例如可否標示「減少抗生素的治療需求及使用」之類似宣稱。新技術

及方法的安全性及效力評估將需要新的評估系統。「全球法規調和」係第三項障礙。VICH 應作為一個平台，讓業者主動定義技術條件及新技術。協會不斷在關注地區性或區域性的法規架構趨勢。動物健康醫療產業（Animal health industry）及所有利害關係者必須支持 OIE 提出現代化及具彈性的法規系統，以管控違法及偽造藥品，落實 OIE 之一般標準。第四項障礙為公眾和消費者是否接受新技術，即使有科學方面的支持，但仍不容易被接受。例如以基因技術複製出的第一個哺乳動物桃莉羊（Dolly），基於道德上並不被社會大眾接受，需要有與利害關係者積極溝通交流的措施。第五項至關重要的障礙為應建立保護創新資料的法規環境，即使此環境具風險及不確定性，但仍可鼓勵及刺激技術的創新，使原創的資料可充分被保護，並使該業者有足夠的時間取得投資報酬。動物健康醫療的市場相較人類的市場，規模小很多。2016 年的資料顯示全球人類醫療健康市場規模達 1 兆 1050 億美元，而全球動物醫療健康市場規模僅 320 億美元。動物健康醫療產業取得投資報酬的時間較長及利潤較小。

動物健康醫療產業已在抗生素領域長期投入資源。自 2017 年起，該協會已律定 5 項「抗生素承諾 （Antibiotics Commitment）」原則，這些指引原則包含謹慎及負責任使用抗生素、疾病預防的推廣、增加產品及專業技能取得的管道、投資產品（包含 ATA 產品）的研發，以預防及治療疾病。「抗生素承諾 （Antibiotics Commitment）」摘陳如下：

(1) 在防疫一體環境（One Health environment）考量下，對 AMR 應具負責任的態度：

A. 抗生素為現代醫療及公共衛生的基礎。

B. 抗生素是治療細菌性疾病唯一的方法。目前沒有替代物質。

C. 於所有領域研發減少醫療重要抗生素需求的動物健康醫療工具。

D. 使用這些工具可改善動物疾病的預防、偵測及治療。

(2) 就動物健康領域，承諾對抗 AMR：

A. 2017 年全球動物醫療健康協會發起 2025 年之前「抗生素承諾」（英文原文及中文翻譯如附件 4）。

B. 2019 年全球動物醫療健康協會訂定「減少抗生素需求的指引」（Roadmap to reducing the need for antibiotics）（如附件 5）。減少抗生素需求以對抗 AMR 分 3 項措施，第 1 項為疾病檢測，即改善疾病監測及診斷工具。第 2 項為改善預防措施，包含改善疫苗、生物安全及健康的工具。第 3 項為改善治療方式，必須負責任使用抗生素。預防及治療疾病措施尚包含主動研發 ATA。

5. 第六節的講師及其他相關學術界、政府機關代表組成專家小組，開放提問。

(1) 美國 Full Circle Science 的 Emma Wall 提問：「主管機關（Regulatory agencies）如何有法規架構定義研討會上所提的 ATA，支持這些 ATA 並落實執行，同時又保護原創者及末端使用者？若有世界衛生組織（World Health Organization, WHO）的計畫整合這些措施（Approaches），WHO 如何使主管機關採納及支持這些措施？如何整合及驗證（Validating）這些傳統措施？當我看到這些法規定義，我看不到可適用於人類或動物傳統藥品（Traditional medicine）的法規空間。」

- 美國 FDA-CVM 的 Joshua Hayes 回應：「老實說，我並不清楚鼓勵這些措施發展的特定修正法規。若有贊助商向 FDA 表示擬申請傳統藥品（Traditional medicine）核准，FDA 將評估其效益及風險，我認為應無障礙」。Emma Wall 回應，資金是很大的障礙。
- 主持人 Peter Borriello（英國獸醫局，Veterinary Medicine Directorate）進一步表示意見，除非產業界能挑選一個產品經營，證實其有效並準備上市，符合市場最大需求，以取得相對報酬，否則產業界不會為之。益生菌在歐盟因無足夠證據而無醫療宣稱，但許多研究顯示其有益無害。是否有產品具很大的效益潛力，而尚未使用於動物或人類，因為其實證所需資金係巨大的障礙，是否有此類產品，Peter 表示不清楚，並請代表產業界的 Erik 表示意見。
- 全球動物醫療健康協會的 Erik 表示：「使業者掙扎是否要研發某傳統或替代藥品的其中一項因素為證實其效力，找出效益，有時很困難。非僅係業者自我闡述，早期階段與法規單位溝通可能會很有幫助嗎？這問題不僅發生於單一國家。因為若業者需要想出一個方法

證實某新類型產品之效力及品質，業者在不同的國家，則需要以不同的方法證實其效力及品質，包含美國、歐盟、日本及中國。業者不僅要證實安全性，尚包含效力及效益。」

- 美國衛生及公共服務部（Department of Health and Human Services）的 Amanda 表示：「我想強調跨領域連結的力量（Strength of networking），研討會不僅針對動物健康，亦考量對抗 AMR 之公共衛生全方位措施。因此，建議進行感染預防討論會議，同時討論其與人類健康的連結性，此可連結立法者（Legislators）關注新產品，某些疾病可能是立法者的姪女或外甥罹患，你永遠不會知道」。Amanda 表示，只是想強調跨領域連結的參與，整合人類健康因素及產品的效益。

(2) OIE 的 Elisabeth 提問：「雖然 OIE 不是法規主管機關（Regulating agency），但是有一項困難是『分類』。我們需要什麼類型的網絡或工作小組，以提供全球性概念？我們在這討論某一產品（Product X），你如何規範它，而產業界能接受，也因此法規主管機關可執行。」

- 美國 FDA-CVM 的 Joshua Hayes 回應，Joshua 認為 VICH 就是一個進行調和的平台，此外，個別的網絡聯繫（Individual network connections）亦可行。
- 歐洲藥品管理局的 Javier 回應，第一點為法律層面，對歐盟而言，不同的分類係由不同的法規主管機關業管，所以機關之間的合作很重要。第二點為協調合作的工作小組可提供分類方面建議。最後，不同的區域可能需要進行調和，所以 VICH 應該是一個很好的交流平台。
- 日本農林水產省的 Takashi Kozasa 回應：「我們每年都有 VICH 會議，這是一個討論『替代物質』分類很好的平台。」
- 美國衛生及公共服務部的 Amanda 回應：「這類型問題建議寄到我們的信箱，我們的委員會將研讀所有資訊並探索未來的可能性。」
- 全球動物醫療健康協會的 Erik 回應，VICH 是一個絕佳的組織，但它會設定一些技術條件及限制。而這需要產業界的專家學者及主管

機關共同合作。產業界強烈支持法規主管機關之間的正式或非正式會議。透過 VICH，全世界的法規主管機關齊聚一堂討論，這非常重要，並非僅有美國、歐盟及日本參與而已。因為產業界注意到有些新設立的機關單位或未成熟的機關單位設定各式各樣的條件，因為它們不敢冒任何風險。因此，若有 VICH 討論平台，將有助於替代物質的發展。

(3) USDA-ARS 的 Cyril Gay 提問，Takashi Kozasa 報告日本有資金誘因鼓勵業者針對 ATA 送件登記上市，所以 Cyril Gay 的提問是，日本政府執行這些措施，是否有看到政策效果？申請 ATA 件數是否增加？

- 日本農林水產省的 Takashi Kozasa 回應：「這個計畫始於 2017 年，我不認為目前它有成功，但日本政府資金援助一些公司進行各種研究，所以未來可能會有新的產品向日本政府申請核准上市。所以我們非常期待業者送件。」

(4) 主持人 Peter Borriello （英國獸醫局）提問，Dr. Kim 有報告抗微生物胜肽（Antimicrobial peptides, AMP）有很好的研究成果。Peter 詢問第六節講師專家及 Dr. Kim，AMP 面臨的法規障礙為何？

- 美國農業部國家農業研究中心的 Dr. Kim 回應，AMP 很難將之「分類」為生物製劑或一般藥品，AMP 與其他化合物或抗微生物的抗生素很不同。
- 美國 FDA-CVM 的 Joshua 回應，AMP 可能具有很多作用，就法規層面，FDA 會檢視產品是否符合法規條件，其標示宣稱是否符合預期效果。

(5) USDA-ARS 的 Cyril Gay 接續向美國 FDA-CVM 的 Joshua 提問，本次研討會的 AMP 應規範為一般藥品或生物製劑？有作用機制及安全性等資料，可預防或治療疾病。這份資料是要送件至 USDA（生物製劑主管機關）或 FDA（動物用藥及飼料添加物主管機關）？

- Joshua 表示他會轉送委員會討論，因為委員會的人員對這些產品很熟悉，像是雷射的免疫刺激療法，委員會亦熟悉 AMP，同時亦期望業者備齊所有研發資料，以辦理核准登記上市。

四、會議結論與後續工作

由 USDA-ARS 的 Cyril Gay 擔任主持人，進行會議總結。

1. **致謝**：Cyril Gay 首先感謝所有專家學者提供本研討會的素材、相關工作人員的努力及贊助商的協助。
2. **本研討會目標是否達成**：Cyril Gay 表示本次研討會的 3 大目標分別為（1）著重於具前景之 ATA 研究結果、（2）評估 ATA 商品化及使用面臨之挑戰、（3）找出法規途徑及鼓勵措施，以發展 ATA。但是 Cyril Gay 認為本次研討並未達達到這些目標。在第六節的研討會留下許多問題，亦耗費很長的時間進行深度討論，Cyril Gay 認為這次可能是最後一次 ATA 研討會，因為這些目標仍未達到，仍有許多研究尚待執行及解決。第六節要強調的是，找尋鼓勵及使 ATA 商品化的法規措施，也因此業者願意投資及發展 ATA。
3. **ATA 的用字及定義**：本研討會每個人都有自己的 ATA 用字及定義，Cyril Gay 認為有些可能有誤或需要做調整，美國 FDA 及歐洲藥品管理局（EMA）的 ATA 用字均採用抗微生物藥品替代物質（Alternatives to antimicrobials, ATA），Cyril Gay 認為 ATA 用字應採用抗生素替代物質（Alternatives to antibiotics, ATA）。在 2012 年第 1 次 ATA 研討會即賦予其定義。Alternatives to antibiotics 廣義為任何可取代治療藥品（Therapeutic drugs）之物質，取代那些對病原細菌、病毒或寄生蟲效果逐漸失效之藥品。而對於此定義有任何想法或建議，都歡迎來信建議，使此定義更周詳。這個定義在未來法規的規範、分類及其作用機制係相當重要的。若沒有法規核准途徑，產業界不會進行投資。
4. **我們學到什麼**：雖然有些疫苗有效力、成本及運輸問題等，疫苗在預防動物疾病仍具舉足輕重地位，此外，生物安全及衛生管理於管理成本上可有效率預防動物疾病。新型藥品、化學物質及酵素方面，Cyril Gay 期待未來有基因療法。植物化學物質方面，中國已使用中草藥（Herbal medicine）數千年，這是一個替代抗生素極具潛力的物質，有些具抗生素活性，而有些具免疫調節活性。但如何適用於法規並使之蓬勃發展，仍是問題。免疫相關產品方面，如研討會所提誘發免疫反應，產生 IgY 對抗病原，Cyril Gay 期待未來有免

疫相關產品可用於預防及治療疾病。在法規主管機關的立場會認為已有法規途徑，請業者及早辦理登記並表示很樂意協助，且不認為有問題。但若以產業界及學術界的角度，則可發現許多缺口，Emma Wall 提到資金缺口是很常見的問題。Cyril Gay 指出，我們真正需要的是公私協力，需要製藥產業界的專家參與，使產品登記核准上市。我們需要投資研究，需要有鼓勵措施填補無法登記上市的缺口。

5. **主持人 Cyril Gay 的結論**：

(1) 定義 ATA 作用機制相當重要，可使 ATA 有效使用，不論其係用於預防、治療；或強化動物健康及動物產能。

(2) 需要整合營養、健康醫療及疾病研究，尚需考量動物遺傳學。

(3) 針對不同動物產品生產系統，可考量結合不同的 ATA，以優化動物健康及疾病管理。

(4) 需要有適當管控的田野試驗，以驗證假說，包含 ATA 的安全性、效力及投資報酬率（Return on investment, ROI）。

6. **研討會之後續行動**：

(1) 公開研討會文章。

(2) 將口頭報告論文及壁報論文上傳至 ATA 網站（https://www.ars.usda.gov/alternativestoantibiotics/）。

(3) STAR-IDAZ 工作坊：ATA 研究缺口及研究優先順序。

(4) STAR-IDAZ 工作小組參與新型 ATA 研發。

(5) 資金募集 （Funding opportunities）。

(6) 公私協力，促進 ATA 研究、發展及商品化。

(7) 第 4 次 ATA 研討會-地點尚待確定。

五、心得與建議

1. 抗生素廣泛使用於人類、動物及植物疾病，使用抗生素時，會形成抗藥性細菌篩選壓力，而篩選出具抗藥性基因之細菌。為解決 AMR 問題，WHO、OIE 及 FAO 在防疫一體（One Health）概念下，透過跨部門及跨國界的共同

合作，在 2015 年積極推動對抗 AMR 全球行動計畫。為減少 AMR 的產生，世界多國陸續刪除動物之抗生素類生長促進劑（AGP）使用許可證，歐盟於 2006 年刪除 AGP 使用許可證，但不包含抗球蟲藥。美國於 2017 年 1 月刪除醫療重要性 AGP 使用許可證，除了不含抗球蟲藥，亦不含動物專用抗生素。例如離子型抗球蟲藥（Ionophores）、Bacitracins、Bambermycins 不被認為是醫療重要抗生素。中國 2019 年公告 AGP 將於 2020 年全部退出，自 2020 年 1 月 1 日起，除了中草藥萃取物（Chinese herbal extracts）之外，停止核發 AGP 許可證，自 2020 年 7 月 1 日起禁止使用 AGP。我國與國外作法接軌，自 2000 年起至今已刪除 36 項含藥物飼料添加物（包含 AGPs），目前尚有 9 種 AGP（不含抗球蟲藥）可供使用，已納入我國防疫一體科技計畫，進行評估，作為後續行政管理之基礎，刪減原則為各先進國家皆不使用、人畜共通使用且影響人體健康風險較高者，及與治療人體疾病之重要藥物具有交叉抗藥性者，逐步刪減 AGP，以減少 AMR 問題。

2. 由於 AMR 興起及世界多國陸續對抗生素進行法規限制，促使產業界及學術界 ATA 的發展，並於本次研討會百花齊放，成果豐碩。美國於 ATA 研發前，新增早期諮詢服務，並建立技術團隊協助 FDA-CVM 及贊助商共同學習新技術，另提供業者資金誘因。歐盟則進行 ATA 法規缺口盤點及提出因應措施。美國及歐盟尚提供「同步科學諮詢」服務。此外，美國與加拿大有法規合作委員會，以進行法規調和。日本則提供資金誘因制度，鼓勵產業界積極研發及申請 ATA 登記。許多國家雖致力於制度改革或提出資金誘因方案，但本次研討會仍未見成效，亦於討論時仍留下許多問題，沒有結論，未解決產業界及學術界所面臨之障礙，尤其是法規及登記所需療效驗證問題。最終 ATA 仍須面對其定義、分類、有效性、毒理安全性及驗證方法為何等問題。於法規面若要與國際接軌與溝通討論，此次會議推薦最佳平台為 VICH。

3. 基於中醫食藥同源概念，有些動物用產品效果雖未達醫療等級，但可能類似人類保健食品，具有保健、生長促進等效果，建議可參考人類「食品」及「保健食品」之分類及標示宣稱方式，作為管理參考依據，考量將動物用產品分類為「飼料/飼料添加物」及「保健飼料添加物」，並給予「保健飼料添加物」有某種程度的彈性空間做標示宣稱，例如可標示具保健及飼料效率之效果。

至於效果之證明、品質管控及用字方面，需與飼料主管機關研議討論。「保健飼料添加物」雖無法完全取代抗生素效果，無法定義為抗生素替代物質（ATA），但可能有助於減少抗生素的使用，進而減少 AMR 問題。

4. 全球動物醫療健康協會於 2019 年訂定「減少抗生素需求的指引」（如附件 5），組織代表 200 家以上動物健康醫療公司及 70 萬名獸醫師，支持「抗生素承諾」（如附件 4）。若參考該承諾，與製藥業者、輸入藥品業者、販賣業者、養殖業者、獸醫師之協會/公會擬定類似之「抗生素承諾」，達成公私協力，可促使謹慎使用抗生素及對抗 AMR 成為產業共識，亦可鼓勵相關業者研發「保健飼料添加物」或 ATA。

5. 預防動物傳染病及對抗 AMR 措施包含生物安全、良好生產及飼養管理、環境溫度管理、基因篩選育種、免疫接種計畫；使用客製化飼料及飼料添加物成分（包含飼料成分篩選易消化、低抗營養因子、低蛋白飲食但可均衡提供胺基酸需求；及補充有益纖維於離乳豬飲食）等，涉及多部門、機關及單位的業管，需跨單位合作推動，國外經驗可為借鏡。

6. 綜觀先進國家之動物用藥品管理單位，包含美國的食品藥物管理署動物藥品中心（FDA-CVM）、歐洲藥品管理局（EMA）及加拿大的衛生部動物用藥處（VDD），透過其人力規模、投入硬軟體及經費資源，可見其對動物用藥品管理重視程度，視為一個獨立及專業的重要產業，我國亦可納入參考，增加管理規模及資源，有助於我國動物用藥品產業發展。

六、附圖

圖 1、本屆 ATA 研討會第六節講師及其他相關學術界、政府機關代表組成專家小組，開放提問。

圖 2、會場走廊展示壁報論文。

七、附件

（一）附件 1：2013 年 3 月 13 日至 3 月 15 日 OIE 於法國巴黎召開的「動物抗微生物藥品謹慎及負責使用之 OIE 全球研討會－對抗 AMR 之國際共識」。

（二）附件 2：2017 年 7 月美國 FDA-CVM 修正版「產業界指引（Guidance for Industry, GFI）」第 170 條之動物用藥品申請費用免付及減免。

（三）附件 3：「歐盟促進抗微生物藥品替代物質核准登記上市」之意見反映報告。

（四）附件 4：2017 年全球動物醫療健康協會發起 2025 年之前「抗生素承諾」（英文原文及中文翻譯）。

（五）附件 5：全球動物醫療健康協會於 2019 年訂定之「減少抗生素需求的指引」（如附件 5）。

OIE GLOBAL CONFERENCE ON THE RESPONSIBLE AND PRUDENT USE OF ANTIMICROBIAL AGENTS FOR ANIMALS

"International solidarity to fight against antimicrobial resistance"

Paris, France, 13–15 March 2013

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RECOMMENDATIONS

CONSIDERING

1. That antimicrobial agents are essential tools for protecting animal health and welfare,
2. That antimicrobial agents also contribute to satisfying the increasing world demand for safe food of animal origin, such as milk, meat, fish and eggs,
3. That antimicrobial resistance is a global human and animal health concern that is influenced by both human and non-human usages of antimicrobial agents,
4. The importance of good governance practices including national legislation and regulatory frameworks for import, marketing authorisation, production, distribution (including transport and storage) and use of quality veterinary medicinal products worldwide,
5. The importance of strong Veterinary Services to promote the responsible and prudent use of antimicrobial agents in animals,
6. The implementation of the OIE[1] PVS Pathway worldwide,
7. The need to increase the capacity of all countries to conduct surveillance of antimicrobial resistance and monitoring of quantities of antimicrobial agents used in food producing animals,
8. The necessity of international solidarity to help all Member Countries to effectively develop and implement measures for responsible and prudent use of antimicrobial agents in animals,
9. The international standards and guidelines developed by the OIE and other international organisations such as Codex Alimentarius to promote the responsible and prudent use of antimicrobial agents in terrestrial and aquatic animals,
10. The OIE List of Antimicrobial Agents of Veterinary Importance and the WHO[2] List of Critically Important Antimicrobials for Human Medicine,
11. The network of OIE National Focal Points for Veterinary Products,
12. The cooperation with Veterinary Statutory Bodies, the veterinary profession, and interested parties to ensure responsible and prudent use of antimicrobial agents in terrestrial and aquatic animals based on OIE standards,
13. The Tripartite mechanisms between FAO[3], OIE and WHO for promoting the "One Health" concept,
14. The active support of VICH[4] by the OIE,

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1 World Organisation for Animal Health
2 World Health Organization
3 Food and Agriculture Organization of the United Nations
4 the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products

THE PARTICIPANTS OF THE OIE GLOBAL CONFERENCE ON THE RESPONSIBLE AND PRUDENT USE OF ANTIMICROBIAL AGENTS FOR ANIMALS

RECOMMEND TO THE OIE

1. To strengthen international cooperation through the Tripartite (FAO-OIE-WHO) approach to promote the responsible and prudent use of antimicrobial agents in humans and animals and to minimise the development and spread of antimicrobial resistance.

2. To continue to develop and update OIE standards, the OIE List of Antimicrobial Agents of Veterinary Importance and policies on the responsible and prudent use of antimicrobial agents with the support of OIE Reference Centres and all relevant OIE experts.

3. To assist Member Countries in strengthening their Veterinary Services and other competent authorities to promote good governance practices including national legislation and regulatory frameworks for import, marketing authorisation, production, distribution (including transport and storage) and use of quality veterinary medicinal products worldwide using, if needed, the OIE PVS Pathway.

4. To encourage Member Countries and OIE Delegates to utilise their OIE National Focal Points on Veterinary Products to identify needs for national capacity building.

5. To continue organising regional training seminars for OIE National Focal Points for Veterinary Products and invite FAO, WHO and stakeholders to participate.

6. To assist Member Countries to conduct surveillance on antimicrobial resistance for animal and human pathogens.

7. To collect harmonised quantitative data on the use of antimicrobial agents in animals with the view to establish a global database.

8. To strengthen cooperation with Veterinary Statutory Bodies and the veterinary profession in Member Countries to implement responsible and prudent use of antimicrobial agents in animals.

9. To explore and promote opportunities for more communication, collaboration and partnerships with relevant public and private interested parties from the human and animal sectors at international, regional and national levels.

10. To encourage intersectoral collaboration and research to better understand and minimise the mechanisms of development of antimicrobial resistance and to develop new molecules.

11. To promote international solidarity and advocate all potential donors to support developing countries to implement OIE standards on the responsible and prudent use of antimicrobial agents.

12. To facilitate the participation of OIE Member Countries in the VICH Outreach Forum with the aim of adopting and utilising harmonised international guidelines related to the technical requirements for registration of veterinary medicinal products, to ensure the quality of these products.

RECOMMEND TO OIE MEMBER COUNTRIES

1. To ensure that the national Veterinary Services fulfil their responsibilities and, where needed, seek assistance through the OIE PVS Pathway to comply with the OIE standards.

2. To implement OIE and Codex Alimentarius international standards and guidelines related to the responsible and prudent use of antimicrobial agents and to follow the recommendations of the OIE List of Antimicrobial Agents of Veterinary Importance including recommendations on fluoroquinolones and on the third and fourth generation of cephalosporins that are considered to be critically important both for human and animal health.

3. To develop and set up an official harmonised national system for collecting data on the monitoring of antimicrobial resistance in relevant animal pathogens and quantities of antimicrobial agents used in food producing animals at the national level based on the OIE standards.

4. To contribute to the OIE initiative to collect data on the antimicrobial agents used in food producing animals (including through medicated feed) with the ultimate aim to create a global database hosted by the OIE.

5. To develop or update appropriate legislation and regulation on import, marketing authorisation, production, distribution (including transport and storage) and use of quality veterinary medicinal products in interaction with other relevant competent authorities and private interested parties, and to ensure their efficient implementation.

6. To encourage the Veterinary Statutory Bodies and the veterinary profession as a whole to develop, implement and ensure compliance with ethics and codes of good veterinary practices, with particular reference to the prescription and delivery of antimicrobial agents by well-trained veterinarians and veterinary para-professional under their authority.

7. To advocate the inclusion in the curriculum for pre-graduate veterinary education (Day 1 competencies) of knowledge on antimicrobial resistance, and of codes of good veterinary practices for the responsible and prudent use of antimicrobial agents in animals.

8. That the Veterinary Statutory Bodies have the capacity and authority to develop and to institute continuing professional development and continuing education programmes directed, in particular, at the responsible and prudent use of antimicrobial agents in animals (including companion animals and wildlife) and at related new technologies that will become available, including diagnostic tests.

9. To nominate, support and maintain national OIE Focal Points for Veterinary Products in their tasks and to ensure close contact with relevant WHO, FAO and Codex Alimentarius Contact Points.

10. To support developing and in transition Member Countries to strengthen their veterinary services, to implement good governance and legislation related to antimicrobial agents in compliance with OIE and Codex Alimentarius standards; and to help them to fight against the use of unlicensed/counterfeit products.

11. To contribute and to participate in global or regional cooperation aiming at developing measures for responsible and prudent use of antimicrobial agents in animals.

12. To promote good agriculture and aquaculture practices including the use of vaccines where applicable and interact with all relevant interested parties while ensuring compliance with OIE and Codex Alimentarius standards to minimise the development and spread of antimicrobial resistance.

13. To support relevant research to improve the understanding of the efficacy of current antimicrobial agents with the aim to prolong their usage while minimising the development of resistance, to develop new molecules and to find alternatives that could be used in animal production for antimicrobial agent substitutions.

14. To facilitate the market authorisation of new molecules and innovative technologies for antimicrobial agent substitutions, and to promote their use.

15. To develop risk assessment and to carefully evaluate practices of use of antimicrobial agents that are not intended to combat animal diseases.

16. To use VICH guidelines to ensure the quality of veterinary medicinal products registered at national level, and to follow closely the VICH Outreach Forum initiative.

#170

Revised
Guidance for Industry

Animal Drug User Fees and Fee Waivers and Reductions

This version of the guidance replaces those made available on March 14, 2004, September 4, 2008, and October 1, 2008. This revision of the guidance document clarifies the criteria for Barrier to Innovation waivers, clarifies the procedures for Small Business waivers, and makes additional clarifying changes.

Submit comments on this revised guidance at any time. Submit electronic comments to https://www.regulations.gov. Submit written comments to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Room 1061, Rockville, MD 20852. Identify all comments with the Docket No. FDA-2004-D-0369.

For further information regarding this document, contact AskCVM@fda.hhs.gov.

Additional copies of this revised guidance document may be requested from the Policy and Regulations Staff (HFV-6), Center for Veterinary Medicine, Food and Drug Administration, 7519 Standish Place, Rockville, MD 20855, and may be viewed on the Internet at either http://www.fda.gov/AnimalVeterinary/default.htm or https://www.regulations.gov.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Veterinary Medicine
July 2017

Table of Contents

Contains Non-Binding Recommendations

Revised Guidance for Industry

Animal Drug User Fees and Fee Waivers and Reductions

This guidance represents the current thinking of the Food and Drug Administration (FDA or Agency) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the FDA office responsible for this guidance as listed on the title page.

I. Introduction

The purpose of this document is to provide guidance on the types of fees the Food and Drug Administration (FDA or the Agency) is authorized to collect under the Animal Drug User Fee Act of 2003 (ADUFA) and how to request waivers and reductions from these fees. This revised guidance describes the types of fees and fee waivers and reductions; what information FDA recommends you submit in support of a request for a fee waiver or reduction; how to submit such a request; and FDA's process for reviewing requests.

Enacted on November 18, 2003, ADUFA (Public Law 108-130) amended the Federal Food, Drug, and Cosmetic Act (the FD&C Act) and requires the FDA to assess and collect user fees for certain applications, products, establishments, and sponsors. It also authorizes the Agency to grant a waiver from or a reduction of those fees in certain circumstances.

The Animal Drug User Fee Amendments of 2008 (ADUFA II) further amended the FD&C Act to revise and reauthorize the ADUFA program for fiscal years 2009 through 2013. Revisions to the ADUFA provisions made by this legislation included refinements to the ADUFA fee structure. In June 2013, the ADUFA program was reauthorized for an additional 5 years from fiscal year 2014 through fiscal year 2018 by the Animal Drug User Fee Amendments of 2013 (ADUFA III).

In general, FDA's guidance documents do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

II. Payment of Fees Pending a Decision on a Waiver/Reduction Request

An animal drug application or supplemental animal drug application submitted by a person subject to ADUFA fees is considered incomplete and will not be accepted for filing by FDA until all fees owed by such person have been paid. Section 740(e) of the FD&C Act. FDA considers fees to be due even if the person has a request for a fee waiver or reduction pending, meaning that either FDA has not yet granted or denied the request or the requestor has asked for agency reconsideration or review of a denial. If FDA grants a waiver or reduction of a fee that has been paid, it will issue a refund as anticipated by section 740(i) of the FD&C Act.

III. Types of Fees

A. Animal Drug Sponsor Fee

An animal drug sponsor is defined in section 739(6) of the FD&C Act as either an applicant named in an animal drug application that has not been withdrawn by the applicant and for which approval has not been withdrawn by the Agency or a person who has submitted an investigational animal drug submission that has not been terminated or otherwise rendered inactive by the Agency. A person who meets this definition within a fiscal year is assessed a sponsor fee in that fiscal year if, after September 1, 2003, the person had pending with FDA an animal drug application, a supplemental animal drug application, or an investigational animal drug submission. Section 740(a)(4) of the FD&C Act. ADUFA requires FDA to collect animal drug sponsor fees on an annual basis; each sponsor will pay only one such fee each fiscal year. Section 740(a)(4) of the FD&C Act. Further guidance regarding animal drug sponsor fees is provided in Guidance for Industry #173, "Animal Drug Sponsor Fees under the Animal Drug User Fee Act (ADUFA)."

B. Animal Drug Application and Supplement Fee

ADUFA requires FDA to collect fees from each person who submits certain animal drug applications or supplements on or after September 1, 2003. Section 740(a)(1)(A) of the FD&C Act.

All animal drug applications submitted under section 512(b)(1) of the FD&C Act are subject to fees. Sections 739(1) and 740(a)(1)(A)(i) of the FD&C Act. Animal drug applications subject to the criteria set forth in section 512(d)(4) of the FD&C Act, and supplemental animal drug applications that request a change for which safety or effectiveness data are required (whether the change is to an application that had been approved under section 512(c)(1) or 512(c)(2) of the FD&C Act), are subject to fees. Sections 739(2) and 740(a)(1)(A)(ii) of the FD&C Act. Supplemental animal drug applications for which safety and effectiveness data are not required and abbreviated new animal drug applications, which are submitted under section 512(b)(2) of the FD&C Act, are not subject to application fees under ADUFA.

If you have paid the fee for an application or supplement which was accepted for filing, but was either not approved or was withdrawn without a waiver or refund, then if you or your licensee, assignee, or successor submit an application or supplement for the same product (i.e., a reactivation), it will not be subject to an application fee. Section 740(a)(1)(C) of the FD&C Act.

An applicant may submit an amendment to an application or supplement. If you amend a pending application or supplement, the unamended application may be considered as withdrawn and the amended application considered resubmitted. 21 CFR 514.6. An amended application that FDA considers to be resubmitted will not result in a new application fee if the unamended application was not approved or was withdrawn without a waiver or refund. FDA intends to accept minor changes without considering the amended application as resubmitted.

C. Animal Drug Product Fee

Under specified circumstances, ADUFA requires FDA to collect an annual product fee for each drug product which has been submitted for listing under section 510 of the FD&C Act. Section 740(a)(2) of the FD&C Act. This requirement applies to approved animal drug products that have been listed or submitted for listing. ADUFA defines the term "animal drug product"[1] to mean "each specific strength or potency of a particular active ingredient or ingredients in final dosage form marketed by a particular manufacturer or distributor, which is uniquely identified by the labeler code and product code portions of the national drug code, and for which an animal drug application or a supplemental animal drug application has been approved." Section 739(3) of the FD&C Act. The product fee is assessed to the person named as the applicant in an animal drug application or supplemental animal drug application for an animal drug product that has been submitted for listing and who, after September 1, 2003, had pending with FDA an animal drug application or supplemental animal drug application. Section 740(a)(2) of the FD&C Act.

D. Animal Drug Establishment Fee

ADUFA requires FDA to collect an annual establishment fee from each person who 1) owns or operates, directly or through an affiliate, an animal drug establishment; 2) is named as the applicant in an animal drug application or supplemental animal drug application for an animal drug product which has been submitted for listing under section 510 of the FD&C Act; and 3) after September 1, 2003, had pending with FDA an animal drug application or supplemental animal drug application. Section 740(a)(3) of the FD&C Act. The person is assessed an establishment fee for each animal drug establishment listed in its approved animal drug application as an establishment that manufactures that animal drug product. Section 740(a)(3) of the FD&C Act. If an animal drug establishment listed in an application does not engage in the manufacture of the animal drug product during a particular fiscal year, an annual establishment fee will not be assessed for that fiscal year on the basis of that product. Section 740(a)(3) of the FD&C Act.

If a single establishment manufactures both animal drug products and human prescription drug products as defined in section 735(3) of the FD&C Act, such establishment shall be assessed both the animal drug establishment fee (under ADUFA) and the prescription drug establishment fee (under PDUFA). Section 740(a)(3) of the FD&C Act.

IV. Types of Fee Waivers and Reductions

This section contains a summary of each of the specific provisions under which a waiver or reduction may be requested for each of the fees described above.

[1] For information on the term "animal drug product" as applied to intentionally altered genomic DNA in animals, including in genetically engineered animals, see Guidance for Industry #187, "Regulation of Intentionally Altered Genomic DNA in Animals" http://www.fda.gov/downloads/animalveterinary/guidancecomplianceenforcement/guidanceforindustry/ucm113903.pdf

A. Significant Barrier to Innovation

Section 740(d)(1)(A) of the FD&C Act provides that FDA shall grant a waiver from or a reduction of one or more of the fees where FDA finds that:

> *the assessment of the fee would present a significant barrier to innovation because of limited resources available to such person or other circumstances.*

FDA interprets this provision to mean that a waiver or reduction is appropriate when: (1) the product for which the waiver is being requested is innovative, or the requestor is otherwise pursuing innovative animal drug products or technology; and (2) the fee would be a significant barrier to the requestor's ability to develop, manufacture, or market the innovative product or technology.

To qualify for a waiver or reduction in user fees under this provision, an applicant must meet both criteria.

1. Is the product innovative?

In evaluating whether a product is innovative, the Agency asks the following questions:

a. Is the product novel or does it represent a medical breakthrough?[2]

i. Does the product have a novel mechanism of action? – Is the biochemical interaction through which the drug substance produces its pharmacological effect different from other approved animal drug products, and does the difference represent advanced "breakthrough" technology (e.g., monoclonal antibodies, RNA inhibitors, gene vectors or altered DNA constructs)?

ii. Does the product have a novel formulation? – Does the drug product's formulation demonstrate an advanced "breakthrough" technology (e.g., nanotechnology) that enhances safety or effectiveness of the drug product?

iii. Does the product employ a novel delivery system? – Does the drug delivery system or drug vehicle demonstrate "breakthrough" technology to improve delivery of the drug (e.g., improves delivery to the target organ, improves bio-distribution of the drug)? Examples are drug carriers with ligand–receptor interactions for target organ delivery; drug-carrying polymers that respond to specific stimuli (e.g., exposure to light, changes in pH or temperature) for cyclic drug release.

[2] What constitutes novel or a medical "breakthrough" may change over time. As new, emerging technology becomes more commonplace, FDA may no longer consider it novel or a medical "breakthrough."

b. Is there sufficient information to suggest the product would likely have the proposed effect? – Is there sufficient information for proof of concept in a validated *in vitro* system or in the target animal or an animal model of the target animal (e.g., laboratory rodent species)?

For the purposes of a Barrier to Innovation waiver, a product is **not** considered innovative if the proposed 'innovation' relates to **any** of the following:

1. An incremental change in manufacturing, dosage form, or route of administration of an approved new animal drug.[3]

2. A drug where the only change is enhanced safety, enhanced efficacy, decreased dosing frequency, or a shorter residue withdrawal time where the same or similar active ingredient is approved, WITHOUT such change utilizing advanced, breakthrough technology.

Additionally, in determining whether a product is innovative, FDA may consider as a factor whether another product using the same technology and for the same indication has been approved, even if the previously approved product is no longer on the market.

2. Does the fee create a significant barrier to the applicant's ability to develop, manufacture, or market innovative products or to pursue innovative technology?

Under section 740(d)(1)(A) of the FD&C Act, an applicant may qualify for a fee waiver if the fee would present a significant barrier to innovation for one of two reasons: "because of limited resources available" to the applicant or due to "other circumstances."

a. Limited Resources

To determine whether a fee would be a significant barrier to an applicant's ability to develop, manufacture, or market innovative products or to pursue innovative technology, the Agency considers the relationship between the annual cost of user fees and the financial resources of the applicant and its affiliates as that term is defined in section 735(11) of the FD&C Act. Under the Act, the applicant is the person who is responsible for payment of the fees and the person who must qualify for a waiver or reduction of user fees. Accordingly, the statute does not allow persons other than those legally subject to user fees, such as a distributor that is not an affiliate, to qualify for or receive waivers or reductions of user fees.

FDA considers the working capital of the requestor and its affiliates in determining whether the requestor has limited financial resources. Working capital is the capital that a company uses for its day-to-day operations, calculated as the current assets minus the

[3] For products related to intentionally altered genomic DNA in animals, an incremental change would include the same or similar genetic changes carrying the same claim (e.g., enhanced growth, different insertional events producing the same product). Please consult the Center if you have questions on this point.

current liabilities. Working capital is frequently used to measure a company's ability to meet current obligations, and is an objective measure of the resources available to the applicant as defined by generally accepted accounting principles. Examples of current assets are cash, marketable securities, accounts receivable, and inventory. Examples of current liabilities are accounts payable, accrued expenses (such as wages, interest, and taxes payable), and short-term notes payable. When calculating working capital, FDA intends to deduct from current liabilities any that reflect marketing costs, including expenses in foreign markets, that are often incurred because of an applicant's marketing decisions, and any dividends payable to investors.

FDA does not intend to consider lack of profitability as evidence of limited resources, given that even a very large requestor may have operating losses.

Beginning with fees assessed for FY 2017, the Agency expects to determine that an applicant with financial resources of less than $20 million (adjusted annually thereafter for inflation[4]), including the financial resources of the applicant's affiliates, has "limited resources available" for purposes of determining whether to grant a barrier to innovation waiver or reduction of fees on financial grounds. An applicant with $20 million or more in financial resources, including the financial resources of the applicant's affiliates, generally will not be considered to have "limited resources available" for purposes of determining whether to grant a barrier to innovation waiver or reduction of fees on financial grounds. The Agency also does not intend to consider product sales figures to be evidence of limited resources, because even a large and profitable company can have low sales figures for an individual product, but not need a waiver on the basis that the ADUFA fees billed to that company would present a significant barrier to innovation.

b. Other Circumstances

Section 740(d)(1)(A) of the FD&C Act authorizes FDA to grant a fee waiver or reduction on barrier to innovation grounds for financial reasons or because of "other circumstances." Consistent with this provision, there are certain circumstances where FDA will consider granting a barrier to innovation waiver when an applicant does not qualify on financial grounds. Those circumstances include, but are not necessarily limited to, the following:

- Public policy or congressional intent. FDA has granted waivers under an analogous provision for human drugs where Congress has indicated its intent to grant certain products or technology regulatory relief (see PET Drugs, 65 FR 13004-5) or where public policy calls for such a waiver (see GFI: HIV Drug Waivers). FDA expects to grant waivers where similar circumstances arise in the context of animal drugs.

B. Fees Exceed Costs

Section 740(d)(1)(B) of the FD&C Act provides that FDA shall grant a waiver from or a reduction of one or more of the fees where FDA finds that:

[4] FDA publishes user fee rates for each fiscal year in the Federal Register on or before August 1st of the preceding fiscal year. FDA will announce the financial resource ceiling for the upcoming fiscal year in this Federal Register notice.

> *the fees to be paid by such person will exceed the anticipated present and future costs incurred by [FDA] in conducting the process for the review of animal drug applications for such person.*

In making this finding, FDA may use standard costs. Section 740(d)(2) of the FD&C Act. FDA interprets "person" in this provision to include the requestor's affiliates,[5] as that term is defined in section 735(9) of the FD&C Act. Therefore, when a person submits a request for a fee waiver or reduction based on the assertion that the Agency's costs associated with the process for review of animal drug applications for that person will be less than the fees, FDA intends to take into consideration all applications submitted by the entity and its affiliates. The term "process for the review of animal drug applications" is defined in section 739(8) of the FD&C Act, and includes activities related to the review of animal drug applications, supplemental animal drug applications, and investigational animal drug submissions. In determining whether the requestor qualifies for a fees exceed costs waiver or reduction, FDA intends to compare the fees paid with the actual and anticipated costs from September 1, 2003, through September 30 of the year for which the request is made.

To evaluate a waiver request on the basis that fees assessed exceed FDA's costs, FDA will need information that will only be available after the end of the fiscal year for which the request is made, likely by the end of March following the close of the fiscal year.

C. Free Choice Feeds

Section 740(d)(1)(C) of the FD&C Act provides that FDA shall grant a waiver from or a reduction of one or more of the fees where FDA finds that:

> *the animal drug application or supplemental animal drug application is intended solely to provide for use of the animal drug in—*
>
> *(i)* a Type B medicated feed (as defined in section 558.3(b)(3) of title 21, Code of Federal Regulations (or any successor regulation)) intended for use in the manufacture of Type C free-choice medicated feeds, or
>
> *(ii)* a Type C free-choice medicated feed (as defined in section 558.3(b)(4) of title 21, Code of Federal Regulations (or any successor regulation)).

Thus, FDA will waive or reduce fees when an application or supplement is intended solely to provide for use of the animal drug in free-choice medicated feed. For the purpose of this provision, FDA intends to define free-choice medicated feeds consistent with 21 CFR 510.455(a) as

[5] An affiliate is a business entity that has a relationship with a second business entity if, directly or indirectly – (a) one business entity controls, or has the power to control, the other business entity; or (b) a third party controls, or has power to control, both of the business entities.

The free-choice administration of animal drugs in feeds involves feeds that are placed in feeding or grazing areas and are not intended to be consumed fully at a single feeding or to constitute the entire diet of the animal. Such methods of administering drugs include, but are not limited to, medicated blocks (agglomerated feed compressed or rendered into a solid mass and cohesive enough to hold its form), mineral mixes, and liquid feed tank supplements ("lick tank" supplements) containing one or more animal drugs.

D. Minor Use or Minor Species

Section 740(d)(1)(D) of the FD&C Act provides that FDA shall grant a waiver from or a reduction of one or more of the fees where FDA finds that:

> *the animal drug application or supplemental animal drug application is intended solely to provide for a minor use or minor species indication.*

For the purpose of this provision, FDA uses the definition of the term "minor use" provided in 21 CFR 516.3(b) which means the intended use of a drug in a major species for an indication that occurs infrequently and only in a small number of animals or in limited geographic areas and in only a small number of animals annually. Small number of animals means equal to or less than: 50,000 horses; 70,000 dogs; 120,000 cats; 310,000 cattle; 1,450,000 pigs; 14,000,000 turkeys; and 72,000,000 chickens.

Likewise, for the purpose of this provision, FDA uses the definition of the term "minor species" that is provided in 21 CFR 516.3(b), which states that minor species are animals, other than humans, that are not major species; that is, animals other than cattle, horses, swine, chickens, turkeys, dogs, and cats.

The Agency intends to waive or reduce the product fee only if the animal drug product is exclusively labeled for minor species or minor use indications. The Agency intends to waive or reduce the establishment fee only if the establishment manufactures products exclusively for minor species or minor use indications.

E. Small Business

Section 740(d)(1)(E) of the FD&C Act provides that FDA shall grant a waiver from or a reduction of one or more of the fees where FDA finds that:

> *the sponsor involved is a small business submitting its first animal drug application to [FDA] for review.*

A "small business" is one that has fewer than 500 employees, including employees of affiliates. Section 740(d)(3)(A) of the FD&C Act. Because section 740(d)(3)(B) states that FDA shall waive the application fee for the first animal drug application submitted, FDA believes that the provision requires a waiver of the entire application fee rather than a reduction, and that the provision pertains to application fees but not to any other type of animal drug user fee. The waiver applies only to the <u>first</u> animal drug application that the small business <u>or its affiliate</u>

submits for review. Section 740(d)(3)(B) of the FD&C Act. FDA believes this means the first application the small business ever submits, regardless of whether it was submitted before or after ADUFA was enacted.

A person who applies for a waiver under this section must certify its qualification for the waiver. Section 704(d)(3)(C) of the FD&C Act. ADUFA requires FDA to periodically publish in the Federal Register a list of persons making such certifications. Section 740(d)(3)(C) of the FD&C Act.

V. Procedures and Timing for Requesting Fee Waivers or Reductions

This section of the guidance document describes the procedures that FDA recommends for submitting a fee waiver or reduction request, and the information that the Agency believes it needs from a person to determine whether it can grant the request for a fee waiver or reduction. Adherence to these procedures will help to minimize time-consuming efforts by the Agency to obtain additional necessary information, and will enable the Agency to grant fee waivers or reductions to qualifying persons in a timely manner. **Please note that you must submit a written request to the Agency for a waiver or reduction no later than 180 days after the fee is due.** Section 740(i) of the FD&C Act. FDA does not intend to consider any requests made later than 180 days after the fee is due.

A. Procedures Applicable To All Requests for Waivers or Reductions

1. Submitting a Waiver or Reduction Request

Because waivers and reductions apply to specific fees due for a specific application or fiscal year, new requests should be submitted for each application or fiscal year. The request must be submitted in writing and must be submitted to FDA by no later than 180 days after the fees are due. Section 704(i) of the FD&C Act. Requests for fee waivers and reductions, other than those made on the basis that fees exceed costs, will be reviewed and granted or denied by FDA's Center for Veterinary Medicine (CVM) ADUFA Waiver Officer. Requests for waivers or reductions made on the basis that fees exceed costs will be evaluated by the ADUFA Waiver Officer and subsequently forwarded, with a recommended disposition, to FDA's Office of Financial Management. The Director of FDA's Office of Financial Management will then review the request and make a decision about whether to grant or deny the request.

To facilitate timely review and processing of requests for a waiver or reduction of one or more types of ADUFA fees, such requests should contain the following information:

a. The name and address of the entity requesting the waiver or reduction (requestor), including the company name and address if the requestor is an agent for the company.
b. The name, telephone number, and e-mail address of a contact person.
c. The specific type(s) of fees for which a waiver or reduction is requested, including:

i. *For application or supplement fees*:

a) the number of the NADA or supplemental NADA for which a fee waiver or reduction is being requested;
b) the trade and established names of animal drug products covered by the application; and
c) the date the application or supplement was submitted.

ii. *For product fees*:

a) the trade and established names of the animal drug product, its applicable National Drug Code (NDC) number, and the number of the NADA or supplemental NADA under which the product was approved;
b) the name of the person holding the approved application for the animal drug product;
c) the specific strength or potency of the product and its final dosage form; and
d) the product fee invoice number and invoice date, if available. A photocopy of FDA's invoice to the person may be submitted to provide this information, though you should clearly state for which specific animal drug product the fee waiver or reduction is requested.

iii. *For establishment fees*:

a) the name, address, and FEI/CFN number of the establishment for which the fee waiver or reduction is requested;
b) the establishment number as listed on the fee invoice, and the invoice number and date, if available. A copy of the invoice is acceptable.

iv. *For sponsor fees:*

a) the name and address of the animal drug sponsor requesting the waiver or reduction;
b) the invoice number and invoice date if available. A copy of the invoice is acceptable.

d. If payment has been made, the date on which payment was made;

e. The particular grounds on which the waiver or reduction is requested (i.e., one or more of the provisions specified in section IV above);[6] and

f. Information and analyses showing why the requestor believes it qualifies for the waiver or reduction.

[6] The Agency intends to waive or reduce sponsor fees only if the sponsor's entire portfolio of files and applications is eligible for a waiver based on one or more of the provisions specified in section IV. Types of Fee Waivers and Reductions.

If submitting a fee waiver or reduction request in advance of the date the fees are due, you should provide any new or updated information as soon as it becomes available.

A request for a fee waiver or reduction should be sent to:

Food & Drug Administration
Center for Veterinary Medicine
Document Control Unit (HFV-199)
Attention: ADUFA Waiver Officer
7500 Standish Place
Rockville, MD 20855

FDA will send a written acknowledgement of receipt of the fee waiver or reduction request to the requestor. The acknowledgement letter will include the date on which the waiver or reduction request was received, and will request any additional information the FDA believes, at the time, will be necessary to evaluate the request. The ADUFA Waiver Officer will evaluate the fees exceed costs waiver or reduction request and forward the recommendation to the Director of the Office of Financial Management.

2. Timing of a Fee Waiver Or Reduction Request

If you plan to request a fee waiver or reduction and wish to minimize the likelihood that you will have to pay the fee and then wait for a refund, FDA encourages you to submit your requests at least 90 days before the fees are due. For animal drug application and supplemental animal drug application fees, this would be 90 days before the expected submission of the application or supplement. For sponsor, product, and establishment fees, this would normally be by November 1 of each fiscal year because these fees are generally due on or before January 31 of the fiscal year. This recommendation does not apply to "Fees Exceed Costs" waivers and reductions since FDA expects that it will not be able to make a decision on them until after the associated fees are due.

3. The Waiver Officer's or Office of Financial Management Director’s Review of the Request

The ADUFA Waiver Officer will review the waiver or reduction request and consult with relevant Agency officials as appropriate. The Waiver Officer may request additional information from, or a meeting with, the requestor during the review period. The Agency expects to notify the requestor of the Waiver Officer’s decision and the reasons for it within 90 days of the receipt of a waiver or reduction request, except for Fees Exceed Costs waiver or reduction requests, which will not be decided by the Director of FDA’s Office of Financial Management until approximately 6 months after the end of the fiscal year for which the waiver or reduction is requested. These time periods may vary depending on, among other things, the number of fee waiver or reduction requests submitted and whether the request contains sufficient supporting information.

4. Requesting Reconsideration of a Decision

If the ADUFA Waiver Officer, or Director of FDA's Office of Financial Management in the case of fees exceed costs requests, fully or partially denies your request for a fee waiver or reduction, you may request reconsideration of that decision. FDA encourages persons to make such requests for reconsideration promptly, and suggests that they be made within 15 days of receiving the decision. A request for reconsideration should state the person's reasons for believing that the decision is in error, and should include any additional information necessary to support the person's position. The ADUFA Waiver Officer or Office of Financial Management Director will issue a decision upon reconsideration, setting forth the reasons for it. A request for reconsideration is decided by the original decision maker, and is different from a request for review under 21 CFR 10.75, which is described in the next two sections. You may, but are not required to, request reconsideration of the initial decision by the ADUFA Waiver Officer or Office of Financial Management Director before seeking review of that decision.

You should send the request for reconsideration to:

Food & Drug Administration
Center for Veterinary Medicine
Document Control Unit (HFV-199)
Attention: ADUFA Waiver Officer
7500 Standish Place
Rockville, MD 20855

5. Requesting Review of the ADUFA Waiver Officer's Decision

If the ADUFA Waiver Officer denies your waiver or reduction request or denies your request for reconsideration, you may seek review by CVM's ADUFA Appeals Officer in accordance with this agency's regulations at 21 CFR 10.75. Please note that this procedure does not apply to fees exceed costs waiver or reduction requests. The procedures that apply to such requests are described separately below. Your request for review should contain a copy of the ADUFA Waiver Officer's original decision, his or her decision upon reconsideration, if any, and the reasons you believe the decisions are in error. The review will be based on information in the administrative file, which includes information and analyses already submitted to the Agency, as provided by 21 CFR 10.75(d).

The request for review should be sent to:

Food and Drug Administration
Center for Veterinary Medicine
Document Control Unit (HFV-199)
Attention: CVM ADUFA Appeals Officer (HFV-1)
7519 Standish Place
Rockville, MD 20855

After reviewing the request, the CVM ADUFA Appeals Officer will issue a written decision.

6. Requesting Review of the CVM ADUFA Appeals Officer's Decision or the Office of Financial Management Director's Decision

If the Director of the Office of Financial Management denies your fees exceed costs waiver or reduction request or denies your request for reconsideration, you may seek review by FDA's User Fee Appeals Officer. You may likewise seek review by FDA's User Fee Appeals Officer if the CVM ADUFA Appeals Officer upholds the ADUFA Waiver Officer's decision. Your request for review should contain a copy of the original decision, any decisions upon reconsideration or review, and the reasons you believe those decisions are in error. The review will be based only on information in the administrative file, which includes information and analyses already submitted to the Agency, as provided by 21 CFR 10.75(d).

The request for review should be sent to:

The Office of the Chief Scientist
FDA White Oak Campus - Building 1
10903 New Hampshire Ave
Silver Spring, MD 20993

After reviewing the request, FDA's User Fee Appeals Officer will issue a written decision.

B. Additional Procedures for Small Business Waiver Requests

In addition to the information requested in section V(A)(1) of this document, a request for a waiver of the application fee on the grounds that the animal drug sponsor is a small business submitting its first new animal drug application to FDA for review must contain a certification that the requestor qualifies for the waiver. Section 740(d)(3)(C) of the FD&C Act. When applying for such a waiver, we recommend that a responsible officer of the entity certify:

a) that the entity has fewer than 500 employees, including employees of affiliates; and
b) that this is the first animal drug application the entity or any one of its affiliates has submitted to FDA for review.

The request for a small business waiver should contain the following certification statement:

> "I certify that to best of my knowledge that 1) [name of company] is a small business within the meaning of 21 U.S.C. 379j-12(d)(3) because [name of company] has fewer than 500 employees, including employees of affiliates; and 2) this is the first animal drug application [name of company] or any of its affiliates has submitted to FDA for review. I further certify that this is an accurate, true, and complete submission of information. This statement is subject to criminal penalties for false statements as set forth in 18 U.S.C. § 1001."

The Agency encourages requestors to time their small business waiver requests carefully to reduce the potential that the information provided as the basis of the request is no longer current when the animal drug application is submitted.

C. Additional Procedures for Significant Barrier to Innovation Waiver or Reduction Requests

In addition to the information requested in section V(A)(1) of this document, a request for a fee waiver or reduction on the basis that the fee would present a significant barrier to innovation should contain the following information:

1. a statement of what the innovation is relating to the animal drug product for which the waiver is being requested, or other animal drug products or technologies the requestor is pursuing);
2. an explanation of why the requestor believes its product or technology is innovative (please see section IV (A)(*1*) above);
3. an analysis of why the fee or fees would present a significant barrier to the requestor's ability to develop, manufacture, or market the innovative product or technology;
4. A statement indicating whether the requestor has any affiliates, and if so, a list of those affiliates[7]; and
5. if the request is based on limited resources:
   a. an estimate of the total fees that the requestor, including its affiliates, would be required to pay in the fiscal year; and
   b. financial statements that show the resources available to the requestor, including its affiliates (please see section IV(A)(*2*)).

D. Additional Procedures for Fees Exceed Costs Waiver or Reduction Requests

In addition to the information requested in section V(A)(1) of this document, a request for a fee waiver or reduction on the basis of fees exceeding costs should include a list of the requestor's "affiliates," as that term is defined in section 735(11) of the FD&C Act, from September 1, 2003, through September 30 of the year for which the request is made. The information will be used to estimate the fees paid and the anticipated costs. For each affiliate on the list, you should include the name, address, and phone number of the affiliate's counsel or head of regulatory affairs so that FDA may contact the affiliate if necessary in reviewing the request.

If a fee waiver or reduction is also requested under other provisions of ADUFA, then FDA intends to evaluate the other waiver or reduction requests first. Only if it denies the other waiver or reduction request(s) would the Agency review the fees exceed costs request.

[7] What is an affiliate? As defined at section 735(11) of the FD&C Act, an affiliate means a business entity that has a relationship with a second business entity if, directly or indirectly – a) one business entity controls, or has the power to control, the other business entity; or b) a third party controls, or has power to control, both of the business entities.

1 10 October 2019
2 EMA/CVMP/461776/2017
3 Committee for Medicinal Products for Veterinary Use

4 CVMP Reflection paper on promoting the authorisation of 5 alternatives to antimicrobials in the EU

6 Draft

7

Adopted by CVMP for release for consultation	10 October 2019
Start of public consultation	18 October 2019
End of consultation (deadline for comments)	30 April 2020

8

Comments should be provided using this template. The completed comments form should be sent to vet-guidelines@ema.europa.eu

9

Keywords	Alternatives to antimicrobials, authorisation, gap analysis, measures, veterinary medicinal products

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Send us a question Go to www.ema.europa.eu/contact **Telephone** +31 (0)88 781 6000 An agency of the European Union

Reflection paper on promoting the authorisation of alternatives to antimicrobials in the EU

Draft

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1. Introduction

Antimicrobial resistance (AMR) is now recognised as a major threat to human and animal health. The European Medicines Network Strategy to 2020[i] highlights that, with respect to veterinary medicines, controlling the risks of AMR arising from the use of antimicrobials, and particularly arising from the non-prudent use, is one of the highest priorities related to animal and public health. The European Commission has published a European One Health Action Plan against AMR[ii] which has as one of its objectives to develop new therapeutics and alternatives. Correspondingly, the CVMP Strategy on Antimicrobials (EMA/CVMP/209189/2015)[iii] has as an objective to encourage and foster the development of alternatives to antimicrobials with the specific action proposed:

> *"The CVMP will reflect further on measures that could be taken to promote the development and access to market of alternatives to antimicrobials, giving particular attention to vaccines (novel and improved) as part of the current initiative to promote availability of products that can reduce the need for antimicrobial treatment within the EU."*

The new veterinary Regulation (EU) 2019/6[iv] that will apply from 28 January 2022, considers AMR to medicinal products for human use and veterinary medicinal products a growing health problem in the European Union and worldwide requiring urgent and coordinated intersectorial action in accordance with the One Health approach. The main aim of the new legislation is to increase availability of veterinary medicinal products in the EU, to tackle AMR, to reduce administrative burden and to strengthen innovation. In this context, stimulating the development of new, alternative medicines to prevent or treat resistant infections is one of the pillars of fighting against the AMR threat and a high priority for EMA and the European medicines regulatory network.

2. Aim

This reflection paper performs a gap analysis by reviewing the measures currently in place to support the authorisation of alternatives to antimicrobials (ATAm) in veterinary medicine, with particular emphasis given to alternatives to antibiotics, and identifying where and how these could be improved.

Potential gaps in the area of authorisation of ATAm were identified through reflection on previous experience with such products at the European Medicines Agency (EMA), discussion with regulators from other regions such as USA, feedback from stakeholders, and review of the outcome of conferences on the subject jointly organised by OIE and the U.S. Department of Agriculture (USDA)[v].

EMA and EFSA published in 2016 a Joint Scientific Opinion on measures to reduce the need to use antimicrobial agents in animal husbandry in the European Union and the resulting impacts on food safety (RONAFA)[vi] . This opinion includes a review of both vaccination and of other alternative measures that formed the basis for the range of ATAm considered for this gap analysis. Appendix 1 presents a non-exhaustive list of examples of ATAm derived from the EMA and EFSA Joint Scientific Opinion on measures to reduce the need to use antimicrobial agents in animal husbandry in the European Union, and the resulting impacts on food safety (RONAFA) to illustrate the range of products and technologies covered within this discussion topic.

In 2015, EMA and Heads of Medicines Agencies established a joint steering group to foster and coordinate the implementation of an action plan to facilitate timely access to the EU market for new or improved veterinary vaccines[i]. In line with the conclusion in the CVMP Strategy on Antimicrobials that vaccination of animals is an effective measure to reduce the need for antimicrobials; this joint action plan is also relevant to the objectives of increasing access to alternatives to antimicrobials.

3. Discussion

Strategic objectives of introducing measures to support the authorisation of ATAm

The use of ATAm represents one way in which to reduce the use of antimicrobials, particularly antibiotics, in veterinary medicine. This reflection paper therefore explores ways by which to ensure that the EU is encouraging the authorisation of ATAm.

This will be achieved by:

- recognising the importance of alternatives to antibiotics as a mean of reducing the use of antimicrobials in veterinary medicines and adopting a pro-active approach to promoting their authorisation;
- ensuring that the EU has the appropriate legal framework and the necessary guidance in place for authorisation of those categories of veterinary medicinal products that can be used as ATAm. It is noteworthy that for some ATAm (e.g. vaccines) the legal framework is well established and adequate guidance is available currently;
- promoting international cooperation and exchange of information with other regulatory regions to assist global development of ATAm and aligning the approach to authorisation where possible;
- providing advice and support to developers and applicants seeking to authorise ATAm within the EU.

3.1. Definition of terms

The terms 'antimicrobial' and 'antibiotic' are defined in the new Regulation 2019/6 as follows:

> *Antimicrobial: 'any substance with a direct action on micro-organisms used for treatment or prevention of infections or infectious diseases, including antibiotics, antivirals, antifungals and anti-protozoals;*
>
> *Antibiotic: 'any substance with a direct action on bacteria that is used for treatment or prevention of infections or infectious diseases.*

However, there is no internationally accepted definition of what constitutes 'alternatives to antimicrobials'. A working definition of alternatives to antimicrobials for the purposes of this document is proposed as follows:

> *'a veterinary medicinal product the use of which provides an alternative approach to the use of antimicrobials in animals or that reduces the need for their use'.*

The definition limits the scope of this document to veterinary medicinal products in line with the mandate of the CVMP. However, any consideration of ATAm will inevitably consider other types of products as the same substances may be used as medicinal products or for another purpose (e.g. as a biocide or feed additive) depending on the way it is presented and the claims that are made.

The RONAFA opinion, although it considers vaccines as an alternative to antimicrobials, does not formally include vaccination as an 'alternative' but rather categorises vaccination as a tertiary prevention measure to reduce the need for antimicrobials through creating more resilient animals. Other sources view vaccines as one of many alternatives to antimicrobials. In practice, the increased uptake of vaccination represents one of the most practical ways in which the use of antimicrobials in general and in particular the use of antibiotics can be reduced, both now and in the future. Ways to promote authorisation and use of effective vaccines are therefore included within the scope of this document.

It is recognised that some of the alternatives that are being developed will themselves fall within this definition of an antimicrobial. Nevertheless, it is appropriate to include such products within the scope of this paper as they represent an alternative to the use of conventional antibiotics which are the main focus of measures to reduce use. Furthermore, the availability of such products has the potential to broaden the choice of active substances that can be used to manage infectious disease in animals.

3.2. Current measures

In general, the same range of support measures is available for applicants seeking to authorise ATAm as for any other new veterinary medicinal product[vii], namely;

- Scientific advice to companies on the appropriate tests and studies in the development of a veterinary medicine.
- Pre-submission meetings for applicants to obtain procedural, regulatory and legal advice from the Agency.
- The Minor Use Minor Species /limited market (MUMS) Scheme to address the lack of veterinary medicines for the treatment of minor animal species and uncommon diseases in major animal species. Where applicants consider that ATAm are intended for a limited market they can seek classification by CVMP of their intended product as MUMS/limited market with the benefits in terms of reduction in data requirements and financial incentives that this may imply.
- The SME (micro, small and medium-sized enterprise) Scheme provides financial incentives and other benefits to companies designated as SMEs. This is particularly relevant for ATAm where initial research and discovery is often carried out by SME companies.

In addition, and particularly as ATAm will be often innovative products that represent novel veterinary therapies, the following groups already generate advice and guidance:

- The Innovation Task Force (ITF) which acts as a forum for early dialogue with applicants on innovative aspects in medicines development. From May 2019, EMA is facilitating early engagement with medicine developers working on therapeutic approaches for the treatment and prevention of bacterial and fungal infections.
- The Ad Hoc Expert Group on Veterinary Novel Therapies (ADVENT) providing advice on the requirements for authorisation of therapies that are new to the veterinary domain.

These groups have complementary roles. ITF provides product-specific advice to applicants at early stages of product development in response to a request. ADVENT identifies priority areas in the field of veterinary novel therapies and publishes general, non-product specific guidance, generally in the form of Question-and-Answer documents.

AMR is a global phenomenon, as recognised by the WHO in the Global Action Plan[viii] and by OIE[iX] in their corresponding strategy. In terms of meeting the need for new products to meet this threat, a global response is therefore required that should involve cooperation between regulators at international level. EMA and CVMP experts participate in relevant international conferences on the topic of ATAm and EMA exchanges information with both the FDA Centre for Veterinary Medicines and the USDA Centre for Veterinary Biologics. More recently, the topic of ATAm in veterinary medicine has been included as specific action item 3.7 in the work plan of the Trans- Atlantic Task Force on AMR (TATFAR).

3.3. Gaps identified and possible additional measures

Table 1 presents the results of a gap analysis between the measures currently available and possible additional measures.

4. Conclusions

This reflection paper has been endorsed by CVMP and represents a reflection on the measures that could be taken to deliver the objective in the CVMP Strategy on Antimicrobials related to ATAm. It is clear that to make meaningful progress on this topic would require not only CVMP, but also the wider European Medicines Regulatory Network, to put in place a set of coordinated actions to promote development, authorisation and use of ATAm in the veterinary domain. Possible activities are proposed for the EMA secretariat, CVMP and its working parties, Coordination Group for Mutual Recognition and Decentralised Procedures - Veterinary, Heads of Medicines Agencies, the European Commission, the animal health industry and national competent authorities. This initial gap analysis clearly shows that making progress on this topic will require considerable engagement of resources across the Network and by industry. A long term approach is therefore supported.

5. Next steps

Following informal consultation within the regulatory network, CVMP considered the responses to the consultation on the draft discussion document and produced this CVMP reflection paper which is released for public consultation. Additional proposals on how to facilitate and incentivise the development and authorisation of ATAm are sought.

6. Potential actions, Actors, Resource and Impact analysis

The gaps identified in the current analysis are categorised in three different areas:

a) Gaps in the EU regulatory framework
b) Gaps in support to developers and applicants of ATAm
c) Gaps in strategic collaboration and communication with stakeholders

Gap	**Activity No**	**Activity**	**Responsible *(and others involved*)**	**Timescale**	**Resource impact**	**Challenges, comments**
a) Gaps in Regulatory Framework						
Lack of consistent terminology causes confusion	1.	Define term 'Alternatives to antimicrobials' in the context of measure to promote their authorisation	CVMP	Short term	Minimal	Definition for ATAm might promote harmonisation of regulatory requirements at EU and international level. The term should include vaccination as vaccines have a major potential for reducing use of antimicrobials in animal husbandry.
Companies developing ATAm are often unsure to which regulatory authority they should apply and what legal framework will apply (e.g. medicine, feed additive, biocide)	2.	Provide clarity to applicants on the classification of borderline products	CMDv Borderline Products Working Group National Competent Authorities (NCAs)	Current	Within the remit of existing Borderline Products Groups NCAs have in place systems to provide advice to applicants on classification of borderline products	In the new Regulation (EU) 2019/6, CMDv is mandated to provide recommendation as to whether a product falls within the definition of a veterinary medicinal product. CMDv readiness and capability to classify ATAm to be confirmed. Possibility of developing guidance with other EU Agencies exists (e.g. EFSA, ECA). Would require mandate from EC and not clear that proactive approach would be better than rapid response to specific queries from applicants. Need for harmonisation with NCAs having already systems in place.

Gap	Activity No	Activity	Responsible *(and others involved)*	Timescale	Resource impact	Challenges, comments
EU legal framework needs to support authorisation of ATAm by establishing appropriate requirements in legislation and providing guidance on the technical requirements that need to be fulfilled	3.	Explore how the new veterinary regulation (NVR) provides framework for authorisation of appropriate ATAm as veterinary medicines and reflect on need for additional guidance	CVMP	Current-Medium term	Within work on NVR	Requirements for VMPs are specified in technical annexes to the NVR, therefore need to ensure that requirements for ATAm are reflected in content of annexes. Reflection necessary on need for additional guidance.
Current lack of guidance increases uncertainty in a number of areas	4.	Generate additional guidance specifically intended to clarify requirements for ATAm. Specific examples are given in the rows below	EMA CVMP	Medium-long term	Would require including in the work programme of relevant CVMP working parties (WPs)	Prioritisation would be essential due to the limited resource available within the veterinary network to generate new guidance. Work in the area of ATAm would be particularly resource intensive as many topics are new to the area of veterinary medicines and would therefore require extensive reflection and consultation before guidance could be produced. Consider need for guidance on GMP requirements for specific ATAm products (e.g. bacteriophages, gene-editing products).

Gap	Activity No	Activity	Responsible *(and others involved)*	Timescale	Resource impact	Challenges, comments
		Explore how benefit risk assessment for VMPs (vaccines and other products) could take into account that a product reduces the use of antimicrobials Explore if/how the beneficial effect of ATAm products on reducing the use of antimicrobials could be reflected in the product information and, if relevant, define data required to support it.	EMA CVMP	Medium-long term	Would require including in the work programme of CVMP.	No specific regulatory framework currently exists for evaluation of claims that relate to products reducing the need to use antimicrobials or how to include evidence as part of B:R for authorisation. OIE list of vaccines that could reduce need for antibiotics may be a useful reference. Attention should be given to providing a regulatory framework for adjunct therapies that are not effective when administered alone but are effective when administered in association with another product (e.g. non-specific immunostimulant that boosts the effect of a vaccine). Likewise the possibility should be evaluated of developing an approach to assess efficacy of ATAm products as their efficacy levels may be lower compared to conventional antimicrobials authorised for the same disease but still show an overall positive benefit:risk balance and a beneficial effect in reducing the need to use conventional antimicrobials.

Gap	Activity No	Activity	Responsible *(and others involved)*	Timescale	Resource impact	Challenges, comments
		Regulatory requirements for bacteriophages	EMA CVMP	Short term	Initial reflection already on work programme for ADVENT	Similar regulatory and scientific challenges exist for authorisation of bacteriophages as human medicines. Need to consider if specific guidance for bacteriophage products, in line with the new Annex II of Regulation (EU) 2019/6, are required.
		Regulatory requirements for novel biologically active molecules that kill bacteria but are not classic pharmaceutical antibiotics (e.g. lysins, peptides, lysozymes and other enzymes), including requirement related to MRLs	EMA CVMP	Medium-long term	Would require including in the work programme of relevant CVMP WPs Relevant topic for ADVENT	ADVENT is working on this topic.

Gap	Activity No	Activity	Responsible *(and others involved)*	Timescale	Resource impact	Challenges, comments
		Regulatory requirements for non-specific immuno-stimulants	EMA CVMP	Medium-long term	Would require including in the work programme of relevant CVMP WPs. Possible relevant topic for ADVENT	To date advice has been given on a case-by-case basis on products/ substances that stimulate innate immunity to enhance resistance to infection or to promote the response to vaccination. The possibility of developing general guidance in this area could be explored. Conditions of use should be defined. Immuno-stimulants are most likely not suitable for extended use. A framework is required to evaluate the impact of ATA on the microbiome of animals and the consequent impact on innate resistance.
		Regulatory requirements/framework for gene editing technology presented as medicinal products (e.g. CRISPR-Cas9)	EMA CVMP	Long term	Unknown Relevant topic for ADVENT	Need to improve knowledge in regulatory domain of potential use of gene editing technology to reduce use of antimicrobials (e.g. to target bacterial pathogens or to restore antimicrobial efficacy by targeting bacterial extrachromosomal genetic elements such as plasmids).

Gap	Activity No	Activity	Responsible *(and others involved)*	Timescale	Resource impact	Challenges, comments
		Regulatory requirements for herbals, phytochemicals and other non-biological active substances presented as alternatives to antimicrobials including establishment of MRLs	EMA CVMP	Medium-long term	Would require including in the work programme of relevant CVMP WPs	Current legislation requires applicants (company, NCA) to submit an MRL application to EMA supported by an appropriate package of safety data with the intention to subsequently seek authorisation of a VMP. Consideration of the MRL requirements for these substances should be given. The efficacy of such substances is not directly comparable to existing antimicrobials and they are frequently presented as reducing the need for antimicrobials without replacing them. The feasibility of developing a framework for evaluation of such substances in support of a claim to reduce use of antimicrobials should be evaluated with a view to reducing the regulatory burden on applicants without compromising on safety, possibly in the context of the new veterinary regulation.
Internationally aligned requirements are needed to promote global development programmes for ATAm	5.	Dedicated exchange of information in the context of TATFAR Action 3. 7 on current activities in the area of ATAm to identify opportunities for further cooperation Harmonisation of requirements for ATAm	EMA (TATFAR) USA Canada Norway VICH	Long term Long term	Planned within TATFAR activities	Objective is to exchange information and thereby reduce duplication of effort. Scope currently limited to exchange of information and does not extend to harmonisation of requirements at this early stage of discussion. Long-term objective to harmonise requirements for ATAm products across regions.

Gap	Activity No	Activity	Responsible *(and others involved)*	Timescale	Resource impact	Challenges, comments
b) Gaps in support to ATAm applicants and developers						
Companies seek early 'upstream' advice to reduce risk related to development of ATAm	6.	Promote ITF as the appropriate forum for scientific, regulatory and procedural advice related to development of innovative VMPs, including ATAm	EMA (V Division; Stakeholders Division) ITF NCA innovation contact points	Current	Additional work required for pro-active communication by EMA Increased workload for ITF related to ATAm	Challenges (i) to identify experts available to the Network with knowledge of ATAm (ii) to identify companies working on ATAm and target communication to them. Explore possibilities to engage the EU Innovations Network on the specific topic of ATAm to engage national innovation offices.
Many companies developing ATAm are SMEs unaware of regulatory requirements and of the assistance provided by EMA	7.	Promote EMA and NCA incentives to SMEs working in the area of ATAm	EMA (SME Office) NCA SME contact points	Current	Additional work required EMA SME office	Challenge would be to identify SMEs working in this area and target communication to them. EMA will include ATAm as a specific topic for consideration within the EMA framework for engagement with academia in the veterinary domain. New Regulation (EU) 2019/6 places an obligation on EU Member States to assist SMEs.

Gap	Activity No	Activity	Responsible *(and others involved)*	Timescale	Resource impact	Challenges, comments
Creation of 'pull' incentives	8.	Financial or other incentives to authorisation of ATAm	TBD	TBD	TBD	Industry has raised the possibility of 'pull' incentives for authorisation of ATAm. The view to date of EMA has been that financial or other procedural, regulatory incentives, over and above those already in place, are not the most significant factor reducing interest in developing ATAm. Furthermore, there is no clear legal basis on which EMA could systematically provide such incentives in the veterinary domain under the current legal framework. The wider EU Medicines Regulatory Network should discuss if there is interest in introducing financial or other "pull" incentives at national or European level to promote authorisation of ATAm and how this could be achieved.

Gap	Activity No	Activity	Responsible *(and others involved)*	Timescale	Resource impact	Challenges, comments
c) Gaps in strategic collaboration and communication with stakeholders						
Communication with stakeholders on ATAm	9.	Create a platform of communication and dialogue with industry on development of ATAm	EMA, CVMP,HMA	Short-medium term	Would require including in the work programme of CVMP, EMA, industry	If agreed that coordinated action is required then communication and engagement of stakeholders from the outset would be important. This would require dedicated resources. In view of the scope and scale of activity required to make progress on this topic options should be explored for the creation of a public private partnership such as was formed for the European Technology Platform for Global Animal Health, including the DISCONTOOLS project. ATAm should be included as a priority topic for the veterinary domain within the EMA Regulatory Science Strategy in terms of both promoting ATAm technologies and the development of new regulatory tools.
Develop objective targets to monitor success of measures to promote ATAm	10.	Draft a roadmap with targets for development of veterinary ATAm in the EU including an impact assessment on potential reduction when reaching these targets	EMA, CVMP HMA	Medium term	Would require including in the work programme of CVMP, WPs, EMA and possibly HMA	Identifying ATAm with the greatest potential for reducing use of antimicrobials and monitoring their progress to authorisation could be an objective measure of success. This would require a substantial investment of resources to achieve.

7. Appendix 1

Examples of alternatives to antimicrobials*:

- Vaccines
- Antibodies
- Immunomodulators
- Bacteriophages (wild-type, engineered)
- Lysins
- Antimicrobial peptides (e.g. bacteriocins, host-defence peptides)
- CRISPR-Cas9-based products
- Probiotic and live organisms (e.g. probiotics, predatory bacteria, competitive exclusion)
- Prebiotics
- Symbiotics
- Postbiotics
- Interferons
- Phytochemicals
- Herbals/Botanicals
- Organic acids
- Biocides
- Teat sealants

*Classification of ATAm products as veterinary medicinal products, feed additives, biocides, etc. will depend on their presentation, intended use and claims made for the product.

8. References

[I] https://www.ema.europa.eu/en/documents/other/eu-medicines-agencies-network-strategy-2020-working-together-improve-health_en.pdf

[ii] https://ec.europa.eu/health/amr/sites/amr/files/amr_action_plan_2017_en.pdf

[iii] https://www.ema.europa.eu/en/documents/scientific-guideline/cvmp-strategy-antimicrobials-2016-2020_en.pdf

[iv] https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32019R0006&from=EN

[v] Seal B.S., Lillehoj H.S., Donovan D.M., Gay C.G. (2013) Alternatives to antibiotics: a symposium on the challenges and solutions for animal production. Anim Health Res Rev. 2013 May 23:1-10.

[vi] https://www.ema.europa.eu/en/documents/report/ema-efsa-joint-scientific-opinion-measures-reduce-need-use-antimicrobial-agents-animal-husbandry_en.pdf

[vii] Ioannou F, Burnsteel C, Mackay DKJ, Gay CG. (2018). Regulatory pathways to enable the licensing of alternatives to antibiotics. Biologicals; 53:72-75.

[viii] http://www.who.int/iris/bitstream/10665/193736/1/9789241509763_eng.pdf

[ix] https://www.oie.int/fileadmin/Home/eng/Media_Center/docs/pdf/PortailAMR/EN_OIE-AMRstrategy.pdf

HealthforAnimals
global animal medicines association

Animal Health Sector Commitments and Actions on Antibiotic Use

Antibiotics are key to treating infections in humans and animals - there are no alternatives to treating life-threatening bacterial infections. The world has recognized that antimicrobial resistance (AMR) is a challenge that costs lives. Our vision is a world where veterinary antibiotics are used responsibly to protect and treat animals, and where they maintain their value as a therapeutic tool. An equally important challenge is maintaining and increasing food safety and security. To address these interconnected challenges, companies and organizations commit to five principles and practical actions, and will proactively encourage others to embrace them.

Principle 1: Protect animal health and welfare in a unified One Health approach.

Actions: We will meet the ethical requirement of protecting animals by providing animal health products of the same high quality as products developed for people. In addressing antimicrobial resistance, we will take a One Health approach to cooperation, equally considering human and animal health, and environmental impact. To address AMR, we will strengthen partnerships between animal health companies, farmers, veterinarians, retailers, authorities and associations. We will reach out to seek new solutions and ideas and are open to explore joint actions with others.

Principle 2: Use antibiotics judiciously and responsibly.

Actions: We promote the use of antibiotics for therapeutic reasons. Under the adage “as little as possible, as much as necessary”, we will continue to promote responsible/judicious use by providing clear labels and good technical advice. We recommend antibiotics be used under veterinary supervision where available. In countries that lack veterinary capabilities, imposing this requirement before addressing the shortage of veterinarians condemns animals to unnecessary suffering. We will increase our efforts to encourage investment in better access to veterinary care. We actively contribute to the promotion of responsible use principles and practices in national responsible use coalitions.

Principle 3: Promote disease prevention and increased access to products and expertise.

Actions: We promote animal husbandry techniques that contribute to disease prevention by sharing our knowledge with producers. We commit to continuing to improve availability of vaccination. We will seek maximum access and affordability of legitimate products to counter the use of illegal, low quality and fake products in some markets, and contribute to education and training on the dangers of their use. We will advocate for programs to increase the number of veterinarians in developing countries.

Principle 4: Invest in development of products for prevention and treatment.

Actions: We will invest in new products that reduce reliance on antibiotics. As animal health companies we will invest between 6%-9% of our annual turnover in the development of new products, diagnostics, genetics and life-cycle management of existing products. Global annual animal health sector revenues are estimated at US$30 billion. Assuming companies not part of HealthforAnimals also invest a similar percentage, this equates to an annual investment of US$1.8 - 2.7 billion for prevention and treatment options for food and companion animals. Investment at these levels for new solutions is commercially viable only if rising cost factors like regulatory cost are reduced. We encourage governments to: increase incentives for new technological advances, facilitate regulatory pathways for all types of products, encourage increased uptake of vaccination, and support availability and access to veterinarians.

Principle 5: Increase knowledge, transparency and communication.

Actions: We support science and evidence-based solutions. Many animal health companies undertake scientific research into AMR pathways, and we support other groups in their research. We will continue to contribute to the collection of national and international antibiotic use data. We will encourage producers and retailers to be transparent about animal health and welfare impacts resulting from antibiotic-free production practices. We will increase our communications efforts about responsible use, vaccination and other prevention methods, while continuing to provide data and advice to our customers on managing diseases.

This Commitment is managed by HealthforAnimals, the global animal medicines association, and our Members. Other organizations or companies are encouraged to join us in affirming these principles.

Contact info@HealthforAnimals.org to learn how to join the Commitment and become a signatory.

This Commitment is supported by organizations representing over 200 companies and 700,000 veterinarians worldwide. View the full list →

Health for Animals
global animal medicines association

HealthforAnimals Member Companies:

Bayer · Boehringer Ingelheim · Ceva · Elanco · MERCK Animal Health · Phibro Animal Health Corporation · vetoquinol Achieve More Together · Virbac · zoetis

HealthforAnimals Member Associations:

Argentina:	Caprove
Australia:	Animal Medicines Australia
Belgium:	PHARMA.BE
Brazil:	Sindicato Nacional da Industria de Produtos Para Saude Animal (SINDAN)
Canada:	Canadian Animal Health Institute
Chile:	Association Gremial de Laboratorios de productos Veterinarios
Denmark:	Danish Association for the Veterinary Pharmaceutical Industry
France:	Syndicat de L'industrie Du Médicaments et Reactif Veterinaires (SIMV)
Germany:	Bundesverband Für Tiergesundheit (BfT)
Europe:	AnimalhealthEurope
India:	Indian Federation of Animal Health Companies (INFAH)
Indonesia:	Indonesian Veterinary Drugs Association
Ireland:	Animal and Plant Health Association (APHA)
Italy:	Italian Animal Health Association (AISA)
Japan:	Japan Veterinary Products Association (JVPA)
Korea:	Korean Animal Health Products Association (KAHPA)
Mexico:	Industria Farmaceutica Veterinaria, Canifarma
Netherlands:	Fabrikanten en Importeurs van Diergeneesmiddelen (FIDIN)
New Zealand:	Association for Animal Health and Crop Protection (AGCARM)
Paraguay:	Cámara de Especialidades Veterinarias del Paraguay
Portugal:	Apifarma
South Africa:	South African Animal Health Association
Asia:	Asian Animal Health Association
Spain:	Veterindustria
Sweden:	Läkemedelsindustriföreningen (LIF)
Switzerland:	Scienceindustries
Thailand:	Thai Animal Health Products Association
United Kingdom:	National Office of Animal Health (NOAH)
United States:	Animal Health Institute (AHI)

Other Animal Medicines Associations:

Greece:	HAVEPHARM
Finland:	Finnish Veterinary Pharma Association (FVPA)
Poland:	Polish Association of Producers and Importers of Veterinary Medicinal Products
Czech Republic:	Czech and Slovak Association of Veterinary Pharmaceutical Companies (CSAVP)
Slovakia:	Czech and Slovak Association of Veterinary Pharmaceutical Companies (CSAVP)
Hungary:	HAIVPMR
Norway:	Norwegian Association of Pharmaceutical Manufacturers (LMI)

Other Companies and Organizations:

Biogenesis
Colorado Serum Company
Coveli
Des-Vet Productos Vet
Dopharma
IDT BIOLOGIKA
Jurox
Kyoritsu Seiyaku
Laboratorio Bio-Vet
LABORATÓRIO Productos Vet
Nippon Zenyaku Kogyo (Zenoaq)
Pharmaxim
Orion
Ourofino
UZINAS CHIMICAS BRASILEIRAS
Vetnil

Support from the Veterinary Community:

WVA

Statement from World Veterinary Association:

The World Veterinary Association (WVA) represents around 500,000 veterinarians around the world. WVA commends HealthforAnimals for the development and launch of the Global Animal Health Sector Antibiotic Commitment WVA supports the initiatives and actions set out in the Commitment; they are in line with the WVA position on responsible use of medicines. WVA wishes HealthforAnimals much success in its endeavors to decrease the development of antimicrobial resistance.

Veterinarians play a primary role in assessing animal health, making a diagnosis, and recommending effective care programs including the use of antimicrobials which must be under veterinary care with a valid veterinarian-client-patient relationship.

The WVA position highlights the global basic principles of antimicrobial use such as:

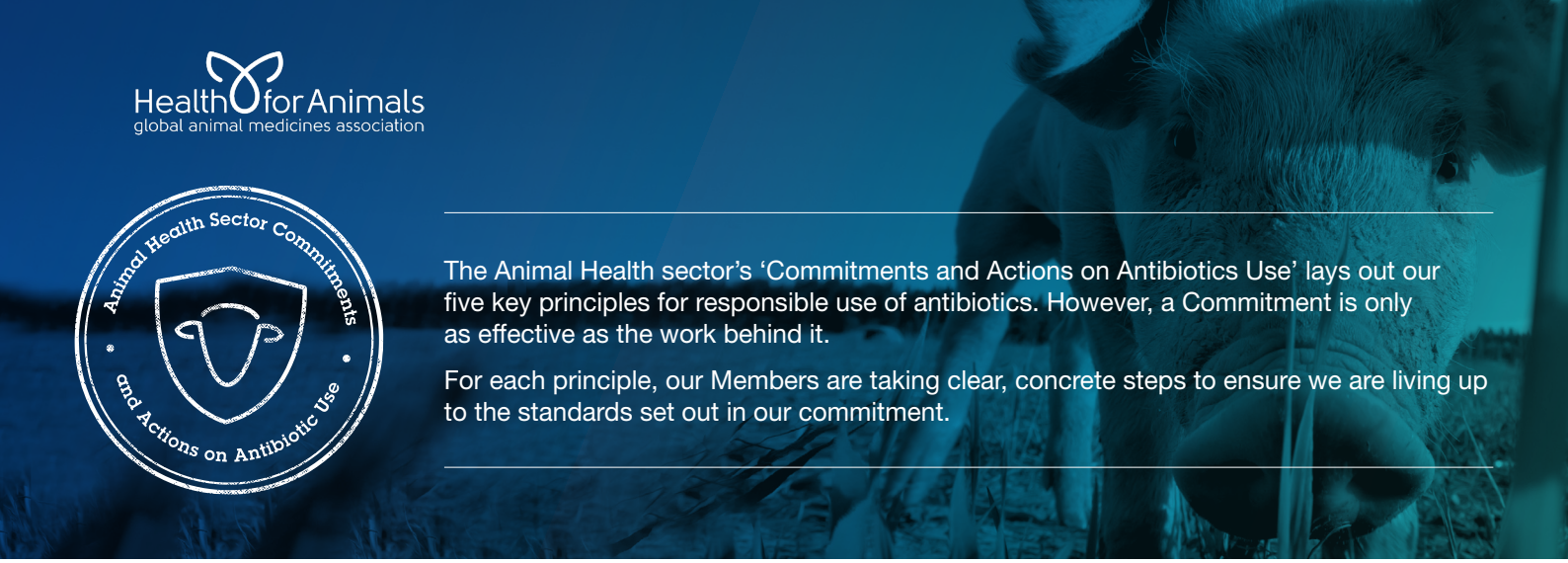
- Therapeutic antimicrobials are licensed or registered for the purposes of disease treatment, control, and prevention.
- The availability of antimicrobials should be based on risk/benefit analysis that considers the importance of the antimicrobial to both veterinary and human medicine.
- Codes of good veterinary practice, quality assurance programs, herd health control and surveillance programs, and education programs should promote the responsible and prudent use of antimicrobials.
- Therapeutic antimicrobials should be used for as long as needed but for the shortest duration necessary, and at the appropriate dosage.

WSAVA Global Veterinary Community

Statement from the World Small Animal Veterinary Association (WSAVA):

The World Small Veterinary Association (WSAVA) represents 200,000 veterinarians worldwide. Based on our active involvement in One Health and the recognition that AMR is very much a One Health issue within which companion animal practice shares responsibility for proper antimicrobial stewardship, the WSAVA endorses the HealthforAnimals antibiotic commitment, and looks forward to working collaboratively to realize its goals.

Health for Animals
global animal medicines association



The Animal Health sector's 'Commitments and Actions on Antibiotics Use' lays out our five key principles for responsible use of antibiotics. However, a Commitment is only as effective as the work behind it.

For each principle, our Members are taking clear, concrete steps to ensure we are living up to the standards set out in our commitment.

Principle 1: Protect animal health and welfare in a unified One Health approach.

Our Members' Work:

- Partnering with the Pan American Health Organization Foundation to support countries in Latin America in creating action plans to combat AMR in line with the World Health Organization's key pillars.
- Building a 'European Platform for Responsible Use of Medicines in Animals' that brings together farmers, veterinarians, pharmacists, manufacturers and more to develop collaborative solutions.
- Working directly with users of veterinary medicines in over 130 countriesto build strong relationships and provide professional guidance.

Principle 2: Use antibiotics judiciously and responsibly.

Our Members' Work:

- Creating GRAM (Guidance for the Rational use of AntiMicrobials), a comprehensive, 500-page global guide to responsible use created by independent experts. The guide improves diagnosis and treatments while providing key advice on managing resistance.
- Training veterinary medicines users in responsible antibiotic use supported by detailed product labels outlining dose, withdrawal periods, and more.
- Investing millions in the next generation of responsible veterinarians through scholarships and grant programs in the U.S., Africa, China and elsewhere.

Principle 3: Promote disease prevention and increased access to products and expertise.

Our Members' Work:

- Launching new apps that help producers in any region optimize vaccinations, identify disease and create prevention strategies specific to their farm.
- Partnering with the Bill and Melinda Gates Foundation to train new veterinarians, develop diagnostic networks and bolster infrastructure in Africa and Southeast Asia.
- Creating new packaging to thwart counterfeiters and working with national governments to warn producers of the risks of illegal medicines.

Principle 4: Invest in development of products for prevention and treatment.

Our Members' Work:

- Creating first-of-its kind, new technologies that allow for herd-specific custom vaccines for animals. These provide effective care targeted to specific animals.
- Developing nutritional supplements that bolster an animal's natural immune system, which can reduce disease and decrease the need for antibiotics.
- Introducing new, animal-only antibiotics that offer veterinarians a way to tackle disease that pose virtually no risk of contributing to resistance in humans.

Principle 5: Increase knowledge, transparency and communication.

Our Members' Work:

- Contributing to antibiotics surveys like the European Medicines Agency's ESVAC report (European Surveillance of Veterinary Antimicrobial Consumption Report).
- Creating web-based tools and training front-line representatives to us integrated analytics to predict disease risk, improve prevention and design sustainable solutions specific to individual farms.
- Collaborating with research organisations and universities to better understand antimicrobial resistance; funding research on AMR in foodborne pathogens.

This work is only a sample of what signatories to the 'Animal Health Sector Commitments and Actions on Antibiotic Use' are doing to support responsible antibiotic use. For more information or to learn about other work being done, contact **Info@HealthforAnimals.org**

View the full 'Animal Health Sector Commitments and Actions on Antibiotic Use' at **HealthforAnimals.org/OurCommitment**

全球動物醫療健康協會之抗生素承諾

- 原則 1：透過防疫一體策略保護動物健康及福祉

1. 行動措施：協會將透過提供等同人用之高品質動物健康醫療產品，以保護動物及符合道德條件要求。在處理 AMR 議題方面，協會將強化動物健康醫療公司、農戶及獸醫師、零售商、主管機關及協會之間的合作。協會將找尋新方案及想法，並公開給其他人，以加入此行動措施。
2. 協會會員的工作：
   (1) 與泛美衛生組織基金會（Pan American Health Organization Foundation）合作，支持拉丁美洲國家建立行動方案以對抗 AMR，符合世界衛生組織（World Health Organization）重要的工作目標。
   (2) 建立「負責任使用動物用藥品」之歐洲平台，以串聯農戶、獸醫師、藥劑師及藥品製造業者等共同發展合作方案。
   (3) 直接與超過 130 個國家的動物用藥品使用者合作，以建立關係及提供專業指引。

- 原則 2：謹慎並負責任地使用抗生素

1. 行動措施：推廣使用治療用抗生素的方式，套句諺語「儘可能少用、必要時才用（As little as possible, as much as necessary）」，協會將持續透過明確標示及良好的技術意見，推廣謹慎並負責任使用抗生素。協會建議抗生素應在獸醫師的監督下使用及取得。針對獸醫師人力不足的國家，處理此問題之前，強制執行此措施，以免動物受苦。協會將鼓勵投資以提供更好的動物醫療照護。協會主動參與推廣負責任使用抗生素的原則及措施。
2. 協會會員的工作：
   (1) 訂定「合理使用抗微生物藥品指引」（Guidance for the Rational use of AntiMicrobials, GRAM），由獨立的專家學者訂定一份全球指引資料。在管理抗藥性議題方面，此指引提供重要意見，改善診斷及治療。

(2) 透過產品標示用法用量、停藥期等注意事項，訓練動物用藥品使用者負責任使用抗生素。

(3) 透過學術研究及補助計畫投資數百萬美金於下一個世代獸醫師，包含美國、非洲、中國及其他國家/區域。

- 原則 3：推廣疾病的預防及增加此類產品及專家。

1. 行動措施：透過分享專業知識給養殖生產者，協會推廣涉及疾病預防的畜牧產業技術。協會承諾持續改善疫苗的取得性。協會將尋找價格平易近人、容易取得的產品，以對抗違法、品質低劣及偽造之產品，並持續教育宣導使用這些產品的危險性。協會提倡增加開發中國家之獸醫師人力。
2. 協會會員的工作：

(1) 推動新的應用軟體，幫助任何區域的養殖生產者優化其牧場的免疫注射、疾病辨別及建立預防策略。

(1) 與比爾與美琳達·蓋茲基金會 （Bill & Melinda Gates Foundation）合作，於非洲及東南亞訓練新進獸醫師、研發診斷網絡及增加基礎建設。

(2) 為對抗偽藥，與政府單位合作制定新包裝，以警告養殖生產者使用違反藥品的風險。

- 原則 4：投資預防及治療疾病的產品

1. 行動措施：協會將投資新產品，以減少對抗生素的依賴。身為動物健康醫療公司，協會將每年營收的 6~9%，投資於新型產品、診斷劑、基因學產品及已存在產品的運作管理。全球每年動物健康醫療領域的收入估計約為 300 億美元。假設非為協會成員公司亦投資類似的比例，則等同於每年投資 18~28 億美元，用於食用動物及伴侶動物之疾病預防及治療技術。協會鼓勵政府機關：（1）增加新技術發展的誘因。（2）推動法規適用所有產品（3） 鼓勵增加疫苗接種率（Uptake of vaccination）（4）支持獸醫師人力資源。
2. 協會會員的工作：

(1) 首次建立一種新型技術，係針對特定動物族群的客製化疫苗（Herd-specific custom vaccines for animals）。此針對特定動物的疫苗可提供有效保護。

(2) 研發營養補給品，增強動物的先天性免疫系統，以降低疾病發生及抗生素需求。

(3) 引進新型，僅動物用之抗生素，供獸醫師處理疾病，並且不會造成人類疾病抗藥性的風險。

- 原則 5：增加專業知識、透明度及溝通交流

1. 行動措施：協會支持以科學實證為基礎的解決方案。許多動物健康醫療公司已著手進行 AMR 研究，而協會支持其研究。協會將持續參與國家及國際抗生素使用量資料收集。協會將鼓勵養殖生產者及藥品販賣業者，使無抗生素飼養生產對動物健康及福利的影響透明化。協會將針對負責任使用抗生素、疫苗免疫及其他預防措施，努力進行溝通交流，並持續提供疾病管理方面的意見給消費者。

2. 協會會員工作：

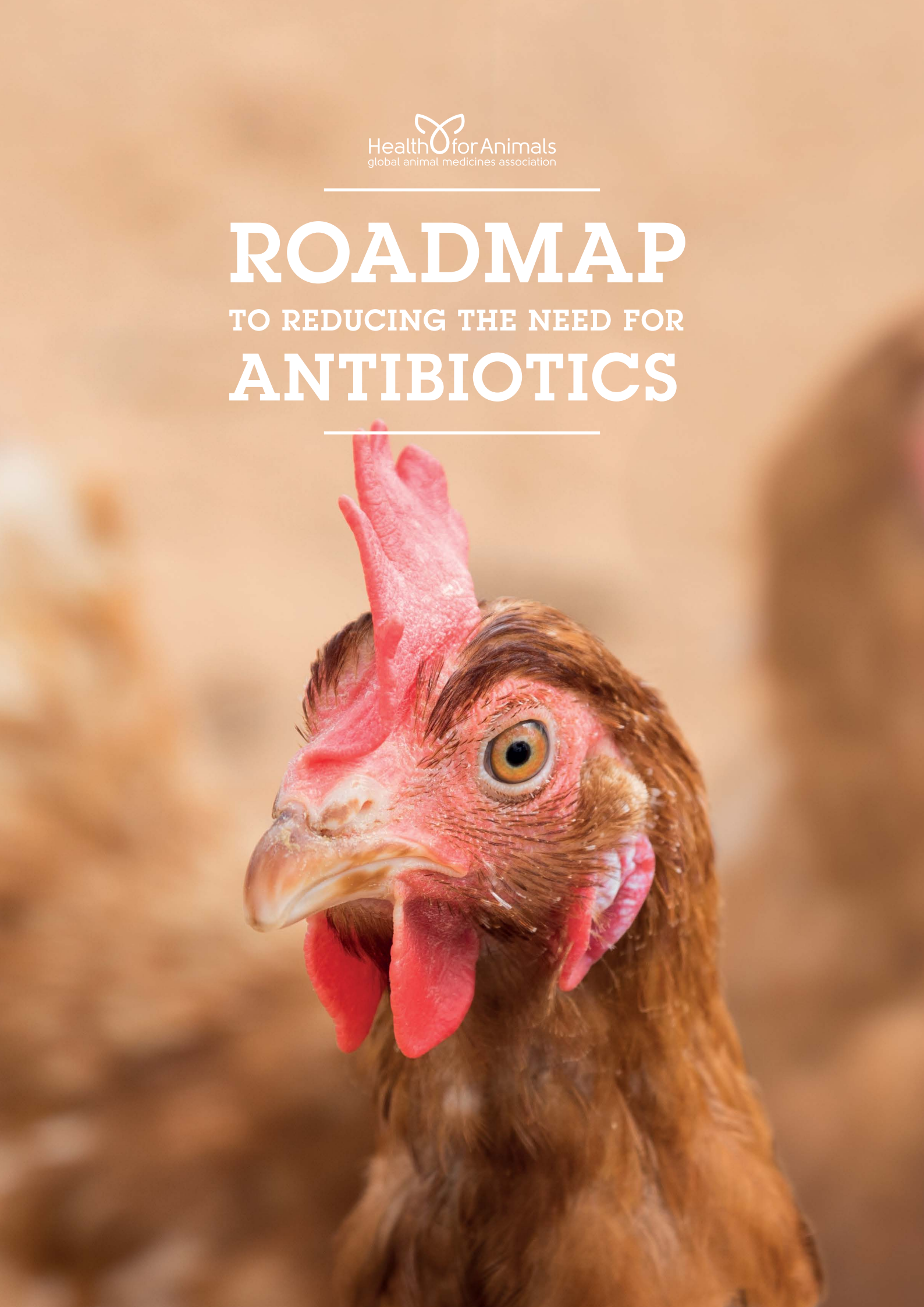
(1) 持續進行抗生素調查，例如歐洲藥品管理局（EMA）的 ESVAC 報告（European Surveillance of Veterinary Antimicrobial Consumption Report, ESVAC report）。

(2) 建立網站工具及訓練第一線代表協會之人員整合分析預測疾病之風險、改善預防措施及針對特定牧場設計永續方案。

(3) 與研究組織及大學合作，以更加瞭解 AMR，另經費資助 AMR 食媒病原研究計畫。

Health for Animals
global animal medicines association

ROADMAP
TO REDUCING THE NEED FOR
ANTIBIOTICS



Contents

This document was produced by HealthforAnimals, the global animal medicines association. HealthforAnimals represents the animal health sector: manufacturers of veterinary pharmaceuticals, vaccines and other animal health products throughout the world, as well as the associations that represent companies at national and regional levels.

1. Introduction

Antibiotics are a cornerstone of modern medicine and public health.

Their importance to human and animal health cannot be understated, which is why antimicrobial resistance (AMR) is such an important global threat. When bacteria develop tolerance or resistance to antibiotics, we risk returning to a time when animals – and people – fell seriously ill or even died from simple, treatable infections.

Antibiotics are the only way to treat a bacterial disease. There is currently no alternative.

As the producers of animal medicines and other health products, our industry equips veterinarians with the tools to manage animal disease. Reducing antibiotic use without first tackling disease rates would mean sick animals go untreated, causing unnecessary suffering and mortality while increasing risk of transfer to other animals and people.

However, we can exploit the full spectrum of animal health tools to reduce the need for antibiotics.

By better protecting animals from the threat of disease, identifying health issues earlier and treating them quickly and responsibly, we can decrease disease levels and with it, the need for antibiotics. This requires maximising the long-term and preventative health benefits of tools such as vaccination, nutrition, antiparasitics, biosecurity, disease surveillance, diagnostics, husbandry and other animal health technologies.

Together, these tools can improve the prevention, detection and treatment of animal disease. This is our roadmap to reducing the need for antibiotics.

The ability to manage and control animal disease has profound consequences for human health and development, from ensuring the safety of meat, milk, fish and eggs to reducing the risk to people of bacterial animal-borne diseases. And while the relationship between using antibiotics in animals and growing levels of resistance in people remains complex and not well understood, AMR affects us all.

Our industry has worked on this challenge for many years, and our 2017 Antibiotics Commitment defined our core principles in approaching AMR. Activities we have undertaken in line with these principles can be seen in Section Five of this Roadmap. But we see more opportunities to reduce the need for antibiotics while also improving animal health.

Our Roadmap to Reducing the Need for Antibiotics offers a clear vision for improving global animal health both in the steps HealthforAnimals and our Members pledge to undertake by 2025, and in the areas where we call on others to take action and support this goal.

2. Our Vision for Reducing the Need for Antibiotics

To preserve antibiotic effectiveness, the animal health industry believes the whole animal health sector – both public and private – must devote more investment, research and energy into three priority areas:

Prevention

Detection

Treatment

Prevention

Disease prevention is our first line of defence and the best way to reduce the need for antibiotics.

Preventing disease outbreaks involves three key elements: vaccination, biosecurity, and overall health and wellbeing. Vaccines are one of the most effective forms of prevention available while biosecurity measures, such as sanitizing equipment or indoor rearing of certain species, can limit bacteria exposure. Strengthening the overall health of an animal also improves their natural resilience against infection and ability to fight off disease, reducing the need for antibiotics.

Improving prevention requires commitments to:

Vaccination

- Improve access to veterinarians and/or paraprofessionals, especially in low- and middle-income countries (LMICs), who can administer vaccinations
- Make government funds for vaccination available to farms, especially in LMICs
- Improve vaccine availability in underserved markets
- Improve the regulatory route for existing vaccines, especially in LMICs
- Enact clear regulations for new types of vaccines
- Deliver new vaccines
- Improve the acceptance of GM/biotech vaccinations
- Strengthen cold chain transportation and the availability of heat-resistant vaccines

Biosecurity

- Increase government funding for farm facilities
- Train animal handlers on good biosecurity practices
- Improve consumer understanding of biosecurity benefits
- Train animal handlers on the cost/benefit of various biosecurity measures
- Increase funding for research on biosecurity practices and adoption

Overall health and wellbeing

- Develop and improve access to in-feed nutritional products
- Develop and improve access to immunostimulants
- Increase research into animal genetics
- Increase public funding for animal nutrition research

Detection

Disease threats and veterinary access vary around the world but sharing information can help treat and contain an outbreak before it spreads.

Early detection of disease can make all the difference in treatment success, allowing for selection of the most appropriate antibiotic from the outset and reducing the risk of the illness spreading throughout herds or flocks.

This relies on two important elements: monitoring and diagnostics. Monitoring can help identify disease threats before an outbreak takes hold and track any emergence of antibiotic resistance, while swift and accurate diagnostics can help ensure appropriate treatment is given at the earliest possible opportunity.

Improving detection levels will require commitments to:

Monitoring

- Improve disease tracking and data collection
- Increase training of veterinarians and/or paraprofessionals on disease identification
- Improve access to veterinarians and/or paraprofessionals in LMICs
- Increase public funding for disease monitoring
- Continue to share antibiotic sales volume data in markets where it is required
- Monitor antibiotic use levels where appropriate
- Monitor AMR levels in food and animals
- Increase research on AMR transfer pathways and the role of the environment

Diagnostics

- Bring new diagnostics to market that can identify disease more rapidly and accurately
- Define legal requirements for farm data protection
- Increase training of veterinarians and/or paraprofessonals on diagnostics tools
- Integrate diagnostics with treatments to allow for rapid identification and care

Treatment

When an animal contracts a bacterial infection, there is currently no viable alternative to antibiotics.

For the times when antibiotic use is necessary, we must support responsible use. This means the right antibiotic, at the right time, at the right dose, administered through the right route.

Improving treatment requires commitments to:

Responsible antibiotic use

- Increase training of veterinarians and/or paraprofessionals on responsible antibiotic use
- Improve access to veterinarians and/or paraprofessionals in LMICs
- Increase veterinary supervision of antibiotic use in LMICs
- Improve understanding of the role of antibiotics in animal care
- Strict enforcement of existing antibiotic use requirements, especially in LMICs
- Foster greater dialogue across the value chain (e.g. suppliers, farmers, vets) on responsible use

Achieving progress under the three pillars of this vision will require dedicated action both by the animal medicines industry and the wider animal and public health sector, which includes governments, international authorities and the private sector.

Read on to **sections 3 and 4**, Our Commitments and Call to Action, to learn about the actions each of these groups can undertake.

3. Our Contribution

Building on our 2017 Antibiotics Commitment, which outlined five key principles to improve animal health and responsible antibiotic use, we see a way forward that addresses AMR through reducing the need for antibiotics.

This Roadmap focuses on the actions the public and private sectors can undertake to reduce the need for antibiotics in animals. This includes better prevention, earlier diagnostics, increased access to innovative treatments, and more.

In this section are the cumulative actions that HealthforAnimals and our Members, will undertake between now and 2025. HealthforAnimals will regularly survey our Members to track progress on the Roadmap and release updates.

However, we cannot achieve this alone. **Read on to section 4** to see how policymakers and international organizations can also take action that can help reduce the need for antibiotics in animals.

Our Actions

Addressing AMR is a difficult, global challenge. But we believe reducing the need for antibiotics is an essential part, and this will require strong action and accountability.

HealthforAnimals and our Members, representing more than 85 percent of the animal medicines industry, pledge to collaboratively undertake the following clear, measurable actions to improve the three areas of our vision – prevention, detection and treatment – by 2025:

Research & development

If we are to continue to maintain and improve animal health as well as reduce the need for antibiotics, we will need new innovations that help keep animals healthier, preserve welfare, diagnose disease earlier and treat illness more accurately. To help achieve this, we will:

- Invest at least $10 billion in research and development
- Deliver at least 100 new vaccines
- Deliver at least 20 new diagnostics tools
- Deliver at least 20 new nutritional enhancement products
- Deliver at least 30 other products that can reduce the need for an antimicrobial by reducing animal stress or boosting the natural immune system (ex. parasiticides, immunostimulants, anthelmintic, etc.)

One Health

In addressing issues such as AMR, we must recognize that this is not an issue limited by species or location. AMR affects animals, people and the planet, and can only be addressed through working across these disciplines. To help achieve this, we will:

- Deliver new tools that reduce the likelihood of human exposure to a resistant pathogen such as *Salmonella*, *Campylobacter*, or *E. coli*
- Conduct an AMR risk analysis for every new antibiotic brought to market

Communications

Reducing the need for antibiotics can only be possible when the importance, benefits, scientific basis, and methods are properly communicated. To help achieve this, we will:

- Strengthen communications on benefits of biosecurity, in-feed supplements, vaccinations, and products that support good animal health
- Participate in forums and public dialogues to help build understanding of risks, benefits, and actions that different stakeholders can take to improve public health outcomes in the fight against AMR
- Issue regular report(s) and/or white paper(s) identifying barriers to adoption of prevention tools (e.g. vaccination, biosecurity, etc) and how they can be addressed
- Issue Roadmap Updates in 2021 and 2023

Veterinary training & access

Veterinarians and veterinary paraprofessionals are on the frontline of the battle against AMR, using their expertise and knowledge to make a difference. They are trained to use antibiotics in a responsible manner that reduces animal suffering while limiting the emergence of resistance. Contributing to greater veterinary training and access can make all the difference in upholding animal health. To do this, we will:

- Provide clear labels on every, single product
- Make technical guidance available to all product users
- Train more than 100,000 veterinarians in responsible use of medicines
- Undertake at least 15 veterinary training partnerships
- Invest at least $5 million in veterinary education scholarships and grants
- Deliver a white paper on opportunities in telemedicine for improving access to veterinarians in high-income and low and middle income nations

Cooperation

The animal medicines industry does not work in a vacuum and we cannot address animal health alone. We will redouble efforts to build partnerships and work across disciplines to reduce the need for antibiotics. To do this, we will:

- Participate in responsible use coalitions in major markets
- Share sales data in every market where it is required
- Undertake five new partnerships that deliver products that help to reduce the need for antibiotics in underserved markets
- Conduct at least 50 audits of active ingredient suppliers to ensure they are meeting appropriate standards
- Encourage medicine users to submit efficacy reports into pharmacovigilance monitoring systems

Knowledge

Addressing AMR will be more successful with greater knowledge and understanding about its origins, development, movement and contributory factors. To support this, we will:

- Provide research grants of at least $1 million
- Publish new, scientific research within peer-reviewed publications which improves understanding of veterinary pathogens or antimicrobial resistance
- Provide data and support to help improve disease tracking to organizations such as the World Organisation for Animal Health (OIE)

HealthforAnimals and our Members commit to undertaking the above actions between now and 2025.

We will issue updates in 2021 and 2023 that evaluate our progress.

4. Call to Action

The animal medicines industry cannot reduce the need for antibiotics alone. Alongside our industry commitments, we also call upon the public sector and international organisations to join us in this effort to reduce the need for antibiotics by improving prevention, detection and treatment of animal disease.

Supportive public policies can drastically change farmer access to new treatments, preventative tools and veterinary expertise, which will allow them to improve animal health and reduce the need for antibiotics.

This will require decisive policy action across the following four areas:

Regulation

Farmers are facing continually evolving animal health threats, with new diseases spreading faster than ever due to natural disasters and global trade. As the animal medicines industry works to deliver new technologies to tackle emerging threats swiftly and effectively, the accompanying regulatory process must also adapt, otherwise the opportunity to respond to a health threat could be lost.

Delivering products into smaller regional markets such as East Africa or South Asia, for example, poses a unique financial challenge. Having a medicine approved for use through the regulatory process and ensuring the infrastructure is in place to deliver it to the market often costs more than a company can recoup. This makes it financially unviable to deliver the newest technologies to many farmers in developing countries who need it the most.

At the other end of the spectrum, animal medicines are advancing faster than ever before with tools like modern vaccinations, stem cell therapies, monoclonal antibodies and more, opening up a world of new prevention and control treatments. Regulations must keep up to allow new products and technologies to be assessed and licensed in a timely manner. Delays can mean veterinarians, livestock farmers and animals must wait longer for an appropriate treatment or product, which could increase disease risk and the need for antibiotics.

Strengthening the regulatory process requires:

- **Regulatory convergence:** Policymakers should support greater regional regulatory harmonization and convergence. This could enable a company to submit a product to one, unified regulatory system and receive a market authorization for multiple countries in a region. This would significantly increase the amount of tools available to veterinarians and farmers, particularly in smaller markets.
- **Modern, flexible regulatory systems:** Ground-breaking products are in the animal medicines pipeline but some may not fit into the current regulatory framework. Regulators must prepare for these situations by offering flexible, collaborative processes that ensure product safety while avoiding unnecessary delay.
- **Controls on illegal medicines:** Policymakers and authorities must crack down on illegal medicines, including counterfeits. Illegal medicines are a $2 billion a year market that threatens farmers, veterinarians, animal safety, and even consumer safety. Actions to improve control could include strengthening enforcement agencies, improving data collection and analysis, facilitating identification of medicine authenticity, and improving general awareness.
- **Support for OIE Standards:** The World Organisation for Animal Health (OIE) offers science-based standards that countries can use to improve prevention and control of disease. Greater adoption of OIE standards can better protect the health and welfare of animals and promote responsible use of medicines.

Consumer acceptance

Consumers are increasingly interested in the provenance and production of their food. The food chain, from retailers to farmers, are working to provide more detail, but there remains a disconnect between marketing campaigns promoting sustainably sourced foods and the threat of disease risk among livestock. This creates confusion for consumers.

For example, indoor rearing of poultry as a biosecurity measure can be an effective way to limit exposure to disease, but consumers are increasingly expecting birds to be raised primarily outdoors. This can significantly increase disease risk as seen in the 2016 outbreak of bird flu in Europe, which was spread by wild birds who swiftly infected outdoor poultry. Policymakers must support public awareness and understanding of animal disease risks, and the necessary measures to prevent outbreaks.

Strengthening this awareness requires:

- **Better public education on biosecurity:** Consumers need to understand that farm conditions and husbandry play an enormous role in preventing disease outbreaks, which affect animals as well as people, their food supplies and their livelihoods.
- **Improved understanding about responsible use and the role of antibiotics in animal care:** The public must understand that, just as in humans, antibiotics are the only way to treat bacterial infections in animals. We can reduce the need for antibiotics, but, they will remain crucial to animal welfare.
- **Greater education about the safety and importance of vaccines:** Consumers should be reassured that vaccines can safely and effectively prevent disease, reducing the need for antibiotics.

Funding for livestock

Livestock contributes 40 percent of global agricultural output, according to the UN's Food and Agriculture Organisation (FAO), yet the percentage of development spending devoted to livestock is less than 0.25 percent. With 1.3 billion people worldwide relying on livestock for their livelihoods and food security, the funding available to support the health of livestock must increase.

Financial assistance is vital to encourage smallholder farmers to invest in preventative medicine such as vaccines. Effective, international disease monitoring also needs crucial funds to limit the risk of a disease emerging that requires antibiotics for treatment.

Finally, investment is also needed for research and development to allow scientists to keep up with emerging disease threats. Strengthening livestock funding requires:

- **Livestock vaccination support:** Investing in preventative medicine is the best way we can reduce the need for antibiotics. Subsidies or other farm-level support is essential for improving uptake of vaccination, especially in low- and middle-income countries.
- **Investment in research and development:** Forming public-private partnerships can be a helpful way of sharing the cost of innovation while investing in health.
- **Funding for disease monitoring across borders:** Disease knows no borders and vigilant surveillance and shared information can help countries limit an outbreak and stop it from spreading, reducing the need for subsequent treatment.
- **Best practice training of farmers:** Livestock producers are on the frontline of animal health. By investing in best practices like good nutrition, husbandry and biosecurity, we can help them reduce the likelihood of disease and the need for antibiotics.

Access to veterinary expertise

It is impossible to reduce the need for antibiotics and tackle AMR without proper access to veterinary expertise. Only with the right expertise can livestock producers improve the prevention, detection and treatment of animal disease.

However we simply do not have enough veterinarians nor veterinary paraprofessionals. This is especially acute in low- and middle-income countries where many animals will never see a veterinarian in their lifetime. This puts farmers in the challenging position where they must make medical decisions for their livestock without adequate training. Increasing access to veterinary expertise must be at the top of the global agenda.

Strengthening access to veterinary expertise requires:

- **Increasing investment in veterinary education:** Veterinarians must be equipped to respond to emerging disease threats and responsibly use antibiotics.
- **Promoting the veterinary profession:** To fill the global shortage of veterinarians, we must redouble efforts to make this an attractive, rewarding career.
- **Investing in veterinary paraprofessionals:** There are simply not enough veterinarians available. Paraprofessionals with some training can make an enormous difference to animal health.
- **Encouraging livestock farmers to seek out veterinary expertise:** This requires promoting trust in the veterinary profession and in the efficacy of animal health products.

Actions by the public sector and international organizations across these four areas can help improve prevention, detection and treatment of animal disease on a national and international scale, reducing the need for antibiotics on farms across the globe. Combined with the actions of our industry, this can make a significant impact in the fight against AMR and improve responsible antibiotic use for all our benefit.

5. Fulfilling our Commitment

Our Roadmap to Reducing the Need for Antibiotics is the product of our experiences and efforts to address AMR, improve responsible use, and bolster the health of animals.

It is also the next step in a process that began in 2017 with the release of our **'Antibiotics Commitment'**. This pledge outlined the five guiding principles for HealthforAnimals and our Members when approaching responsible use and AMR:

Principle 1:	**Protect animal health and welfare in a unified One Health approach**
Principle 2:	**Use antibiotics judiciously and responsibly**
Principle 3:	**Promote disease prevention and increased access to products and expertise**
Principle 4:	**Invest in development of products for prevention and treatment**
Principle 5:	**Increase knowledge, transparency and communication**

These principles, and the actions we have undertaken to meet them, have informed our vision and helped guide our way forward. Since our Antibiotics Commitment is the foundation of our Roadmap to Reducing the Need for Antibiotics, it's essential that we demonstrate how we embody this Commitment.

Activities in this section have been provided by HealthforAnimals Members, which includes 10 company members and associations around the world:

The activities demonstrate how our industry is fulfilling the principles of our Commitment and provided us with the learnings necessary to develop this Roadmap. This list not exhaustive, but demonstrates the wide breadth of work we undertake to address AMR and responsible use.

Principle 1:

Protect animal health and welfare in a unified One Health approach

Selection of activities by HealthforAnimals Members

Memorandum of Understanding with World Organization for Animal Health (OIE)

In 2017, HealthforAnimals officially renewed their Memorandum of Understanding with the OIE. This agreement calls for both organizations to work together towards common goals in “responsible and prudent use of antimicrobials and anthelmintics with the aim of tackling resistance,” alongside other areas including regulatory frameworks, information sharing and development of new medicines.

How does this address AMR and responsible use?

The OIE is the premier global body for animal health and a leader on AMR and responsible antibiotic use. With 182 Member Countries, OIE’s ability to affect change across the world is unprecedented. Working with OIE is essential to tackling the global challenge of antibiotic resistance, and this cooperation agreement enables HealthforAnimals and our industry to directly contribute to their efforts.

Global responsible use coalitions

Around the world, HealthforAnimals and our Member Companies and Associations participate in value chain coalitions that work to improve responsible use of antibiotics, such as RUMA (Responsible use of Medicines in Agriculture) in the UK, EPRUMA (the European Platform the Responsible Use of Medicines in Animals) across Europe, ALIANÇA in Brazil, and others. These coalitions allow the entire value chain – from producer to medicine developer to retailer – to work together towards responsible use. Each is an essential collaborative platform that offers a unified approach to the challenge.

How does this address AMR and responsible use?

Resistance cannot be solved by just one link in the value chain. Working together means the effort of each link (whether the developer, producer, retailer, etc.) builds upon one another to become greater than the sum of their parts. In addition, close collaboration ensures the efforts of the animal health sector remain focused, working towards a common goal.

HealthforAnimals' Antibiotics Commitment

Ten of the largest animal health companies in the world are united through the global association, HealthforAnimals. Members of the Association work together on common issues affecting veterinary medicine and the wider public health sector. In 2017, HealthforAnimals and its Members agreed to an industry-wide Antibiotics Commitment, outlining the five principles that underpin their work on responsible use and AMR.

How does this address AMR and responsible use?

The Commitment publicly sets out the industry's ability and responsibility to support the responsible use of antibiotics and provides principles against which it can be measured.

Online training for farmers to support responsible use of antibiotics on UK farms

The National Office for Animal Health (NOAH), RUMA, the Veterinary Medicines Directorate (VMD), the British Retail Consortium (BRC) and leading academics developed a robust and trusted online training programme for all those working in the sheep, dairy, beef and pig sectors in the UK. The Animal Medicines Best Practice (AMBP) Programme gives farmers and vets access to new resources, enabling a coordinated and consistent approach to farmer training in the responsible use of antibiotics.

How does this address AMR and responsible use?

The programme aims to raise awareness, knowledge and understanding of AMR and helps drive best practice in a consistent manner across UK farms when it comes to using antibiotics. Training modules are available for farmers via the NOAH website or directly through an online Lantra e-learning platform. Veterinarians can also access resource materials, enabling them to deliver training directly to their farmer clients.

Participation in global veterinary associations

HealthforAnimals is an official Member of the World Veterinary Association (WVA) and World Small Animal Veterinary Association (WSAVA), which represent a combined 700,000 veterinarians in over 100 nations worldwide. The two associations set clear defined standards and guidelines for proper animal treatment, including how to use antimicrobials responsibly. HealthforAnimals is an active contributor to working groups in each association and strongly supports their efforts to encourage responsible use and improve access to veterinarians.

How does this address AMR and responsible use?

By working with global veterinary associations, we can support the efforts of veterinarians to tackle AMR and improve responsible use. HealthforAnimals has done this by offering technical expertise, forming partnerships, and participating in working groups with each association.

Training veterinarians on responsible use of antibiotics in Spain

Spanish veterinary medicines association Veterindustria hosted a web seminar on responsible use of antimicrobials for over 1000 veterinarians at the 2018 launch of their product compendium Gui@Vet. The compendium is renewed every two years and these responsible use webinars are organized for each publication.

In collaboration with the Spanish Medicines Agency and the Board of Deans of Spanish Veterinary Faculties, Veterindustria provides further training on responsible use of animal medicines at all veterinary faculties across Spain, including veterinary university hospitals.

How does this address AMR and responsible use?

Providing clear guidance for the correct use of antibiotics directly to those entrusted to use these products, the veterinarians, is one of the best ways to ensure their responsible use. Raising awareness through training sessions at student level and practicing veterinarian level, and furthering knowledge about the challenges of antibiotic resistance helps to ensure better understanding of what is at stake and what role the veterinarians play in addressing the challenge of AMR.

Surveillance of disease outbreaks

Launched on 27 January 2016, the STAR-IDAZ International Research Consortium (IRC) aims to maximize funding for coordinated animal health research strategies for at least 30 priority diseases, infections and issues. HealthforAnimals and several Members are partner members of the IRC, while AnimalhealthEurope, the association for animal medicines companies in Europe, is a secretariat member.

How does this address AMR and responsible use?

Among the outcomes of international research are candidate vaccines, diagnostics or other therapeutic health products, all of which help to prevent or better control disease and reduce the need for antibiotics.

VICH support

VICH is a trilateral (EU-Japan-USA) program aimed at harmonizing technical requirements for veterinary product registration. This makes bringing products to market more efficient and predictable, which puts innovative products that can reduce the need for antibiotics in the hands of users quicker. Through its Members, observers and outreach forum participants, VICH represents approximately 100 countries. HealthforAnimals has been an active member of VICH for many years.

How does this address AMR and responsible use?

VICH activities help products that can reduce the need for antibiotics in animals reach users in a quicker, more efficient manner. This helps tackle challenges earlier and more effectively.

Principle 2:

Use antibiotics judiciously and responsibly

Selection of activities by HealthforAnimals Members

Promoting best practices to companion animal veterinarians in Europe

Vetoquinol recently launched a campaign to educate veterinary clinics about the prudent use of antibiotics. Vetoquinol provided an electronic sales aid for all company territory managers to help explain best practices to veterinarians. Brochure and webinars outlined to veterinarians the prudent use approach in areas such as dermatology, respiratory infections and urinary tract infections.

In-clinic meetings with practising veterinarians shared methods in the field of prudent use and “lunch & learn” training sessions were organized.

How does this address AMR and responsible use?

These types of campaigns raise awareness on AMR, responsible use, and antibiotic stewardship for veterinarians and pet owners.

'Cevolution'

With increasing global concern about the impact of antibiotic resistance on health and welfare of people and animals, Ceva Santé Animale undertook an extensive, company-wide change called 'Cevolution.' Ceva’s aim was to make a wide portfolio of antibiotics available, encouraging veterinarians to make the right diagnosis, prescribe the right antibiotic, at the right time and only for individual infected animals. This includes extensive education and training program for vets and farmers. A comprehensive library of high-quality, authoritative print and online resources, produced in partnership with international opinion leaders, and a regular newsletter are freely available to veterinary practitioners.

How does this address AMR and responsible use?

‘Cevolution’ helps veterinarians to choose the most appropriate antibiotic for the diagnosed infection. Extensive education, training and information ensures vets and farmers are aware of best practice and latest developments in the control of AMR.

Eight-point antibiotic stewardship plan

Elanco Animal Health has committed to an eight-step antibiotic stewardship plan that promotes responsible use of antibiotics and greater research into new treatment technologies. One year after launching the plan, Elanco convened more than 200 global animal protein industry leaders, intergovernmental organizations, NGOs, and experts at a One Health Antibiotic Stewardship Summit to address critical challenges and establish pathways forward.

In 2018, Elanco further refined the eight-point plan with commitments in three key areas of stewardship: combating antimicrobial resistance through responsible antibiotic use, reducing the need for medically important antibiotics in livestock, and significantly investing in new research.

How does this address AMR and responsible use?

The eight-point plan promotes responsible use practices, reducing the need for antibiotics, and development of alternatives. These actions can improve use of existing antibiotics and spur development of new treatment or prevention tools.

Promoting best practices to livestock veterinarians in Europe

Vetoquinol recently launched a communications campaign to promote best practices in antibiotic use, which included materials such as a in-depth brochure outlining the differences among the four categories of antibiotics and the varying needs for prescription.

Vetoquinol also ran workshops, in cooperation with universities, for veterinarians to explain the relevant legislation and prudent use of antibiotics.

This was supported by a farmer case study on mastitis prevention, which showed how to reduce the need for antibiotics through targeted mastitis treatments, rapid diagnostics, and sensitivity testing methods.

The company also developed sanitary audits, performed by Service Implementation Consultants, and worked with digital partners to help practices understand their current antibiotic usage and discuss appropriate usage.

Vetoquinol territory managers have also received specific training about the National Plan about Antibiotic Resistance and the company position on the topic.

How does this address AMR and responsible use?

The Vetoquinol campaign helps European users better understand and fundamentally improve their use of antibiotics in livestock. This means more effective animal care that ultimately reduces the need for antibiotics.

Guidance for the rational use of antimicrobials (GRAM)

Several years ago, Ceva Santé Animale began work to address the veterinarian's need for a pragmatic guide to rational prescribing, which can be used under the time pressure of a consultation. This resulted in the 2016-2017 launch of Ceva’s ‘Guidance for the rational use of antimicrobials’ (GRAM), a comprehensive practical and easy-to-use guide to help reduce the development of antimicrobial resistance in pets.

Since its launch, GRAM has been released in various languages and is freely available to veterinary practitioners. With over 500 pages, it was developed over six months by an independent panel of 10 experts from seven European countries, all recognised leaders in antibiotherapy.

GRAM aims to synthesise what already exists, reach consensus and simplify the material so as to provide clear, practical answers to the questions in relation to rational use of antimicrobials in canine and feline surgery and medicine. It includes 37 disease factsheets, 29 detailed recommendations and six synopses dealing with major topics, e.g. ‘key questions before initiating any antibiotherapy’.

How does this address AMR and responsible use?

GRAM emphasises proper diagnosis before treatment and the use of options other than antibiotics; e.g. use of suitable topical antiseptics as initial choices for the treatment of superficial dermatological conditions.

Digital support for treatment of BRD

In 2016, a meta-analysis comparing antibiotic options for treating bovine respiratory disease (BRD) was published in Preventative Veterinary Medicine. In 2017, Bayer Animal Health's technical veterinary team in conjunction with IT programmers began the creation of a web application to present the results of this study in a clear, concise and useable format. The goal was to support practitioners searching for data on BRD treatment options by providing them with data from this meta-analysis.

The iCOWNT web application allows practitioners to compare the relative risk of retreatment between two products and view a ranking of products based on efficacy (from highly efficacious to not efficacious) based on the published data. This assists practitioners in antimicrobial selection for BRD treatment and helps prevent them from choosing products that have a low likelihood of success.

How does this address AMR and responsible use?

Practitioners using the iCOWNT web application and underlying data to support BRD treatment protocols can reduce their retreatment rate as well as their likelihood of selection of inappropriate antimicrobials for BRD treatment. This can result in a reduction in overall antibiotic use and assist in preventing the selection of antimicrobials that could lead to the selection of resistant BRD pathogens.

Increasing veterinary supervision of use

As part of efforts to improve the responsible use of antibiotics, Elanco Animal Health has committed to new partnerships in countries with limited resources that aim to increase veterinary and professional oversight of antibiotic use.

In addition, Elanco has completed submission of 67 labels for five shared-class molecules that moves products from over-the-counter use to under the oversight of a veterinarian in all countries where over-the-counter uses remained and veterinary infrastructure exists. Unfortunately, veterinary infrastructure doesn't exist in all parts of the globe to allow for this move completely. In places where veterinarian oversight is not available, Elanco is working to educate farmers and others on the responsible use and administration of antibiotics.

How does this address AMR and responsible use?

Increasing access to veterinarians promotes preventative medicine which improves overall animal health and reduces the need for antibiotics. In situations where antibiotics are needed, veterinarians are best positioned to use antibiotics correctly and responsibly. Increasing access to their expertise promotes better use of antibiotics at the right time, in the right amount, for the right duration. Where veterinary expertise is unavailable, increasing farmer education promotes better use of antibiotics.

Individual Pig Care program

Individual Pig Care from Zoetis is an educational, in-barn training program that helps caregivers assess pig populations and support farmers antibiotic use. The program can help producers spot sickness sooner. When illness is addressed sooner, treatment success and well-being can be improved.

The program also helps personalize health protocols and reduce treatments. By using the classification system, caregivers can communicate a pig's condition to managers and veterinarians. This helps veterinarians prescribe the correct product for the pig's condition.

How does this address AMR and responsible use?

When illness is spotted and treated sooner, pigs can return to full health sooner. This reduces the need for additional antibiotic treatments and stops the illness from spreading to more animals, who may then require treatment.

Raising awareness of responsible use through non-product advertising materials

To help educate veterinarians on the implication of antimicrobial resistance and responsible use of antibiotics, Zoetis developed microsite and webinar series for veterinarians. The objective was to provide a proactive campaign to raise awareness about the responsible use of antibiotics. Over 4,000 veterinarians engaged in the campaign on sites multiple languages, including English, Dutch, French, German, Portuguese and Spanish.

How does this address AMR and responsible use?

Proactively promoting the responsible use of antibiotics as a topic under the slogan "As much as necessary, as little as possible" supports overall awareness of this topic to the wider veterinary audience and greater adoption of best practices.

Principle 3:

Promote disease prevention and increased access to products and expertise

Selection of activities by HealthforAnimals Members

Advancing accessibility of quality medicines, knowledge and education in Sub-Saharan Africa

The Zoetis ALPHA initiative, sponsored by the Bill & Melinda Gates Foundation, aims to advance livestock health and productivity in Sub-Saharan Africa through the increased availability of veterinary medicines, services and education.

Expected results include increase availability of veterinary medicines, services and education; implementation of disease diagnostics infrastructure; and development of veterinary laboratory networks and outreach services into business hubs in Ethiopia, Nigeria, Tanzania and Uganda.

How does this address AMR and responsible use?

Better access to medicines and expertise can significantly improve livestock management. Veterinarians and farmers will be able to better prevent and manage health problems, which can reduce disease risk and the need for antibiotic treatments. This is especially needed in areas of Africa that lack veterinary capacity.

CEVA Lung Program

In 2014, the Ceva Lung Program was launched in Asia and has been subsequently rolled out worldwide. The Program, which runs as a user-friendly app on Android and iPad mobile devices in multiple languages, assists in the correct diagnosis of respiratory diseases by providing a methodology and guidelines for scoring lesions at slaughter. The Program calculates the incidence, severity and impact of enzootic pneumonia and pleuropneumonia and reveals the presence of subclinical infections. The results can be used to evaluate the efficacy of control measures, including vaccination protocols, flag changes in disease dynamics and benchmark effectiveness of respiratory disease management in comparison to other farms.

Use of the Program has grown rapidly. In 2018, data was collected and analysed from more than 500,000 lungs. In addition to being useful as a strategic tool on individual farms, this unique, 'big data' set is being used to identify factors associated with high or low prevalence of respiratory disease to help design better preventive programs.

How does this address AMR and responsible use?

By providing vets and farmers with a simple but effective tool to help them improve the management of respiratory diseases through more effective vaccination regimes, use of antibiotics can be reduced to a minimum, while enhancing productivity and welfare. This reduces the chance of AMR strains developing in the animal population.

Convenience program evaluation for poultry

The ‘Convenience Program Evaluation’ is an initiative by Merck Animal Health, known as MSD Animal Health outside the US and Canada, designed to help poultry producers

protect chickens against various diseases while achieving optimal vaccination standards, bird quality and performance goals.

Through the Convenience Program suite, producers receive vaccination support in the form of laboratory services and field visits, and also staff training and scientific seminars. These services enable them to remain highly proficient in poultry health practices.

How does this address AMR and responsible use?

This suite of services empowers poultry producers to protect their birds from disease and decrease the need for antimicrobials for disease treatment.

Disease prevention and control for profitable livestock production in Nigeria

In January 2019, the Veterinary Teaching Hospital of the University of Ibadan hosted a seminar on disease prevention and control. Several topics were discussed, ranging from biosecurity, disease symptoms, identification and also disease reporting.

The Nigeria Zoetis/ALPHA Initiative supported this seminar with writing materials and learning resources. The Initiative also officially launched a Learn & Grow microsite at the event, which provides free educational modules on livestock health and business courses on the microsite. 300 people attended, ranging from students, veterinarians from both public and private sectors, veterinary students, lecturers and paravets.

How does this address AMR and responsible use?

Veterinary oversight of antibiotics can improve responsible use. By improving access to veterinary expertise in areas like Nigeria where it is lacking, we can subsequently improve responsible use of antibiotics.

Educational scholarships

Each year, Zoetis provides USD$500,000 in educational scholarships to over 200 veterinary students around the world. These are offered to students focused on species, diseases or regions which may be underserved in animal agriculture or pet heath.

How does this address AMR and responsible use?

More veterinarians and greater access to veterinary care improves responsible use.

Farmer and veterinary training academies

Boehringer Ingelheim organizes training academies on all continents, open to both internal and external professionals to be trained on the impact of diseases and how to prevent them. These academies are developed by universities or experts in the field. These training sessions provide the knowledge and support that producers and veterinarians require to identify and understand the dynamics of diseases, minimize disease transmission and maximize immune response while still running a profitable farm.

An example is the Boehringer Ingelheim Swine Academy (BISA®), organized in collaboration with international experts, such as the Iowa State University of Science and Technology and the University of Illinois, for Boehringer Ingelheim employees and

practitioners. It provides the participants with hands-on training run by scientists and industry professionals.

For our customers in the ruminant segment, Boehringer Ingelheim organizes the Milk Quality Academy, focusing on mastitis prevention, through useful advice and guidance.

How does this address AMR and responsible use?

Increased knowledge and support for prevention helps animal health practitioners avoid diseases, which can ultimately reduce the need for antibiotics. In addition, demonstrating that prevention works without compromising on profit offers an important business case for practitioners.

Handling and treatment practices in Mexico

Bayer recently collaborated with cattle farmers in Mexico to improve on-farm handling and treatment practices. This led to a reduction in the overall rate of morbidity, mortality and improved weight gain in cattle.

How does this address AMR and responsible use?

Improvements in handling and treatment practices reduced respiratory morbidity without the need for antibiotic treatments.

Leptospirosis vaccine

In 2019, Merck Animal Health, known as MSD Animal Health outside the US and Canada, released a leptospirosis vaccine effective for dogs against four of the five known serovars that cause canine leptospirosis infection, Nobivac EDGE LEPTO4. Leptospira bacteria are widespread in the environment and are zoonotic, with up to 10 million people infected every year and a fatality rate in humans of up to five per cent. In dogs, leptospira infections cause serious damage to the liver and kidneys and can cause fever, loss of appetite, shivering, muscle pain, weakness, and urinary symptoms.

How does this address AMR and responsible use?

By limiting the spread of leptospirosis among dogs and in turn reducing the chance of human infection, the vaccine ultimately reduces the need for antimicrobials to treat leptospira infections.

Innovation in vaccination devices

Ease of administration can be a significant factor in the adoption of a vaccine by veterinarians and animal caretakers. In 2018, Merck Animal Health, known as MSD Animal Health outside the US and Canada, developed a needle-free vaccination device for pigs, the IDAL 3G.

The device aims to help professionals administer vaccinations more quickly and at greater scale because they are more easily maintained and cleaned, are capable of injecting into multiple injection sites, allow for comprehensive record-keeping, and ensure the proper dose is administered during each injection.

How does this address AMR and responsible use?

Increasing the ease of vaccination for pigs and their handlers can improve the adoption of robust vaccine protocols, allowing for more pigs to be vaccinated, and encourage more handlers to vaccinate. This can decrease the need to use antimicrobials treat disease over the lifetime of the pig, and reduce the possibility of AMR developing in swine bacteria.

Developing new vaccines and delivery systems

Phibro Animal Health has had 77 new vaccine licences granted in 18 different countries over the last three years. Examples of new products include a live virus vaccine in an effervescent tablet, sealed in sterile aluminium blister packaging. The user-friendly tablet is convenient and safe to handle so it allows vaccines to be used in locations without access to equipment or refrigeration.

Phibro has also invested in autogenous animal vaccines, which are herd-specific vaccines (also referred to as custom vaccines) and can be effective against illnesses such as BRD, Pinkeye (IBK), and enteric diseases like Salmonellosis. Animal death rates from these diseases are well documented, and the use of autogenous vaccines to combat these conditions can be effective.

How does this address AMR and responsible use?

Vaccination and herd immunity are key parts of antimicrobial stewardship and two principle ways to reduce the need for antimicrobials, reducing the chance of AMR pathogens strains developing in the population.

Prevention and diagnostic toolbox

Effective vaccination programs help keep livestock healthy and productive, which helps reduce the need for antibiotics. Availability of vaccines is not a guarantee for success though. Perfect management may not always avoid exposure to pathogens and a vaccine may not prevent every outbreak from occurring. This is why Boehringer Ingelheim offers a full suite of tools that can work alongside vaccination, such as early warning tools can help to identify the dynamics of a disease and enable initiation of the correct treatment.

For example, the ‘SoundTalks’ tool can measure coughing in a pig barn as an early indicator of Mycoplasma infections. This will be followed by an onsite diagnostics tool which can help obtain a fast detection of pathogens and diseases. This promotes quicker detection and more accurate treatment selection.

How does this address AMR and responsible use?

Prevention is key to reducing the need for antibiotics. When a bacterial disease is avoided, the need for antibiotic treatment falls. However, disease cannot always be prevented. Early detection tools can help stop bacterial disease before it spreads widely and increases the need for antibiotic treatments across a herd.

'Time to Vaccinate' Campaign

The 'Time to Vaccinate' campaign is an initiative by Merck Animal Health, known as MSD Animal Health outside the US and Canada, intended to provide farmers with information and shared experiences about vaccination as a preventive tool. 'Time to Vaccinate' connects beef and dairy farmers who want to learn about vaccination for preventable diseases with farmers who’ve already adopted a preventive approach to managing their herds. The objective is to increase awareness and ultimately vaccination rates.

Time to Vaccinate is expected to increase the number of farmers and veterinarians who implement a preventative vaccination protocol on their farm which could lead to continuous improvement in overall animal health, well-being and productivity.

How does this address AMR and responsible use?

Increased implementation of vaccination programs, in conjunction with other farm management best practices such as quality nutrition, biosecurity, and animal handling, will help to prevent infectious diseases from negatively impacting ruminant health and productivity. Quality implementation of vaccination protocols may result in less bacterial infectious bacterial disease on farms and enhance antimicrobial stewardship.

Developing a vaccine against ileitis in swine

Ileitis is a bacterial disease that infects the intestines of an animal. Once an animal is infected, an antibiotic is the only treatment. If untreated, ileitis can cause pain, suffering and even death for an animal.

In 2000, Boehringer Ingelheim developed a vaccine that could prevent ileitis. In the 20 years since then, more than 700 million pigs have been vaccinated against ileitis. Researchers have tracked field use of the ileitis vaccine and found evidence it reduces the need for antibiotics.

How does this address AMR and responsible use?

Vaccines against ileitis can reduce the need for antibiotic treatment in swine production by preventing the disease, while also increasing awareness of the need of preventive care.

Vaccines in Norwegian aquaculture

In the early 1990s, a vaccine against furunculosis – a salmon skin disease – was released by Pharmaq. Later it was made effective against three types of vibriosis infections in addition to furunculosis. The vaccine enabled the aquaculture industry to shift from antibiotic treatments to prevention through routine vaccination.

How does this address AMR and responsible use?

Through the introduction of predictable vaccines, the Norwegian aquaculture industry has reduced its use of antibiotics by 99.8 percent per ton of trout and salmon produced, compared to 1987 level. Norwegian aquaculture also grew from 57,000 tons in 1987 to 1.25m tons in 2012.

Principle 4:
Invest in development of products for prevention and treatment

Selection of activities by HealthforAnimals Members

Alternative topical solutions to support innate immune system in dogs

Virbac recently developed a new technology based on plant extracts, (boldo and meadowsweet) which promotes natural secretion of antimicrobial peptides (AMPs) by keratinocytes (a cell type in the skin) that can treat bacterial infections, especially in atopic dogs. These AMPs naturally produced by the body were shown to also successfully treat resistant bacteria.

How does this address AMR and responsible use?

The use of topical therapies, especially in the case of superficial skin infections, can stimulate natural defences for treatment, which reduce the need for an antibiotic.

Developing diagnostics and monitoring tools

Boehringer Ingelheim has recently introduced two platforms that give farmers and veterinarians additional information to improve preventive healthcare.

SoundTalks is technology designed to detect early symptoms of respiratory disease. The system includes devices that continuously and objectively monitor the herd via automated analysis of sound. The devices are the 'ears – continuously listening to the pigs – and algorithms are the 'brain' – interpreting what is heard.

Mobinostics is a point-of-care system that can be operated on farm, from a veterinarian's vehicle or in clinic – reducing the need to ship samples to a central testing laboratory. Mobinostics is simple to use – no need for a trained lab technician – and will allow testing of various types of samples (e.g. nasal swabs, blood, oral fluids) for targeted diseases in less than 60 minutes.

How does this address AMR and responsible use?

Earlier detection of disease and rapid diagnosis mean farmers and veterinarians can intervene sooner, enabling an improved treatment response with the potential to shorten and reduce the number of treatments, including antibiotics.

Education and Digital Tools

Bayer encourages taking a practical and holistic approach to mitigating infectious diseases. Ongoing scientific and educational outreach to livestock professionals encompasses topics such as good biosecurity and the importance of detecting diseases early. Digital tools such as BCS Cowdition and BCS SowDition smartphone applications can help simplify accuracy and tracking of body condition scores for dairy cows and sows, respectively.

How does this address AMR and responsible use?

Better livestock management improves detection, control and treatment of bacterial disease, which can lead to fewer and more targeted antibiotic treatments.

Integrated Health Ecosystems for Precision Livestock Farming

Boehringer Ingelheim has recently launched an 'Integrated Health Ecosystem' central data-management platform that integrates technologies and tools within and across swine farms into one ecosystem. This system can provide better insights and decision-ready information to veterinarians and producers enabling them to take more informed decisions that increase health, performance and profitability. This approach brings together multiple technologies that can enable more effective monitoring and detection of diseases, fast diagnosis of causative agents, and precise intervention.

How does this address AMR and responsible use?

Effective utilisation of precision livestock farming enables earlier and more precise intervention and disease prevention plans, which can reduce the need for antibiotics. It also improves responsible use through improved effectiveness and continuous accountability where antibiotic use is needed for the well-being of animals.

Investment in new prevention and treatment tools

In its 2017/22 strategic plan, Vetoquinol plans to invest up to 20 percent of its R&D pot in tools that can reduce the need for antibiotics.

This can include solutions that span across genetics, prevention, hygiene and biosafety, vaccines, immunostimulants and efficient diagnostics. This will target the use of anti-infectives still available for animal health and new targeted treatments that do not cross-react with critical antibiotics.

How does this address AMR and responsible use?

The innovations produced will either substitute or reduce the use of antibiotics.

Proteobiotic use in pigs and poultry

In 2018, Bio Agri Mix, a member of the Canadian Animal Health Institute (CAHI is an Association Member of HealthforAnimals), launched a novel proteobiotic for the Canadian livestock industry. Proteobiotics represent a new class of anti-virulent products providing an alternative to conventional antimicrobial preventative programs. More than 300,000 pigs have received preventative therapy with NUVIO to control *E. coli* K88 and the poultry sector has recorded rapid uptake for Necrotic Enteritis control.

How does this address AMR and responsible use?

Through reducing the prevalence of animal disease and providing an alternative to antimicrobial use in the Canadian livestock industry, proteobiotics can reduce the need for antimicrobial use and the chance subsequent AMR strains emerge.

Maintaining intestinal integrity in poultry flocks

With the growing consumer demand for lean protein that is produced without the use of antibiotics, Phibro Animal Health has worked to address gut health challenges in poultry raised without antibiotics. Such gut health problems are a major concern, as they often have an impact on animal welfare, product quality, and affordability.

Phibro's Magni-Phi helps maintain overall intestinal health in poultry, which may lead to a reduction in diseases and decrease the need for antibiotics. This natural product is made from quillaja extract and yucca powder and is listed by the Organic Materials Review Institute (OMRI).

How does this address AMR and responsible use?

Protecting and improving overall animal health bolsters an animal's natural defences against illness. When an animal can naturally fight off an infection, it reduces the need for animal antibiotics in the future.

Targeting improved nutritional health

In 2016, Elanco announced the creation of a nutritional health division, which focuses on functional nutrition products, including enzymes, probiotics and prebiotics, which impact animal microbiomes and other dietary factors to reduce disease incidence, improve gut health and enhance feed digestibility. The organization recently launched Correlink – a novel direct-fed microbial (probiotic) product outside the U.S – and announced a global, exclusive in-licensing agreement to launch an in-feed antibody product focused on reducing and controlling coccidiosis.

How does this address AMR and responsible use?

Improving nutrition bolsters an animal's natural defences against illness. When an animal can naturally fight off an infection, it reduces the need for animal antibiotics in the future.

Poultry immunostimulants to reduce *E. coli* incidence

Bayer's Victrio is an immunostimulant that stimulates the innate immune system in poultry, providing a rapid, nonspecific, protective response to infectious agents. This offers a non-antibiotic option to help reduce mortality associated with *E. coli* in embryonated eggs and newborn chicks. The treatment is registered in a number of countries including USA and Canada.

How does this address AMR and responsible use?

As a non-antibiotic option, Victrio can help poultry producers reduce the need for antibiotics when addressing the challenge of mortality associated with *E. coli*, especially in the early stages of life when chicks are highly susceptible to *E. coli* infections. Reducing antibiotic use at hatchery is an important step in reducing AMR in chickens.

R&D into new treatment options

In 2018, Elanco Animal Health announced it was investing at least half of its food animal research and development budget in projects dedicated to developing alternatives to shared-class antibiotics. This builds off Elanco's 2015 'Eight-Point Antibiotic Stewardship Plan,' which restructured its work in this area, creating two new research and development teams focused on advancing antibiotic alternatives.

How does this address AMR and responsible use?

Providing alternative treatment options for farmers can help limit development of resistance in existing antibiotic treatments.

Principle 5:
Increase knowledge, transparency and communication

Selection of activities by HealthforAnimals Members

Educating consumers with ExploreAnimalHealth.org

Created by Phibro Animal Health, ExploreAnimalHealth.org is a website intended for consumers that delivers clear, credible and easily understood information about animal antibiotic use, vaccines, nutritional products and the One Health approach. The site features shareable content for use on websites and social media channels, infographics, blogs, resource links, and a video library.

How does this address AMR and responsible use?

Improving consumer understanding of animal antibiotic use and the challenges we face in tackling AMR helps them become advocates for responsible use.

Horizontal AMR transfer research

In 2018, Bio Agri Mix, a member of the Canadian Animal Health Institute (CAHI is an Association Member of HealthforAnimals), completed an initial AMR gene study in litter samples from 10 poultry production systems. Results are being used in follow-up research to further develop AMR gene PCR panels and create a pilot study in conjunction with the Chicken Farmers of Canada (CFC). While the project is still underway, initial results provided encouraging signs that the final research could assist veterinarians in making prudent antimicrobial decisions.

How does this address AMR and responsible use?

Monitoring AMR and transfer genes will enable veterinarians to make prudent antimicrobial use decisions without hampering animal welfare, thus reducing the potential for AMR strains developing in animals.

Monitoring antibiotic sensitivity in New Zealand

In 2017, Bayer introduced DairyAntibiogram, an antibiotic sensitivity test for mastitis bacteria on dairy farms, in New Zealand. The test is performed on bulk milk samples obtained directly from the milk processors. Armed with results of the DairyAntibiogram, dairy professionals have knowledge of the antibiotic resistance status of a herd, enabling them to better select the most effective, responsible and sustainable antibiotic treatment. The test now includes 10 antibiotics and a website for enhanced tracking and results management.

How does this address AMR and responsible use?

DairyAntibiogram equips dairy professionals with knowledge of the antibiotic sensitivity status of a herd and enables them to better select effective, responsible and sustainable antibiotic treatment for mastitis. This helps veterinarians ensure that they are using antibiotics in the most responsible and effective way possible, when needed. This subsequently reduces the risk of new AMR strains emerging in the animal population.

'One Health Antibiotic Stewardship Summit'

In 2016, Elanco organized a 'One Health Antibiotic Stewardship Summit', convening more than 200 global animal protein industry leaders, including company chief executives and livestock owners, intergovernmental organizations, NGOs, and experts to discuss critical challenges. Topics included increasing global veterinary training and capacity, enhancing metrics and monitoring of responsible use globally increasing incentives for innovation and working to enhance predictability of regulatory pathways.

How does this address AMR and responsible use

Building momentum for One Health approaches to responsible use and AMR helps promote long-term, sustainable solutions. These issues cannot be solved by one sector, it requires communication across the full livestock value chain as well as with human health.

One Health education series on AMR

In 2018, Merck Animal Health, known as MSD Animal Health outside the US and Canada, in partnership with the National Institute for Animal Agriculture (NIAA), initiated the One Health educational series. This is a video series that explores AMR and the collaborative efforts between ranchers, animal health and human health experts to address the issue. The series continues in 2019.

How does this address AMR and responsible use?

Through education and increased understanding of the challenges producers face related to the prevention and treatment of animals, the proper use of antibiotics can be better implemented.

Pradofloxacin sensitivity discs

Since 2015, Bayer Inc. has provided Pradofloxacin sensitivity discs free of charge to any Canadian diagnostic laboratory or veterinary clinic conducting culture and sensitivity testing.

How does this address AMR and responsible use?

Accurate culture and sensitivity testing helps ensure the selection of the most effective antimicrobial for the pathogen being tested. This improves treatment outcomes, reduces the chance of relapses and reduces the potential for selecting resistant bacteria.

PROHEALTH Consortium

The PROHEALTH Consortium was a collaboration between 22 academic, industry and private enterprise organisations – including HealthforAnimals Members – from 11 countries to explore new ways to ensure the sustainability of modern animal production.

The project focused on disease threats associated with the intensity of production in swine herds and poultry flocks. It recommended innovative prediction, prevention and detection solutions to improve animal health and increase productivity, while limiting environmental impact and preserving profitability for livestock farmers.

The project presented scientific evidence about the multifactorial dimension of animal pathologies linked to modern farming.

The findings of PROHEALTH's research addressed these issues and provided the foundations for practical guidelines to help farmers.

How does this address AMR and responsible use?

Through comprehensive research on pressing issues pertaining to farming practices, antimicrobial usage, and AMR, the PROHEALTH Consortium provided steps that policymakers, researchers, and farmers could undertake to mitigate the development of AMR strains and promote responsible use of antimicrobials.

Research antibiotic treatments in canines

Otitis externa is an inflammation of the ear canal and/or pinnae, and can represent up to 20 percent of consultations in dogs. Acute otitis externa management is often handled through topical treatments which comprise a mixture of corticosteroids, antibiotics and antifungal molecules.

Frequent recurrences and use of antibiotics to treat it may lead to resistance, so, identification of the underlying cause is key to decrease frequencies of flare ups.

Virbac has collaborated with ONIRIS veterinary school to gather recent epidemiologic data regarding microbial identification and their sensitivity to antibiotics in otitis externa cases. This will result in academic papers and clinical studies, which will explore if an ear cleanser – either alone or coupled with the right antimicrobia – could better treat, cure and prevent recurrence of otitis with *Pseudomanas* spp.

How does this address AMR and responsible use?

Reducing the recurrence of chronic otitis externa may decrease the use of antibiotics to treat those otitis cases, which is cases frequently associated with resistant Pseudomonas aeruginosa

Monitoring antibiotic susceptibility of pathogens in livestock.

The antibiotic susceptibility monitoring programs of CEESA are an ongoing collaboration among veterinary pharmaceutical companies for twenty years.

CEESA conducts two types of monitoring: the EASSA program, which collects zoonotic and commensal bacteria at slaughter from healthy food-producing animals, and the target pathogen programs (VetPath, MycoPath and ComPath), which collect bacterial isolates from diseased animals prior to antibiotic treatment.

The latter programs are the only long-standing pan-European projects in veterinary medicine where antibiotic susceptibility data for a large variety of target pathogens are generated.

Through valuable support by external laboratories and veterinary practitioners, CEESA has meanwhile generated a collection of more than 55,000 non-duplicate bacterial isolates.

How does this address AMR and responsible use?

Understanding the evolution of pathogens and their susceptibility to treatment is essential to the long term efficacy of antibiotics. It allows veterinarians, farmers and medicine manufacturers to adapt protocols to limit resistance development.

The list of activities in this section are only a selection of the work by HealthforAnimals members.

To discover more materials, such as our Antibiotics Commitment, or request information about a specific activity listed above visit **HealthforAnimals.org** or contact us at **info@HealthforAnimals.org**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ATA 2019

3rd International Symposium on Alternatives to Antibiotics

Challenges and Solutions in Animal Health and Production

Bangkok, Thailand

December 16-18, 2019

Programme and Book of Abstracts

PROGRAMME & BOOK OF ABSTRACTS

3rd International Symposium on Alternatives to Antibiotics (ATA):

Challenges and Solutions in Animal Health and Production

The Berkeley Hotel, Bangkok, Thailand
16-18 December 2019

Organised by

USDA

Faculty of Veterinary Science · Chulalongkorn University

Department of Livestock Development

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IMPORTANT NOTES

Preregistration badge pick up

Date:	December 15, 2019
Time:	13:00-17:00
Venue:	The Berkeley Hotel Pratunam lobby

Main conference

Date:	December 16-18, 2019
Time:	December 16, 08:00 -17:00; December 17-18, 08:30 -17:00
Venue:	Mayfair Grand Ballroom, 11th floor

Registration

Date:	December 16-18, 2019
Time:	December 16, opens at 07:30
	December 17-18, opens at 08:00
Venue:	Mayfair Grand Ballroom, 11th floor

Opening ceremony

Date:	December 16, 2019
Time:	8:00
Venue:	Mayfair Grand Ballroom, 11th floor

Lunch

Date:	December 16-18, 2019
Time:	12:00-13:30
Venue:	The Palladium Hall, 10th floor

Welcome cocktail reception

Date:	December 16, 2019
Time:	18:00-20:00
Venue:	The Palladium Hall, 10th floor

The 3rd International Symposium on Alternatives to Antibiotics wishes to thank the following for their generous support

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WELCOME TO

The 3rd International Symposium on Alternatives to Antibiotics

Challenges and Solutions in Animal Health and Production

The Berkeley Hotel, Bangkok, Thailand

16-18 December 2019

In view of the continuing global concerns with antibiotic resistance, there is a pressing need to have a scientific forum to assess the scientific advancements made since the 2nd International Symposium on Alternatives to Antibiotics held at the World Organisation for Animal Health (OIE) in Paris, France, in 2016. The objectives of this 3rd International Symposium are to highlight promising research results and novel technologies that provide alternatives to antibiotics for use in animal health and production, assess challenges associated with their commercialization and use, and provide recommendations to support their development. The symposium will focus on the latest scientific breakthroughs and technologies that provide new options and alternative strategies for preventing and treating diseases of animals and reduce the use of medically important antibiotics in agriculture. Although some of these new technologies provide the means for implementing a One Health approach and have direct applications as medical interventions for human health, the focus of the symposium is on animal health and production and food safety.

The following six areas will be explored in detail through scientific presentations and expert panel discussions:

1. Vaccines that could reduce the use of medically important antibiotics
2. Microbial-derived products, such as probiotics and bacteriophage gene products
3. Non-nutritive phytochemicals, including prebiotics
4. Immune-related products, such as antibodies, microbial peptides and cytokines
5. Innovative drugs, chemicals, and enzymes
6. Regulatory pathways to enable the licensure of alternatives to antibiotics and incentives from stakeholders to support their development.

Rungtip Chuanchuen
Chair – organizing committee

Hyun Lillehoj
Chair – scientific committee

Cyril Gay
Chair – steering committee

GREETINGS FROM CU VET, THE LOCAL HOST

Dear Delegates of the 2019 ATA Symposium,

We warmly welcome you to Bangkok, the great city of angles. We are very happy that you decided to travel to this wonderful city to participate the great symposium devoted to alternatives to antibiotics. The ATA 2019 symposium is the result of the hard work of the exceptional team who has intended to increase knowledge of alternatives to antibiotics to veterinary fields. The scientific program has been carefully designed to provide topics that are directly useful to veterinary sectors as well as human and environmental sectors. We have been fortunate to recruit world-renown speakers who have agreed to share their expertise and professional knowledge to all of us. The oral and poster presentations were carefully reviewed and the presenters have been enthusiastic to share the results of their newest research. This symposium is also a platform for all delegates to communicate and learn from each other.

Thanks to all volunteers, speakers, the support of our partners from industry and in particular, participations of all delegates. This symposium will not be successful at this magnitude without all the contributions.

We are confident that you will enjoy the symposium and the scientific contents. Most importantly take your time to enjoy and experience the unique blend of traditional and modern cultures of Bangkok.

Prof. Roongroje Thanawongnuwech
Dean
Faculty of Veterinary Science, Chulalongkorn University

COMMITTEE

Scientific Committee

- Cyril Gay, USDA-ARS
- Hyun Lillehoj, USDA/ARS
- Rungtip Chuanchuen, Chulalongkorn University
- Elisabeth Erlacher-Vindel, OIE
- Dennis M. Dixon, NIAID, NIH
- Filip Van Immerseel, Ghent University
- Chengbo Yang, University of Manitoba
- Henk P. Haagsman, Utrecht University
- Peter M. H. Heegaard, Technical University of Denmark
- Hong Dong, Beijing University
- Jaap Wagenaar, Utrecht University

Steering Committee

- Cyril Gerard Gay, USDA, ARS
- Rungtip Chuanchuen, Chulalongkorn University
- Hyun Lillehoj, USDA, ARS
- Charles Li, USDA-ARS
- Chengbo Yang, University of Manitoba
- Ron Cravens, Cravens Life Sciences

Organizing Committee (Chulalongkorn University)

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- Kaywalee Chatdawong
- Sasi Jaroenpoj
- Saharuetai Jeamsripong
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- Pattrarat Chanchaithong
- Taradon Luangtongkum
- Anudep Rungsipipat
- Navapon Techakriengkrai
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- Suphot Wathnaponsark
- Rangsiya Prathan
- Suwanee Chalermchainukul
- Shabbir A Simjee, CLSI subcomittee

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INVITED SPEAKERS

Elisabeth Erlacher-Vindel

- OIE, France
- The global objectives for AMR and alternatives for the animals

Dennis Dixon

- NIH, United States
- Global strategies for developing alternatives to antibiotics for human health

Hyun Lillehoj

- USDA-ARS, United States
- Antibiotics, germs and antibiotic alternatives for animals

John Prescott

- University of Guelph, Canada
- Immunization of broiler chickens against necrotic enteritis: Progress and possibilities

Filip Van Immerseel

- University of Ghent, Belgium
- The future of *Salmonella* vaccines in a geographically diverse and changing epidemiological environment

Tun, Hein Min

- University of Hong Kong, Hong Kong
- Microbiome for gut health: A modern tool and a target in the effort to address antimicrobial resistance

Todd Callaway

- University of Georgia, United States
- Non-antibiotic strategies to modify the microbial population of dairy cattle: impacts on milk production, animal health and food safety

Tom Rehberger

- Church & Dwight Co., Inc., United States
- Managing the gut microbial populations: From science to practice

Inkyung Park

- USDA-ARS, Korea
- Small molecular weight metabolites regulating growth and immunity as postbiotic antibiotic alternatives

Junjun Wang

- China Agricultural University , China
- Strategies to reduce antibiotics in swine production in China

Judy Chen

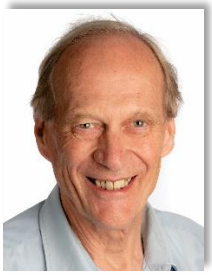
- USDA-ARS, United States
- Non-antibiotic treatments for honey bee diseases in the era of omics

Pietro Celi

- DSM, Switzerland
- Fighting AMR by optimizing gastrointestinal functionality: A holistic approach

Chengbo Yang

- University of Manitoba, Canada
- Organic acids as antibiotic alternatives in monogastric animals



John Furness

- University of Melbourne, Australia
- Sensing and reacting: micronutrients and phytochemicals in gut health

Hong Dong

- Beijing Key Laboratory of Traditional Chinese Veterinary Medicine, China
- Mechanisms of Baitouweng Decoction in the treatment of diarrhea caused by *Escherichia coli*

Emma Wall

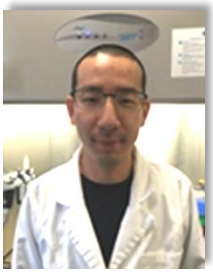
- Full circle science, USA
- Phytonutrients: The Next Generation

Prashant Mishra

- AVT Natural, Mexico
- Science-based use of plant extracts to improve animal health in post-antibiotic era: where are we?

Peter Heegaard

- Technical University of Denmark, Denmark
- Passive Immunity and IgG-like antibodies as an alternative to antibiotics



Woohyun Kim

- Gyeongsang National University, Korea
- Host defence peptides with anti-microbial and immunomodulatory activities as antibiotic alternatives

Douglas Korver

- University of Alberta, Canada
- Making the transition from research trials to field application

Henk Haagsman

- Utrecht University, The Netherland
- Reprogramming the innate immune system as an alternative

Joshua Hayes

- Center for Veterinary Medicine, Food and Drug Administration, United States
- US FDA's Regulatory Pathway for Alternatives to Veterinary Antimicrobials

Javier Pozo

- European Medicine Agency, Amsterdam, The Netherlands
- Promoting the authorization of alternatives to veterinary medicinal antimicrobials in the European Union

Takashi Kozasa

- Ministry of Agriculture, Forestry and Fisheries, the Government of Japan
- Legal framework for the approval designation of alternatives to antibiotics.

Erik De Ridder

- Health for Animals
- Industry perspective on the registration of alternatives to antibiotics.

SCIENTIFIC PROGRAM

Day 1: Monday 16 December 2019

07:30	**REGISTRATION BEGINS**	
08:00-08:05	Welcome address from Professor Dr. Roongroje Thanawongnuwech, Dean, Faculty of Veterinary Science, Chulalongkorn University, Thailand	
08:05-08:10	Objectives and expected outcomes for the 3rd International Symposium on Alternatives to Antibiotics - Dr. Cyril Gay, Senior National Program Leader, USDA-ARS, United States of America	
08:10-08:30	**Keynote Presentation:** The global objectives for AMR and alternatives for the animals • *Elisabeth Erlacher-Vindel*, The Antimicrobial Resistance and Veterinary Products Department, World Organisation of Animal Health (OIE), France	
08:30-09:00	**Keynote Presentation:** Global strategies for developing alternatives to antibiotics for human health • *Dennis Dixon*, NIH, United States of America	
09:00-09:30	**Keynote Presentation:** Antibiotics, germs and antibiotic alternatives for animals • *Hyun Lillehoj*, USDA-ARS, United States of America	
09:30-10:00	**COFFEE BREAK / POSTER SESSION**	
SESSION	**1. VACCINES**	
CHAIRS	*John Prescott*, Canada and *Cyril Gay*, United States of America	
10:00-10:25	Immunization of broiler chickens against necrotic enteritis: Progress and possibilities • *John Prescott*, University of Guelph, Canada	
10:25-10:50	The future of *Salmonella* vaccines in a geographically diverse and changing epidemiological environment • *Filip Van Immerseel*, University of Ghent, Belgium	
10:50-11:05	Novel vaccine antigens identified by chicken monoclonal antibodies against apicomplexans • *Kazumi Sasai*, Osaka Prefecture University, Japan	
11:05-11:20	Development of a subunit vaccine targeting *Clostridium perfringens* enzymes for the control of necrotic enteritis in broilers • *Lisa Bielke*, The Ohio State University, United States of America	
11:20-11:35	Immunization with recombinant subunit vaccines from virulent *Clostridium perfringens* field strains confers partial protection against necrotic enteritis in broiler chickens • *Charles Li,* BARC/ARS/USDA, United States of America	
11:35-12:00	**Roundtable discussion**	
12:00-13:30	**LUNCH AT THE PALLADIUM HALL / POSTER SESSION**	
SESSION	**2. MICROBIAL-DERIVED PRODUCTS**	
CHAIRS	**Advances in the research and development of alternatives to antibiotics**	**ACADEMIA**
	Tun, Hein Min, Hong Kong and *Todd Callaway*, United States of America	
13:30-13:55	Microbiome for gut health: A modern tool and a target in the effort to address antimicrobial resistance • *Tun, Hein Min*, University of Hong Kong, Hong Kong	
13:55-14:20	Non-antibiotic strategies to modify the microbial population of dairy cattle: impacts on milk production, animal health and food safety • *Todd Callaway*, University of Georgia, United States of America	
14:20-14:45	Small molecular weight metabolites regulating growth and immunity as postbiotic antibiotic alternatives • *Inkyung Park*, USDA-ARS, Korea	
14:45-15:00	Microbiological quality and possible role as a source of antimicrobial resistance genes of commercial probiotic products for livestock and aquatic animals • *Rungtip Chuanchuen,* Chulalongkorn University, Thailand	
15:00-15:15	Swine-derived probiotic *L. Plantarum* modulates porcine intestinal endogenous HDP synthesis • *Jing Wang,* Beijing Academy of Agriculture, China	
15:15-15:45	**COFFEE BREAK / POSTER SESSION**	
CHAIRS	**Research results from commercially available alternatives to antibiotics**	**INDUSTRY**
	Tom Rehberger, United States and *Judy Chen*, United States of America	
15:45-16:10	Managing the gut microbial populations: From science to practice • *Tom Rehberger*, Church & Dwight Co., Inc., United States of America	
16:10-16:25	Responses of *Bacillus* amyloliquefaciens CECT 5940 supplementation in weaned pig diets • *Thammakit Thammathipborworn,* Nutrition & Care, Evonik (Thailand) LTD.	

16:25-16:40	Fermentate Bioactives Impact on SARA and a mastitis *Stretoccocus uberis* challenge to reduce AIF use in bovines • *Leon Samuel Barringer,* Diamond V, United States of America
16:40-17:00	**Roundtable discussion**
18:00-20:00	**WELCOME COCKTAIL RECEPTION THE PALLADIUM HALL**

Day 2: Tuesday 17 December 2019

SESSION	**3. INNOVATIVES DRUGS, CHEMICALS AND ENZYMES**	
CHAIRS	**Advances in the research and development of alternatives to antibiotics**	**ACADEMIA**
	Chengbo Yang, Canada and *Junjun Wang*, China	
08:30-08:55	Strategies to reduce antibiotics in swine production in China • *Junjun Wang*, China	
08:55-09:20	Non-antibiotic treatments for honey bee diseases in the era of omics • *Judy Chen*, USDA-ARS, United States of America	
09:20-09:35	In vitro and in vivo characterization of a Gly-substituted DLP4 cationic peptide against *Staphylococcus aureus* CVCC 546 • *Bing Li,* Chinese Academy of Agricultural Sciences, China	
09:35-09:50	Afterlife of bacterial cell debris: Peptidoglycan in the gastrointestinal tract • *Christian Nyffenegger,* Novozymes A/S, Denmark	
09:50-10:20	**COFFEE BREAK / POSTER SESSION**	
CHAIRS	**Research results from commercially available alternatives to antibiotics**	**INDUSTRY**
	Hyun Lillehoj, United States and *Pietro Celi*, Switzerland	
10:20-10:45	Combating AMR by optimizing gastrointestinal functionality: A holistic approach • *Pietro Celi*, DSM, Switzerland	
10:45-11:10	Organic acids as antibiotic alternatives in monogastric animals • *Chengbo Yang*, University of Manitoba, Canada	
11:10-11:25	25-OH-D3: An indispensable tool to managing antibiotic free feeding programs for commercial broilers • *Thau Kiong Chung,* DSM Nutritional Products Asia Pacific, Singapore	
11:25-11:40	Alternatives to Veterinary Antimicrobials (AVANT): A new EU project focused on diarrhoea in pigs • *Poul Jesper Baekbo,* SEGES, Denmark	
11:40-12:00	**Roundtable discussion**	
12:00-13:30	**LUNCH AT THE PALLADIUM HALL / POSTER SESSION**	
SESSION	**4. PHYTOCHEMICALS**	
CHAIRS	**Advances in the research and development of alternatives to antibiotics**	**ACADEMIA**
	Hong Dong, China and *John Furness*, Australia	
13:30-13:55	Sensing and reacting: micronutrients and phytochemicals in gut health • *John Furness*, University of Melbourne, Australia	
13:55-14:20	Mechanisms of Baitouweng Decoction in the treatment of diarrhea caused by *Escherichia coli* • *Hong Dong*, Beijing Key Laboratory of Traditional Chinese Veterinary Medicine, China	
14:20-14:35	An anthocyanin-rich purple potato extracts reduce high fat diet and lipopolysaccharide (LPS) induced obesity and low-grade gut inflammation • *Hua Zhang,* Jiangxi University, China	
14:35-14:50	Dietary resistant potato starch alters immunological status and microbial populations in swine to limit *Salmonella* • *Crystal L Loving,* USDA-ARS, United States of America	
14:50-15:20	**COFFEE BREAK/ POSTER SESSION**	

CHAIRS	**Research results from commercially available alternatives to antibiotics**	**INDUSTRY**
	Prashant Mishra, Mexico and *Emma Wall,* United States of America	
15:20-15:45	Phytonutrients: The Next Generation • *Emma Wall*, Full Circle Science, United States of America	
15:45-16:10	Science-based use of plant extracts to improve animal health in post-antibiotic era: where are we? • *Prashant Mishra*, AVT Natural, Mexico	
16:10-16:25	*In-vitro* antibacterial activity of phytobiotic against *Eschericia coli* and *Mycoplasma gallisepticum* • *Elvina Jonas Jahja,* PT. MEDION FARMA JAYA, Indonesia	
16:25-16:40	*In vitro* and *in vivo* evaluation of therapeutic effects of neutrapath™ against *Salmonella Typhimurium* • *Hongyu Xue,* Amlan International, United States of America	
16:40-17:00	**Roundtable discussion**	

Day 3: Wednesday 18 December 2019

SESSION	**5. IMMUNE-RELATED PRODUCTS**	
CHAIRS	**Advances in the research and development of alternatives to antibiotics**	**ACADEMIA**
	Peter Heegaard, Denmark and *Woohyun Kim*, Korea	
08:30-08:55	Passive Immunity and IgG-like antibodies as an alternative to antibiotics • *Peter Heegaard,* Technical University of Denmark, Denmark	
08:55-09:20	Host defence peptides with anti-microbial and immunomodulatory activities as antibiotic alternatives • *Woohyun Kim,* Gyeongsang National University, Korea	
09:20-09:35	Innovative enterobactin-specific egg yolk antibodies for controlling Gram-negative pathogens • *Jun Lin*, University of Tennessee, United States of America	
09:35-09:50	High throughput screening for natural host defense peptide-inducing compounds as alternatives to antibiotics • *Glenn Zhang,* Oklahoma State University, United States of America	
09:50-10:20	**COFFEE BREAK / POSTER SESSION**	
CHAIRS	**Research results from commercially available alternatives to antibiotics**	**INDUSTRY**
	Douglas Korver, Canada and *Henk Haagsman*, The Netherlands	
10:20-10:45	Making the transition from research trials to field application • *Douglas Korver,* University of Alberta, Canada	
10:45-11:10	Reprogramming the innate immune system as an alternative • *Henk Haagsman*, Utrecht University, The Netherland	
11:10-11:25	Efficacy of dried egg product administered to male broiler chickens during experimental necrotic enteritis • *Jeffery Escobar*, Elanco Animal Health, United States of America	
11:25-11:40	Yeast cell wall immunomodulatory and intestinal integrity effects on broilers challenged with *Salmonella* Enteritidis • *Ekachai Jenwitheesuk,* ICC Brazil, Brazil	
11:40-12:00	**Roundtable discussion**	
12:00-13:30	**LUNCH AT THE PALLADIUM HALL / POSTER SESSION**	
SESSION	**6. REGULATORY PATHWAYS TO ENABLE THE LICENSING OF ALTERNATIVES TO ANTIBIOTICS AND INCENTIVES FROM STAKEHOLDERS TO SUPPORT THEIR DEVELOPMENT**	
CHAIRS	*Joshua Hayes,* Food and Drug Administration, United States and *Peter Borriello,* Veterinary Medicine Directorate, United Kingdom	
13:30-13:55	US FDA's Regulatory Pathway for Alternatives to Veterinary Antimicrobials • *Joshua Hayes*, Center for Veterinary Medicine, Food and Drug Administration, United States of America	
13:55-14:20	EU approach to authorization of novel technologies with particular emphasis on alternatives to antibiotics • *Javier Pozo*, European Medicine Agency, Amsterdam, The Netherlands	
14:20-14:45	Legal framework for the approval/designation of alternatives to antibiotics. • *Takashi Kozasa*, Ministry of Agriculture, Forestry and Fisheries, the Government of Japan	

14:45-15:10	Industry perspective on the registration of alternatives to antibiotics. • *Erik De Ridder*, Health for Animals, Belgium
15:10-15:40	**COFFEE BREAK / POSTER SESSION**
15:40-16:30	**Roundtable discussion** • *Scientist perspective - Filip Van Immerseel*, University of Ghent, Belgium • *Regulatory perspective - Pete Borriello, Joshua Hayes, Javier Pozo, Takashi Kozasa* • *Industry perspective - Erik De Ridder*, Health for Animals, Belgium • *Policy perspective - Jomana F. Musmar,* U.S. Department of Health and Human Services, United States of America • *Funder perspective -Renée Larocque,* International Development Research Centre (IDRC), Canada **Roundtable questions** • What novel scientific data that are relevant to ATAs could be used to effectively support their licensing and registration with regulatory authorities? • What scientific information is critical for industry to know prior to sponsoring a product/idea for regulatory review? • What level of return must a product promise for it to be commercialized? • What are potential incentives to promote and maintain stakeholder interest in early to advanced R&D of alternatives to antibiotics? • Are there funding incentives for research to develop ATAs for livestock and aquaculture production in low- and middle-income countries?
16:30-17:00	**CONCLUSIONS AND NEXT STEP** Dr. Cyril Gerard Gay, Senior National Program Leader, USDA-ARS, United States of America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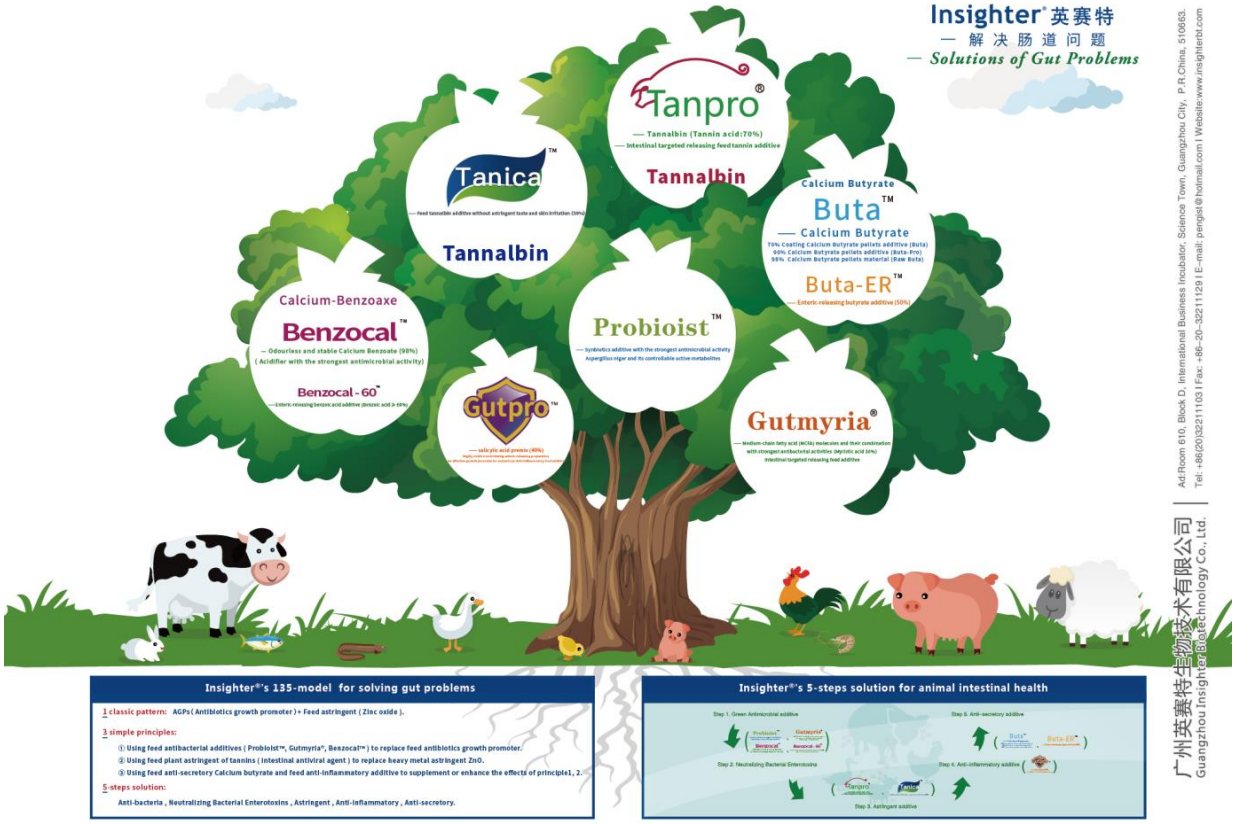

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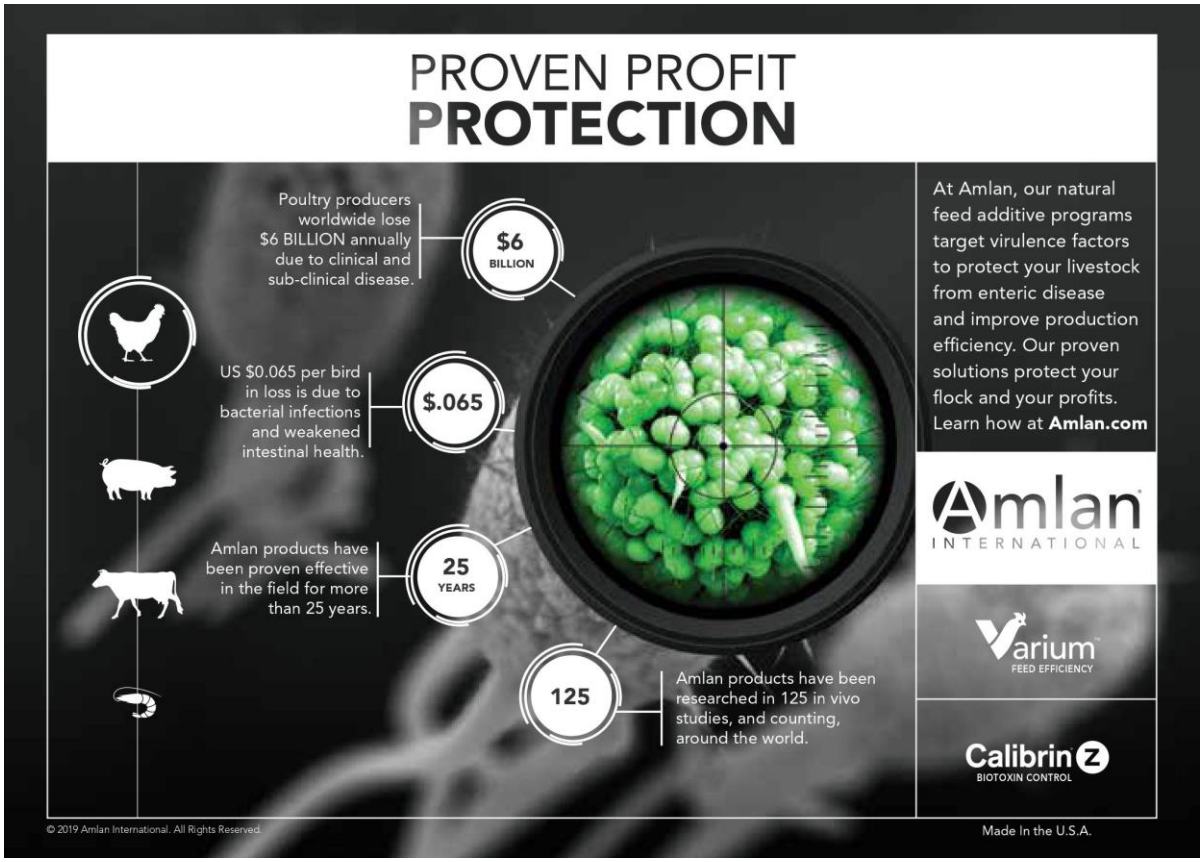
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LIST OF ABSTRACTS

Programme Overview

Objectives and expected outcomes

C. G. Gay

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In view of the continuing global concerns with the loss of medically important antibiotics, either due to regulatory restrictions or the emergence of antimicrobial resistance, this symposium provides a scientific forum to assess the scientific advancements made in the research and development of alternatives to antibiotics. The key objectives of this symposium are to highlight promising research results and novel technologies that provide alternatives to antibiotics for use in animal health and production, assess challenges associated with their commercialization and use, and provide actionable strategies to support their development. The symposium will focus on five product categories that could reduce the use of medically important antibiotics in animal health and production: 1) vaccines; 2) microbial-derived products; 3) phytochemicals; 4) immune-derived products; and 5) innovative drugs, chemicals, and enzymes. The issue of antimicrobial resistance is a priority 'One Health' issue with important ramifications for public health and agriculture. It is recognized that one of the fundamental challenges of the 21st Century will be to augment agricultural production to feed an increasing world population, which is wholly dependent on the availability of interventions to prevent and control animal and plant diseases. Importantly, the success of the global agricultural enterprise in preventing and controlling diseases will directly impact global food security and the Global Health Security Agenda, key initiatives identified by the World Organisation for Animal Health (OIE) and the United Nations Food Agriculture Organization (FAO) and the World Health Organization (WHO). To be clear, this symposium is not intended to be a venue to eliminate the use of antibiotics in animals as there is a specific need for antibiotics to treat diseases. Nor is this a venue to advocate strategies that use scientifically unproven approaches that will also eventually fail against documented pathogen adaptability and resistant strain development. Rather, the topics that have been selected for this symposium are the research of innovative products for the prevention and treatment of diseases, as well as the enhancement of animal production, that do not result in the creation of selection pressure favoring the development of antimicrobial resistance. As such, the research and development of innovative drugs and antibiotic alternatives are included as key strategic objectives in the United States National Action Plan for Combating Antimicrobial Resistant Bacteria (CARB). The global increase in antibiotic resistance among bacterial pathogens is believed to be due to the over- and misuse of antibiotics in human and animal health. One of the key public health concerns linked to agriculture is the potential development of antibiotic resistant strains within food animal production facilities and among food-borne bacteria that could seriously compromise therapeutic options and medical interventions. Thus, stewardship programs and alternatives to the continued reliance on antibiotics in agricultural production need to be developed. There is also increasing scientific evidence that implicates certain antibiotics with disrupting the normal flora of the gut, yielding negative consequence on the immune system, disease tolerance and health. As we move into the 21st Century and the demands for food products increase to meet the nutritional needs of a growing world population, finding alternative strategies to improve animal health and production has become a global issue, and a critical component of efforts to alleviate poverty and world hunger.

Keynote Presentation

The global objectives for AMR and alternatives for the animals

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Antimicrobial resistance (AMR) is a global human and animal health concern, which is influenced by the use of antimicrobial agents in human and veterinary medicine, and the plant sector as recognised at the highest political level by the UN General Assembly resolution 71/3, adopted in 2016. To combat antimicrobial resistance, the World Organisation for Animal health (OIE) develops science-based intergovernmental standards and guidelines covering terrestrial and aquatic animals.

The OIE contributed to the development of the WHO Global Action Plan on Antimicrobial Resistance, that was endorsed by the OIE's 180 Member Countries through a Resolution unanimously adopted in May 2015. On November 2016, following the request from its World Assembly of Delegates, the OIE compiled its AMR activities into a strategy.

The OIE Strategy on Antimicrobial Resistance and the Prudent Use of Antimicrobials, outlines goals and activities to support Member Countries in their fight against AMR, and to encourage the national ownership and implementation of international Standards. The structure of the OIE Strategy supports the objectives established in the Global Action Plan, and reflects the mandate of the OIE, through four main objectives: i) Improve awareness and understanding; ii) Strengthen knowledge through surveillance and research; iii) Support good governance and capacity building; and iv) Encourage implementation of international standards.

Following these objectives, the OIE provides guidance and support to research into alternatives to antibiotics including vaccines, by working alongside partner organisations to encourage the development, uptake and registration of new tools, and validated products that will provide alternatives to the use of antibiotics and reduce the emergence and spread of AMR. In this framework, the OIE supported the United States Department of Agriculture (USDA) in the organisation of the first two "International Symposia on Alternatives to Antibiotics, Challenges and Solutions in Animal Production" (September 2012 and December 2016), that were held at the OIE Headquarters in Paris. In addition, the OIE supports the efforts of the STAR-IDAZ International Research Consortium (IRC) into improving the coordination of research programmes at international level to contribute to the development of new and improved animal health strategies for several priority diseases/issues, including on AMR and on the development of alternatives to antibiotics.

Keywords: global, objectives, AMR, alternatives, animals

Keynote Presentation

Global strategies for developing alternatives to antibiotics for human health

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Antimicrobial resistance is one of the top public health problems worldwide. Especially concerning is the loss of effective antibiotics for bacterial infections that were previously highly effective and led to major advances in human health. Continued exposure to existing classes of antibiotics with few new classes emerging, coupled with failure of pharmaceutical companies to recoup return on investment relative to other classes of drugs for use in modern medicine have created a complicated mix of challenges. The NIAID is a key component of the US national and international effort to address these challenges. Approaches include basic, translational and clinical research for better means of diagnosis, prevention and treatment, as well as preclinical and clinical resources to decrease the risks to antibiotic companies. NIAID antibiotic resistance research plans have included emphasis on exploring alternatives to traditional antibiotics. Examples of efforts to incentivize research in this area, including special funding announcements, along with selected examples of projects moving forward will be summarized.

Keywords: Antimicrobial, resistance, antibiotics, bacterial, infections

Keynote Presentation

Antibiotics, germs and antibiotic alternatives for animal

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Dietary antibiotics have been used in the food animal industry for more than 60 years, not only to control infectious diseases, but also to increase feed efficiency and improve growth performance. In chickens, subtherapeutic, in-feed antibiotics can increase body weight gain up to 8% and decrease the feed conversion ratio (feed intake/body weight gain) up to 5%, both compared with an antibiotic-free diet. Antibiotics overuse and abuse on a global scale have led to the emergence of multi-drug resistant "superbugs" from food animals and humans. The United States Food and Drug Administration has requested that agriculture producers discontinue sub-therapeutic dosing of antibiotics into animal feed, which for over 60 years, was the common practice to promote their economic value by increasing feed efficiency and growth. Therefore, development of novel antibiotic alternatives that can replace growth promoting drugs is timely and critical for sustainability of animal agriculture. At this third international symposium on alternatives to antibiotics, various strategies for developing novel alternatives to antibiotics for agriculture animal production will be discussed and we will learn more on the mode of action of novel antibiotic alternatives. In this talk, I will review our current knowledge on the underlying mechanisms of action of growth promoting drugs, the effects of antibiotics on gut microbiota and alternative alternatives to antibiotics. Antibiotics were originally thought to improve animal growth through reductions in the number and diversity of the normal bacterial flora present in the gut, which in turn, increased the bioavailability of nutrients available to the host and/or reduced the production of microbial metabolites deleterious to animal growth. Alternatively, antibiotics were suggested to improve growth performance through an anti-inflammatory effect directed toward the intestinal epithelium. With the advent of novel molecular biology and bioinformatics techniques, it is now clear that changes in the host intestinal inflammatory response, as well as the structure and diversity of the gut microbial community, occur when antibiotics are introduced into animal diets. Because there is a close cross talks that influence gut microbiota, immune system and brain function, understanding these interactions is critical to develop novel antibiotic alternatives. Current technological advances in "omics" technology is enabling global gene expression and metabolomic studies in commercial food animals to obtain better understanding of biochemical processes for the development of novel compounds for alternative ways of promoting growth and immunity. These approaches should provide the framework for future studies to identify natural chemical compounds to improve poultry growth performance without the use of in-feed antibiotics.

Keywords: Antimicrobial resistance, Microbiota, Antibiotics, Alternatives to antibiotics

SESSION 1

Vaccines

ORAL PRESENTATIONS

Immunization of broiler chickens against necrotic enteritis: Progress and possibilities

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Immunization of broiler chickens against necrotic enteritis (NE) caused by *Clostridium perfringens* is an important approach for control of this globally important disease. Although the last decade has seen considerable advances in understanding NE, including the 2008 discovery of NetB, a critically important pore-forming toxin, much remains to be discovered about the detailed pathogenesis and the basis of immunity to NE on which a scientifically designed immunization strategy must be based. The challenges include: 1. Understanding the basis and extent of immunity; 2. Induction of active immunity in newborn chicks in the face of maternal antibodies; 3. Identification and characterization of key protective antigens; 4. Defining experimental models to evaluate the protective efficacy of candidate antigens and the relevance of such models to naturally occurring NE; 5. Developing a vaccine that is safe, easy to deliver, robust under field conditions, and inexpensive; 6. Evaluating candidate vaccines under field conditions that do not compromise the welfare of the chicken.

Considerable progress has been made in identifying antigens of importance in immunity to NE using experimental immunization-challenge models. Passive immunity in chicks obtained through breeder vaccination using bacterial supernatant that includes the alpha toxin is short-lived; however, this vaccine has been currently withdrawn from the market. In inducing an active immunity, it is striking as to how many antigens of *C. perfringens* can provide some level of protection against experimental NE. These include (abbreviated names): Alpha toxin, EFtu, FBA, GPD, HP, NetB, PFOR, PGM and certain pilus proteins. It seems also clear that no one antigen can provide complete protection and that a combination of antigens or artificial hybrid protein constructs seem to offer superior protection against NE. It is clearly possible to immunize actively against NE but it is also clear that the level of protection by immunization depends on the severity of the experimental challenge.

How immunity to these antigens works is not defined. There is some evidence that antibodies may interfere with the growth of *C. perfringens* by binding to the bacterium itself and thus preventing their multiplication rather than, for example, by toxin neutralization or opsonization. The surprising protective efficacy of some "housekeeping" rather than just of virulence-associated proteins, suggests that bacterial growth inhibition might be a plausible explanation for their effect. Nevertheless, there seems to be a general agreement that oral immunization using recombinant vectors (example, *Eimeria*, *Lactobacillus*, *Salmonella*) can be a feasible option and that inducing an effective mucosal immunity is of great importance. An unresolved issue is whether NE evoked by coccidial infection requires that the *C. perfringens* strain be NetB-positive. If it does not, then the focus on NetB in immunization studies may be misleading, and research investment on the currently known most useful protective immunogens (alpha toxin, FBA, HP) would be more appropriate.

It is clear that immunization will be a useful but probably not perfect adjunct approach for controlling NE and that a search for the perfect antigen(s) and their delivery will likely delay progress in introducing immunization.

Keywords: Necrotic enteritis, immunization, review, progress, possibilities

The future of *Salmonella* vaccines in a geographically diverse and changing epidemiological environment

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Most important global *Salmonella* serotypes that cause food poisoning in humans are Enteritidis, derived mainly from eggs and egg-derived products, and Typhimurium, derived from porcine (and poultry) meat. *Salmonella* vaccination programs in laying hens, using inactivated and live vaccines, have been shown to be efficient in reducing egg contamination, while vaccination of broilers and pigs is still not commonly done, although there are some vaccines marketed and used in some regions. Apart from Enteritidis and Typhimurium, different serotypes can be of local and regional importance. Novel trends are the global emergence of *Salmonella* serogroup C strains in poultry, including multi-resistant clonal strains worldwide (e.g. *Salmonella* Infantis clones). Vaccines for poultry that are commercially available can be inactivated and live vaccines, the latter mostly based on spontaneous mutations, and mainly derived from Enteritidis and Typhimurium strains. Vaccines for the host-specific serotypes Gallinarum and Choleraesuis, that cause systemic infections in poultry and pigs, respectively, also are in use. In poultry, inactivated vaccines have been used for parent and layer flocks, and live vaccines mainly for layer flocks. Live vaccines are stimulating cell-mediated and humoral responses, and mucosal innate responses, as they mimic a natural infection, while inactivated vaccines mostly result in antibody responses. Both have been shown to be able to confer (at least partial) protection. Egg contamination in layers is well under control using vaccines. The most important challenges for *Salmonella* vaccines are the production of efficient vaccines for pigs and broilers, and the constant changes in serotype distribution, and thus the development of cross-protecting vaccines against a range of serotypes in broilers. For broilers, the difficulty is the build-up of active immunity in the short life span of the bird. An early bacteriological colonization-inhibition effect conferred by live vaccines has been described in the intestine but this is mainly efficient within the same serotype, so there is a lack of cross-protection using this method. For pigs, lymph node colonization seems difficult to control using vaccines. In addition, often serology is used for monitoring of *Salmonella* in pigs, and there can be interference with monitoring when piglets are vaccinated. Although there are no safety issues with current vaccines, the detailed knowledge on the molecular pathogenesis of *Salmonella* infections should result in attenuated and highly characterized deletion mutant vaccines, and add markers to differentiate vaccine and field strains and serological responses against vaccine and field strains. Regulatory aspects that are of importance in this regard are the faster acceptance of live attenuated mutant strains for emerging serotypes that contain identical gene deletions as one that are already marketed for other serotypes. Other challenges are early protection as chicks are highly susceptible during the post-hatch period, and extension of the duration of protection, considering the trend to extend the productive cycle in layers.

Keywords: *Salmonella*, poultry, vaccinaton, Enteritidis, Typhimurium

Novel vaccine antigens identified by chicken monoclonal antibodies against apicomplexans

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The phylum Apicomplexa comprises obligate intracellular parasites that infect vertebrates. All invasive forms of Apicomplexa possess an apical complex, a unique assembly of organelles localized to the anterior end of the parasite and involved in host cell invasion. The chicken antibodies have been demonstrated to be useful for immunochemical research and clinical applications. In contrast to mammals, chicken antibody diversity is mostly generated by somatic mechanisms. It may be possible to produce antibodies in chickens that are difficult or impossible to produce in mammals. Previously, we have developed chicken monoclonal antibodies (mAbs) raised against *Eimeria acervulina* (Protozoa, Apicomplexa) and demonstrated that the chicken mAb, 6D-12-G10, recognized the conoid of *E. acervulina* sporozoites as the apical cytoskeleton and significantly inhibited sporozoite invasions of T lymphocytes *in vitro*. This antigen was highly conserved among Apicomplexan parasites, including other *Eimeria* spp., *Toxoplasma*, and *Neospora*. In further analyses using this mAb, we identified the apical cytoskeletal antigen of *Cryptosporidium parvum,* a pathogen of increasing clinical significance in livestock, birds, and wildlife as well as humans. Here, we characterized this antigen in *C. parvum* to assess its potential as a vaccine against cryptosporidiosis. Indirect immunofluorescence demonstrated that the reactivity of 6D-12-G10 with *C. parvum* sporozoites was similar to those of anti-β- and anti-γ-tubulins antibodies. Immunoelectron microscopy with the 6D-12-G10 mAb detected the antigen both on the sporozoite surface and underneath the inner membrane at the apical region of zoites. The 6D-12-G10 mAb significantly inhibited *in vitro* host cell invasion by *C. parvum*. MALDI-TOF/MS and LC-MS/MS analysis of tryptic peptides revealed that the mAb 6D-12-G10 target antigen was elongation factor-1α (EF-1α). These results indicate that EF-1α plays an essential role in mediating host cell entry by the parasites and, as such, could be a candidate vaccine antigen against the apicomplexans.

Keywords: *Eimeria*, Cryptosporidium, Apical cytoskeletal antigen, Chicken monoclonal antibody, elongation factor-1α

Development of a subunit vaccine targeting Clostridium perfringens enzymes for the control of necrotic enteritis in broilers

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Necrotic enteritis (NE) is a pervasive enteric disease responsible for large scale economic losses within the global poultry industry. The etiologic agent of NE is *Clostridium perfringens* (CP), an opportunistic pathogen that utilizes numerous extracellular toxins and glycoside hydrolases (GH) as key virulence and nutrient acquisition factors. Notably, some GH, mucinases, degrade components of mucin in the gastrointestinal tract as an energy source. Targeting this mechanism may serve to reduce the incidence of disease associated with CP. Two experiments were completed that evaluated mucinase vaccine targets sourced from conserved peptide sequences of carbohydrate binding module 32 (CBM32) of CP mucinases. In experiment 1, 37 antigen peptides were synthetically generated and used to produce hyper-immune sera which was then evaluated for ability to obstruct CP growth *in vitro*. Total CFU of CP were measured at 4h, 6h, and 8h incubation to determine growth rate. Peptides 4, 5, 22, 24, and 30 were selected for further *in vivo* testing based on conservation or the ability to inhibit CP growth by over 50% at 6h and 8h. In experiment 2, the aforementioned peptides were conjugated to an agonistic, CD40-targetting antibody and evaluated *in vivo*. Broilers were given an *Eimeria maxima* (EM) and CP in order to induce NE and assess vaccine efficacy. Treatments included a non-vaccinated non-inoculated control (NVNC), non-vaccinated inoculated control (NVIC), vaccination with peptide 4, 5, 22, 24, or 30 (VP4-VP30), or a combination of all five peptides (MC). There was a significant increase ($p<0.05$) in the percent change in BWG (%ChangeBWG) relative to NVIC for VP22 and MC of 18.54% and 17.43%, respectively. MC vaccinated group had the lowest lesions with a mean score of 0.63±0.18. These results suggest the MC combination was the most successful in alleviating overall performance losses associated with NE-infected broilers and encourage future testing of MC in the development of an NE vaccine.

Keywords: vaccine, *Clostridium*, necrotic enteritis, *Eimeria*, subunit vaccine

Immunization with novel vaccine candidate recombinant antigens from virulent *Clostridium perfringens* field strains confers partial protection against necrotic enteritis in broiler chickens

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Necrotic enteritis (NE) is one of the top enteric infectious diseases in commercial broiler chickens that is caused primarily by *Clostridium perfringens* (CP) A/G strains and responsible for around $6 billion economical loss worldwide. Coccidiosis is the major predisposing factor for NE. With gradual reduction and eventual withdrawal of antibiotic growth promoters from animal feed due to public and regulatory pressures, alternatives to antibiotic approaches assume top- priority for global poultry industry. Vaccination should be an ideal approach for mass prevention. However, there is no effective vaccine commercially available for NE. In this study, the recombinant proteins: chimeric NetB and alpha-toxin (NA), chimeric Fructose-1,6-bisphosphate aldolase and a hypothetic protein (FBA/HP), truncated TpeL, and Collage adhesion protein (Cna) were evaluated for their vaccine efficacy against severe NE challenge with *netB*$^+$*tpeL*$^+$ CP strain using two different NE challenge models which were developed at ARS. Young broiler chicks were immunized twice subcutaneously with adjuvanted CP proteins on days 4, and 15 and various disease parameters were evaluated. Optimum protection was seen when CP proteins mixed with MONTANIDE™ ISA 71 VG (Seppic Inc., France) were given twice intramuscularly using a dual infection NE model (*E. maxima*/CP) and a CP alone NE model. Immunization with all pooled antigens provided better protection against virulent challenges in both models. Immunization with these immunogens merits further investigation in the future, especially in mucosal delivery route.

Keywords: Necrotic enteritis, vaccine, *Clostridium perfringens*, *ii* recombinant proteins

SESSION 1

Vaccines

POSTER PRESENTATIONS

VA1
Combination of multiple antigens are essential for the development of a novel vaccine against *Staphylococcus aureus* infection

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Staphylococcus aureus (S. aureus) is a common pathogen found in the community and in hospitals. Most notably, Methicillin-resistant *S. aureus* is resistant to many antibiotics, which is a growing public health concern. The emergence of drug-resistant strains has prompted the search for alternative treatments such as immunotherapeutic approaches. Prophylactic vaccination is the best approach to combat against MRSA since it can provide protection without any concerns regarding antibiotic resistance. To date, most clinical trials of vaccines or passive immunization against *S. aureus* have ended in failure. In this study, we investigated multiple proteins as possible targets for a vaccine. Mice vaccinated with these purified proteins elicited high titers of specific antibodies as well as Th1- and Th17-biased immune responses in mice. Animal test indicated a protection rate over 90% in several animal models against multiple strains of *S. aureus*. Interestingly, gdT cells transferred from the vaccinated mice to naïve mice can confer protection to the naïve mice against *S. aureus* challenge in skin infection models. These findings raise the hope that the candidate antigens could be developed into multivalent and serotype-independent vaccines against *S. aureus* infection.

Keywords: *Staphylococcus aureus,* Th17, Gamma-Delta T cells, MRSA

VA2
Montanide™ ISA 71 R VG for efficient vaccines against infectious coryza

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Infectious Coryza (IC) is a poultry disease caused by *Avibacterium paragallinarum* which affects respiratory system and causes economic losses (decrease of laying production and increase of culling rate in growing chickens). *Av. paragallinarum* is classified into three serovars with endemic presence in countries like India. Development of trivalent inactivated IC vaccines is a strategy to protect chickens. Adjuvants are required to improve their efficacy. MONTANIDE™ ISA 71 R VG (ISA 71R) is an adjuvant designed to resist to destabilizing antigenic media such as bacteria. In this study, we evaluated the performance of ISA 71 R formulated in a trivalent IC vaccine.

Firstly, 30 layers were injected at D0 and D21 with a trivalent vaccine based on ISA 71R. Vaccine stability was tested and efficacy was assessed by a virulent challenge at D35. The vaccine was stable and induced 100% protection against all three serovars.

Then approximately 100,000 layers distributed in 4 farms with high infectious coryza prevalence in Southern India were vaccinated with the same ISA 71R vaccine in a prime/boost protocol. Injection sites were inspected and mortality was monitored during 7 days post vaccination. Egg production and mortality were recorded weekly up to 17 months of age and compared to standard mortality and egg production. No untoward effects were observed after vaccination. No incidences of infectious coryza were observed during production period. Egg production and the mortality rate were similar to what is observed in a healthy flock.

The vaccine adjuvanted with ISA 71 R VG is efficacious in controlling IC in field conditions.

Keywords: Infectious Coryza, egg production, vaccine, adjuvant, Montanide ISA 71 R VG

VA3
DIVA vaccine provides cross-protection against *Salmonella* serovars in food animals

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Current limitations of available *Salmonella* vaccines include the lack of cross-protection due to serovar-specific protection and interference with *Salmonella* surveillance programs to identify *Salmonella*-positive herds or flocks. A major hurdle to overcoming these limitations is the highly variable, immunodominant lipopolysaccharide (LPS) that 1) differentiates the >2,600 *Salmonella* serovars by their differences in LPS (and flagella), thereby contributing to serovar specific immunity, and 2) is the antigenic focus of *Salmonella*-specific antibody detection ELISAs for herd level monitoring for *Salmonella* exposure. To address these limitations, a live attenuated *Salmonella enterica* serovar Typhimurium vaccine (BBS 866) was designed to dramatically reduce LPS by deleting the *rfaH* gene, thereby decreasing serovar specific immunity, generating an attenuated strain, and creating a DIVA vaccine (differentiate infected from vaccinated animals). Two vaccine trials were performed in swine. In the first vaccine trial, pigs were administered two doses of the vaccine (vaccination and booster) and challenged with wild-type *S.* Typhimurium UK1 that causes gastroenteritis. The swine rectal temperatures, plasma IFNγ levels, fecal shedding and tissue colonization with wild-type *S.* Typhimurium UK1 were significantly reduced in vaccinated pigs compared to mock-vaccinated swine. In the second vaccine trial, pigs were administered a single dose of the vaccine and challenged with virulent multi-drug resistant (MDR) *S.* Choleraesuis that causes systemic disease in swine. Compared to the mock-vaccinated group, the vaccinated pigs exhibited significantly reduced rectal temperatures, serum IFNγ levels, and tissue colonization. Furthermore, during the challenge period, the isolation of *S.* Choleraesuis from blood cultures was significantly greater in mock-vaccinated pigs compared to vaccinated swine. In both vaccine trials, the vaccine strain did not induce a serological response to *Salmonella* LPS and therefore an ELISA can be used to differentiate infected from vaccinated animals (DIVA). Because the *Salmonella* vaccine was designed to reduce serovar specificity to provide cross-protection against diverse *Salmonella* serovars, we evaluated its applicability in another food animal commodity, turkeys. The vaccine reduced systemic and intestinal colonization of vaccinated turkeys following challenge with MDR *Salmonella* Heidelberg. The data from these vaccine trials indicate that the live, attenuated *S.* Typhimurium vaccine can both protect food animals from *Salmonella* that cause systemic disease and also reduce the potential for transmission of *Salmonella* that cause foodborne disease in humans.

Keywords: *Salmonella*, vaccine, poultry, swine, DIVA

VA4
Survey of Avian Pathogenic *Escherichia coli* (APEC) in the Asia-Pacific Region

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Avian pathogenic *Escherichia coli* (APEC) is one of the leading pathogens economically affecting the poultry industry. It is commonly associated with the syndromic disease colibacillosis which can manifest itself in many different forms such as airsacculitis, cellulitis, pericarditis, perihepatitis and respiratory distress. Over the years, many virulence-associated genes (VAGs) have been discovered which contribute to this avian disease such as *hlyF*, *iroN*, *iss*, *iutA*, and *ompT*. Many of these VAGs are plasmid encoded and as a result, *E. coli* strains can acquire varying numbers and combinations. To gauge the prevalence of this poultry pathogen, we conducted a survey across the Asia-Pacific region over the span of two years to observe the APEC levels and profiles of selected VAGs. A total of 1,621 broiler gastrointestinal tracts were collected from 11 different countries: Australia, Bangladesh, Brazil, India, Japan, Malaysia, Myanmar, Philippines, Taiwan, Thailand, and Vietnam. Total *E. coli* was enumerated from the small intestine, and up to five colonies from each sample were isolated and cultured for down-stream characterization. A panel of 5 APEC VAGs (listed above) were used to distinguish APEC from non-APEC isolates, with any isolate possessing 2 or more of these VAGs considered to be APEC. Isolates were grouped by the number of VAGs possessed. In total, 9,625 *E. coli* isolates were isolated from all the samples, of which 4,012 (42%) were found to be APEC. APEC levels ranged from <1.0E+02 CFU/g to 1.8E+08 CFU/g. There was a wide range of counts in all countries, but Australia and Malaysia tended to have the lowest APEC level, while India and Taiwan had the highest. India and Taiwan also had the highest ratios of APEC to commensal *E. coli*, while Bangladesh and Australia had the lowest. In conclusion, we observed varying levels and distributions of APEC and the VAGs they carry across regions. Continued monitoring of the prevalence and severity of APEC worldwide, especially as producers continue to reduce antibiotic usage, will help assess the need for alternatives to antibiotics to combat this poultry pathogen.

Keywords: Avian Pathogenic *Escherichia coli*, colibacillosis, virulence-associated genes

VA5
Multi-drug resistance pump gene deletion strains cannot survive in egg white because of ovotransferrin-derived antimicrobial activity, and are safe and effective oral live attenuated vaccines

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Multi-drug resistance (MDR) pumps are of vital importance for microbial survival in a variety of environments as they can export antimicrobial molecules, including host-derived antimicrobial peptides. In this way bacteria can withstand the antimicrobial peptides at mucosal surfaces, and colonization in specific host niches can be achieved. MDR pumps thus constitute a virulence trait as a protection mechanism against innate host defenses. The outer membrane channel TolC, used by Salmonella MDR pumps to export antimicrobial compounds, was shown to be crucial for survival of *Salmonella* in egg white, using deletion mutants and agar spot and egg white survival assays. MDR pump mutants had an identical phenotype, indicating that strategies of *Salmonella* to survive in egg white are likely based on protection against egg white antimicrobial components. The *tolC* gene promoter was shown to be activated by egg white, pointing to an active upregulation of defense mechanisms when encountering harsh environments. Testing of egg white fractions, derived by chromatographic methods, showed that ovotransferrin was the main driver of the antimicrobial activity against the TolC deletion mutant, and not against the wild type. Either the TolC channel thus aids in pumping out siderophores to compete with ovotransferrin for iron, or TolC pumps out ovotransferrin-derived antimicrobial molecules. Triple oral vaccination of layer pullets (day 1, week 6, week 16) with a ΔTolC outer membrane channel mutant or a *ΔacrABacrEFmdtABC* MDR pump mutant was shown to protect against organ colonization and egg contamination by the wild type challenge strain. In the challenge control group, intravenous challenge with *Salmonella* Enteritidis at week 24 resulted in high colonization levels in the gut and internal organs, including the reproductive tract, and in high egg contamination levels, while vaccinated animals had significantly lower challenge strain bacteria in their organs. Egg contamination was completely prevented. These data show that the deletion specific genes, based on knowledge of the pathogenesis of the infection, can generate potential safe and effective vaccine strains for *Salmonella* in poultry.

Keywords: *Salmonella*, vaccines, MDR pumps

VA6
Lipopolysaccharide and lipopolysaccharide modification gene deletions affect TLR-4 mediated inflammatory signals in chicken oviduct cells and are potential safe live vaccines in production animals

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Salmonella Enteritidis is the world's most common cause of salmonellosis in part because it has the ability to colonize the oviduct and contaminate eggs, while *Salmonella* Typhimurium is mainly a contaminant of porc and poultry meat. One of the important aspects of vaccine development is differentiation of infected from vaccinated animals. This can be achieved either by differentiation with regard to the serological response (DIVA concept) or else by the phenotype of the strain on bacteriological media. LPS mutants have been described as potentially being of value as live oral vaccine strains for both poultry and pigs, because they can be attenuated and have defects in TLR-4 signaling and thus induction of inflammation. For chickens, using the *in vivo* expression technology, it was shown that the rfbH gene, involved in lipopolysaccharide O-antigen synthesis, is transcriptionally induced during growth in whole eggs at room temperature. A *S.* Enteritidis ΔrfbH strain was unable to multiply in eggs at room temperature and did not survive in egg white at 42 degrees C. The attenuation was most likely caused by an increased susceptibility of the ΔrfbH mutant to yet undefined antibacterial components of the egg albumen. Knock-outs of specific LPS modification genes, that alter the LPS structure by adding small molecules to the lipid A and LPS core structure, had an egg white survival defect and showed not to activate TLR-4 mediated downstream mediators (IL-1, IL-6, TNF-alpha). These gene deletions can thus be added to the list of potential targets when producing live attenuated vaccines. Gene deletions would also alter serologic responses against the LPS so that vaccinated birds can be differentiated from challenged birds. This was shown for piglets, in which immunization with ΔrfaJ and ΔrfaL mutants resulted in the induction of a serological response lacking detectable antibodies against LPS. The strains protected mice against *Salmonella* Typhimurium infection when orally administered.

Keywords: *Salmonella*, LPS, vaccines

VA7
Eimeria maxima vaccination via *Pichia pastoris* recombinant vector for coccidia protection in broiler chickens

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Coccidiosis remains one of the most devastating protozoal diseases faced by the global poultry industry, and multiple drug options for control are classified as antibiotics, which limits treatment options. Moreover, coccidiosis vaccines are known to contribute markedly to bacterial enteropathies such as necrotic enteritis. Many strategies, including vaccination, are used to control this illness, yet outcomes are variable. Here we describe the application of a novel recombinant vaccine targeting *Eimeria* spp. in *Eimeria maxima* (EM) inoculated broilers. A new *Pichia pastoris* vaccine-vector expressing thrombospondin-related adhesive protein (TRAP) family, rhomboid protease (ROM5) and high mobility group box 1 (HMGB1) protein has been previously developed. In experiment 1, we sought to compare live and killed forms of this vaccine. The comparison involved high (1x107 cell/mL) or low (1x106 cell/mL) doses using either oral or subcutaneous routes. Our previous vaccine with a Bacillus-vectored TRAP-ROM5-HMGB1 showed protection against EM M6 innoculation. Experiment 2 compared the *Bacillus* and *Pichia*-vector efficacy against EM M6 innoculation. In experiment 3, timing and the delivery of the vaccines were compared. However, this vaccine was carrying the zeocin gene as the marker. To evaluate the vaccine when this gene was removed, a fourth experiment was conducted. Results showed that there were no significant differences in body weight gain (BWG) or percent change in BWG (%ChangeBWG) relative to the positive control in experiments 1 or 2. In experiment 3, BWG was significantly higher in chickens that were vaccinated via drinking water at day-of-hatch or at d5 then boosted with the same vaccine via same route at d14. No differences were observed for lesion scores (LS) in any of the experiments. Most importantly, quantification of oocysts per gram (OPG) of feces was significantly lower in all groups vaccinated with a form of the *Pichia*-vectored vaccine especially at the level of accumulative oocysts shedding or oocysts shedding per bird in experiments 2 and 3. In experiment 4, the differences in BWG, %ChangeBWG, lesion scores and OPG were all non-significant. Overall, this approach to vaccination, or augmentation of live oocyst-based vaccines, appears promising.

Keywords: *Eimeria,* recombinant, avirulent, vaccine, *Pichia*

VA8
Evaluation of a Universal Subunit *Eimeria* spp. Vaccine

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Diseases associated with animal production and presently controlled by antibiotics represent a critical area for research and development. Coccidial infections in poultry have long been controlled by ionophores and/or coccidiostats and today these treatments have come under increased scrutiny by regulatory agencies and consumers. Additionally, traditional coccidia vaccines are limited to combining individual strains of attenuated oocysts; individual strains typically provide no cross-protection against other *Eimeria* strains. Therefore, there is a critical need for the development of new technologies to control *Eimeria* spp. Optimally, these new technologies should address and overcome concerns of both cross-protection and the one vaccine strain per species protection model. We have developed a novel vaccine platform, BTVCx, that incorporates a subunit/epitope sequence, common for all *Eimeria* spp. (broad spectrum), into an inactivated orally administered vaccine that protects poultry against coccidiosis by inducing mucosal immunity.BTVCx was evaluated in two separated mixed-*Eimeria* spp. challenge trials at Southern Poultry Research (Athens, GA). For each experiment, 1000 day of hatch chicks (Cobb 500) were randomly assigned to either the control non-treated group (Ctl) or the treated group (n=50/pen 10 replicate pens/group) that received BTVCx (0.2ml/bird/oral gavage) on d2 and 16 of life. On day 28 (exp1) or d21 (exp2), birds were challenged with a combination of *E. acervuline* (EA), *E. maxima* (EM), and *E. tenella* (ET). Six days post challenge, 5 birds/pen birds were sacrificed, group weighed, and coccidial lesion scored according to the Johnson-Reid scale wherein 0 is normal and 1, 2, 3, or 4 indicate increasing severity of infection. On d28 (experiment 1) or d27 (experiment 2), fresh fecal samples were collected from each pen determine the degree of oocysts shedding/cycling. Results showed significant reductions in lesions scores in both experiments 42% for experiment 1 and 45% for experiment 2 (36/39%EA, 43/39%EM, 60/66%ET, respectively) and total oocyst shedding was reduced 42% in experiment 1 and 65% in experiment 2 (40/75%EA, 68/85%EM, 40%ET, respectively). In a third challenge experiment (see experimental design above), BTVCx was compared to a Ctl group and commercial coccidia vaccine group (Vx): Productive parameters (Adjusted Feed Conversion Rate (FCR) and Average Weight Gain (DWG)) were measured throughout the course of the experiment. At the conclusion of the experiment (d42) statistical differences were observed in DWG and FCR when comparing the BTVCx group and the Vx group with the Ctl group; birds receiving BTVCx weighed an average of 87g/bird more than the Ctl group and feed conversion improved by 84 points. These data taken together indicate the potential of BTVCx as an alternative control strategy for coccidiosis.

Keywords: Vaccine, Coccidia, Alternative, *Eimeria*, Poultry

SESSION 2
Microbial-derived products

ORAL PRESENTATIONS

Microbiome for gut health: A modern tool and a target in the effort to address antimicrobial resistance

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The emergence and spread of antimicrobial resistance (AMR) is a global concern, and it has become a major political, social, and economic burden of our time. The use of antimicrobials in livestock agriculture has been a major focus of this issue since it is one of the potential contributing factors causing human AMR infections. Concerning AMR, projections suggest that by 2050, more people will die of bacterial infections than cancer due to the fact that currently available antimicrobials will no longer be as effective in treating bacterial infections. This will not only affect the health outcomes of humans, but it will also affect those of animals, including production yields of food-producing animals. Therefore, control and prevention of AMR require the exigent adoption of a "One Health" approach through the integration of human, animal, and environmental health. Research on AMR in both human and animals has focused mainly on pathogenic bacterial species which are readily cultured in the laboratory. Recent advances in next-generation sequencing of complex microbial communities (microbiomes) improved our understanding of the ecology of AMR in One Health. This cutting-edge technology enables to track the fate of AMR genes. On the other hand, our understanding is growing in the aspect of reciprocal, intimate relationships between microbiome and host immune system that are orchestrated by preceding microbial encounters and prepare the host for future ones. Antimicrobials alter the structure of the microbiota, expand the host-specific pool of AMR genes and bacteria, degrade the protective effects of the microbiota against invasion by pathogens, and may impair vaccine efficacy. Other strategies including manipulation of the gut microbiome to eliminate antimicrobial resistant bacteria or to boost host immune responses to vaccines may prove valuable in addressing antimicrobial resistance. In recent years, manipulation of the microbiome using microbial-derived products (including fecal transplantation) to improve gut health is becoming a promising alternative to antimicrobials in animal agriculture. Fecal microbiota transplant (FMT) has shown effectiveness in treating certain human diseases such as *Clostridium difficile* infection, however, the fundamental science behind the application of FMT is still not yet fully understood. With this notion, the application of FMT in livestock agriculture should be cautious and more research efforts are needed. Understanding the role of gut health in achieving optimal production is of essential to discover the most reliable and sustainable alternatives to replace antimicrobial compounds used in livestock.

Keywords: Microbiome, Gut Health, Antimicrobial Resistance

Non antibiotic strategies to modify the microbial population of dairy cattle: impacts on milk production, animal health, and food safety

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In the United States, the dairy industry includes approximately 9 million cattle that produce on average 19,000 pounds of milk a year, and comprise 50% of the ground beef supply. Cattle are ruminant animals that depend on a symbiotic relationship with the microbial population of their gastrointestinal tract to convert forage and grain to high quality meat and milk. The gastrointestinal microbial population of dairy cattle is extremely dense and diverse, and is a complex natural ecosystem that can be utilized to improve animal production efficiency, sustainability, animal and human health, as well as food safety. For example, a decreased ruminal microbiome diversity and increased lactate utilizing bacterial populations in beef and dairy cattle have been linked with increased milk production efficiency. While antibiotics have been used for many years to shift microbial populations to increase production efficiency, the mode of action of antibiotics on the gastrointestinal microbiome and host animal physiology (both positive and negative) remain largely unknown and unreplaceable. Non-antibiotic strategies have been devised to modify the microbial population of dairy cattle on the farm. A large number of approaches, including management practices, dietary changes, organic acid inclusion, probiotic and prebiotic feed additives, and vaccination have been widely used worldwide in the dairy industry to enhance milk production and feed efficiency, as well as to improve animal health. Many of these strategies rely upon harnessing the natural competitive nature of bacteria and specific microbial ecological factors to eliminate pathogens that negatively impact animal production, health, or food safety which may have unintended consequences of which we need to be aware. In this presentation we explore the ecology behind the efficacy of alternatives to antibiotics and how they may impact dairy production efficiency and can be used to improve both human and animal health.

Keywords: antibiotic alternatives, prebiotics, organic acids, microbiome, animal efficiency

Microbiological quality and possible role as a source of antimicrobial resistance genes of commercial probiotic products for livestock and aquatic animals

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The reduction of antimicrobial use is one of the most important action to combat antimicrobial resistance (AMR) crisis. Probiotic products are among alternatives to antibiotics that have been increasingly used in food animal production. Therefore, this study aims to determine the microbiological quality and the presence of resistance genes in some probiotics available for livestock and aquatic animals in Thailand. Nine commercial-probiotic products for livestock and aquatic animals were examined for the number of probiotic bacterial strains as indicated on the product labels. Confirmation of species was performed by multiplex PCR for *Lactobacillus* and *Enterococcus* and Amplified Ribosomal DNA Restriction Analysis (ARDRA) for *Bacillus*. The contamination of pathogenic bacteria (i.e. *Escherichia coli* and *Salmonella enterica*) and the presence of 56 genes that encode resistance to clinically-important antibiotics were determined. The results showed that none of the products tested were contaminated with *E. coli* and *Salmonella*. Inaccurate labelling in either numbers or species of bacteria was a common issue among the probiotic products tested. Some products did not contain the species as claimed on the label. *B. licheniformis* and *B. sphaericus* were commonly misidentified as *B. subtilis*. Of seven products claimed to contain *B. subtilis*, six were found to contain *B. subtilis* cluster consisting *B. pumilus*, *B. amyloliquefaciens* and *B. atropheus* and one was positive to *B. licheniformis*. Contamination of *L. rhamnosus* and *L. casei*-group was frequently found. *E. faecium* was mislabeled as *Streptococcus faecium*. Resistance genes encoding resistance to sulphonamides, streptomycin and tetracycline were observed in three products. One product contained both *sul1* and *aadA2* and one carried *tetA* and *tetM*. The study is currently being undertaken to find horizontal transfer of genes in probiotic samples. While the use of probiotics in food animals may generate beneficial effects, it can also pose risks as a source of resistance genes. The results highlight the need to regulate the production and the use of probiotics to assure their quality and reduce their potential contribution to the spread of AMR.

Swine-derived probiotic *L. Plantarum* modulates porcine intestinal endogenous HDP synthesis

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Weaning stress renders piglets susceptible to pathogen infection, which leads to post-weaning diarrhea, a severe condition characterized by heavy diarrhea and mortality in piglets. Probiotics exert beneficial health effects, mainly by reinforcing the intestinal barrier function and modulating the gut microbiota. However, the mechanisms of action, and especially, the specific immunomodulatory effects of probiotics on porcine have not yet been elucidated. Host defense peptides (HDPs) have antimicrobial as well as immunoregulatory activities and are involved in epithelial innate immune defense. Dietary modulation of endogenous HDP synthesis is an effective way to boost the host innate immune system. This study aimed to investigate the role of the swine derived probiotic *Lactobacillus plantarum* strain ZLP001 in porcine HDP induction and the underlying mechanism. To this end, we evaluated the stimulatory effect of *L. plantarum* ZLP001 on HDP expression in piglet intestinal tissue *in vivo* and porcine IPEC-J2 cells and 3D4/31 cells *in vitro*, and we examined the underlying intracellular signaling pathway in IPEC-J2 cells. Quantitative real time polymerase chain reaction (qPCR) analysis showed that *L. plantarum* ZLP001 treatment increased the mRNA expression of jejunal and ileal HDPs in weaned piglets. In IPEC-J2 and 3D4/31 cells, *L. plantarum* ZLP001 stimulated HDP expression, but different HDP induction patterns were observed, with the various HDPs exhibiting different relative mRNA levels in each cell line. In addition, *L. plantarum* ZLP001 induced HDP secretion, which enhanced the potential antimicrobial activity of IPEC-J2 cell-culture supernatant after incubation with *L. plantarum* ZLP001. *L. plantarum* ZLP001 induced porcine HDP expression through TLR2 recognition as evidenced by the fact that HDP expression was suppressed in TLR2-knockdown IPEC-J2 cells. Further, we found that *L. plantarum* ZLP001 activated the extracellular signal-regulated kinase (ERK)1/2 and c-jun N-terminal kinase (JNK) signaling pathways, as indicated by enhanced phosphorylation of both ERK1/2 and JNK and the fact that HDP expression was suppressed upon inhibition of ERK1/2 and JNK. Furthermore, *L. plantarum* ZLP001 activated c-fos and c-jun transcription factor phosphorylation and activity. We conclude that *L. plantarum* ZLP001 induces porcine HDP expression *in vivo* and in *vitro*, and the induction seems to be regulated via TLR2 as well as the ERK1/2/JNK and c-jun/c-fos signaling pathways. Modulation of endogenous HDPs mediated by *L. plantarum* ZLP001 might be a promising approach to improving intestinal health and enhancing diarrhea resistance in weaning piglets.

Keywords: *Lactobacillus plantarum* ZLP001, host defense peptide, weaning piglet, induction effect, underling mechanism

Small molecular weight metabolites regulating growth and immunity as postbiotic antibiotic alternatives

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Mounting regulatory pressure, consumer concerns about antibiotic resistant bacteria, and competition from alternative protein sources have prompted the poultry producers to search for novel antibiotic alternatives. Recently, *Bacillus* spp. as a direct fed microbial (DFM) is gaining in popularity as an antibiotic alternative. A metabolomic approach was used to characterize and identify host- and microbiome-derived biochemical compounds in the ileal content of broiler chickens which were fed dietary *Bacillus subtilis* as DFM. Fourteen-day-old broiler chickens (n = 196) were fed a basal diet or a diet supplemented with *Bacillus subtilis* 1781 or 747 as DFM. The chickens and the amount of feed that the broiler chickens consumed were weighed at 21 days of age for growth performance measurement. Eight chickens per group were euthanized and their ileal content harvested for metabolomic profiling. From 14 to 21 day of age, body weight gains of chickens fed diets supplemented *Bacillus subtilis* 1781 and 747 were significantly increased compared to those of chickens fed basal diet. Compared with unsupplemented controls, the levels of 83 biochemicals were significantly altered (25 increased, 58 decreased) in chickens given the *Bacillus subtilis* 1781 DFM-supplemented diet, while 50 were significantly altered (12 increased, 38 decreased) with the *Bacillus subtilis* 747 DFM-supplemented diet. The changes in the levels of intestinal biochemicals provided a distinctive biochemical signature unique to each *Bacillus subtilis*-supplemented group. These results provide the framework for future studies to identify natural chemical compounds that can be used for improving poultry growth performance.

Keywords: antibiotic alternative, *Bacillus subtilis*, gut metabolite

Managing the gut microbial populations: From science to practice

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Colonization and succession of the gastrointestinal microbiota in avian species is not well characterized; however as in other systems, it is recognized that the microbiota influences the health and production efficiency of poultry. Numerous studies have revealed that the gut microbiota of commercial poultry flocks is highly variable within and between flocks. We believe this is influenced by the initial colonizers of the gastrointestinal tract, as the small intestinal microbiota between producers differ significantly in poults and chicks at day-of-hatch. Our data suggests that horizontal transmission from the environment is greater than vertical transmission in poultry production due to common commercial management practices. We have shown that successional changes in the small intestinal microbiota start *in ovo* and are affected by management practices such as disinfection of the setter and the hatcher as well as administration of antibiotics and/or vaccines *in ovo* leading to hatchery-specific bacterial populations. The early establishment of lactic acid bacteria essential for stabilizing intestinal homeostasis, digestion and nutrient absorption, and nurturing mucosal conditions for immunological protection is disrupted in commercial birds. In addition, our research has documented significant populations of avian pathogenic *Escherichia coli* (APEC) in broiler chicks at the day of hatch. APEC is a causative agent for colibacillosis in birds in the form of airsacculitis, cellulitis, pericarditis, or perihepatitis. A high population of APEC can disrupt gastrointestinal homeostatis in the young bird and impact early growth and performance. If left unchecked, these isolates can translocate to the blood stream and cause colibacillosis.

In order to, promote colonization by beneficial bacteria and reduce the levels of APEC, without the use of antibiotics, a probiotic was developed comprised of two lactic acid bacteria selected for their immunomodulatory capabilities and two *Bacillus* strains that produced secondary metabolites inhibitory to APEC strains. This probiotic was administered to chicks in a single dose at the hatchery. A study comparing the probiotic against the antibiotic gentamycin, indicated that both the probiotic and the antibiotic reduced APEC levels in two-week old broilers compared to untreated birds. A second study showed a reduction in levels of APEC at day 7 as well as more uniform flocks as the coefficient of variability at harvest was reduced. The probiotic is therefore an effective alternative for antibiotics to establish a healthy microbiota and control APEC when applied at the hatchery.

Responses of *Bacillus amyloliquefaciens* CECT 5940 supplementation in weaned pig diets

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At weaning, piglets suffer from immunological, environmental, nutritional and social stress, consequently affecting the gut health and growth performance. Supplementation of probiotics is considered as one of the strategies to maintain the intestinal health and minimize the negative effects of the weaning stress in piglets. Probiotic, *Bacillus amyloliquefaciens* CECT 5940 is a natural spore - forming bacteria, which supports gut health and improves growth performance in poultry. However, there are limited studies conducted to delineate effects of dietary supplementation of probiotics *B. amyloliquefaciens* CECT 5940 in piglets. A study was therefore conducted to evaluate the effects of probiotic (*B. amyloliquefaciens* CECT 5940) supplementation on growth performance, plasma urea nitrogen and fecal microbiota in weaned piglets. A total of 180 mixed-sex pigs weaned at 28 d were randomly distributed to 3 dietary treatments consisting 6 replicate pens (10 pigs per replicate pens). The duration of the study was 62 d, which included pre-starter (28 to 60 d; 7 to 18 kg BW) and starter (61 to 90 d; 18 to 35 kg BW) phases. All the diets were corn-soybean meal-based and pigs were provided *ad libitum* access to feed and water throughout the study. The dietary treatments were i) control (without AGP and probiotic), ii) supplemented with AGP (Colistin sulphate at the inclusion of 20 ppm/kg of feed), iii) supplemented with probiotic (*B. amyloliquefaciens* CECT 5940 @ $1x10^9$ cfu per kg of feed). Data were analyzed as completely randomized design and each pen was considered as an experimental unit. Data on growth performance showed that during starter and overall periods, piglets fed probiotics had better (P<0.05) ADG compared with those fed AGP (481 g versus 468 g). The overall feed efficiency of piglets fed probiotics was improved (P<0.05) compared with AGP supplemented group (2.02 versus 2.07). The final BW of probiotics group (37.54 kg) was higher than AGP (36.75 kg) and control group (36.20 kg) pigs. There were no significant differences in plasma urea-nitrogen levels and microbial profiles between the dietary treatments. In conclusion, supplementation of probiotics (*B. amyloliquefaciens* CECT 5940) had better growth performance than AGP supplemented pigs, which implies that probiotics could be beneficial in improving performance of young pigs fed AGP-free diets.

Keywords: AGP, probiotics, piglets, performance

Fermentate Bioactives Impact on SARA and a Mastitis *Stretoccocus uberis* Challenge to Reduce AIF Use in Bovines

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The "normal" process for evaluating interventions for prevention of syndromes impacting production animals is to develop a molecule specific against the disorder. This is followed by controlled trials and finally large epidemiologic surveillance data to support widespread adoption. This presentation highlights the use of epidemiologic surveillance data to reverse engineer direct controlled trials. Following the controlled trials, proof of concept trials was designed and conducted to illustrate the impact of unique fermentate bioactives against sub-acute ruminal acidosis (SARA) and mastitis.

Data will be presented in two proofs of concept trials demonstrating the potential for reducing anti-infectives (AIF) used to prevent: 1. SARA, and 2. Bovine Mastitis.

1. Study: A 5x5 Latin square study was designed in which cannulated steers were fitted with indwelling pH monitors and placed on a high grain diet. Control animals were fed the same diet and received tylosin and rumensin whereas the treated animals received the high grain diet and only *Saccharomyces* bioactives. A negative control treatment received the high grain diet only. Results: The steers in the antibiotic and negative control treatments spent significantly more time below the SARA (< 5.6 pH for 180 minutes) threshold. The fermentate bioactive prevented induction of SARA and reduced the amount of rumen LPS.
2. Study: 20 multiparous Holsteins were randomized into a treatment and control group. A direct challenge of *Streptococcus uberis* was administered through the teat canal and data captured. Results: The treatment group demonstrated improved resistance within the mammary gland to mastitic events via: 1. Improved local, but not systemic, immune cell function; 2. Increased tissue response to the pathogen; 3. A priming effect on body tissues via HSPs (Heat Shock Proteins) activation; and 4. Gene up-regulation of key innate immune system compounds/cells.

Taken together, these studies demonstrate favorable shift in microbiome and up-regulation of the innate immune system as a result of the feeding of these novel bioactives, will reduce the dependence and need for AIF in bovine production settings.

Keywords: microbiome, fermentate bioactive, gene up-regulation, SARA, *Saccharomyces*

SESSION 2
Microbial-derived products

POSTER PRESENTATIONS

MI1
Effect of *Bacillus* spp. probiotic supplementation on performance, immune response and gut health of broilers challenged with *Salmonella* Enteritidis

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Inclusion of antibiotics in poultry diet as a growth promoters (AGPs) can reduce the prevalence of enteric pathogen such as *Salmonella* spp. With the emergence and dissemination of antibiotic resistance *Salmonella*, increasing consumer demand on use of antibiotics as a feed additive in U.S. Therefore, search for alternative strategies to replace antibiotics as a feed additive has gained interest in animal agriculture. In addition, continuing circulation of multiple serovars of *Salmonella* in poultry flocks, along with increasing reports of human Salmonellosis, warrants the necessity of developing control methods to decrease *Salmonella* load in poultry production. *Bacillus subtilis* and *Bacillus licheniformis* are potential probiotics and are currently used as a probiotic in poultry production. The overall objective in this proposal is to determine the effects of *B. subtilis* and *B. licheniformis* probiotic supplementation on performance, cecal *Salmonella* load, immune response, and gut morphology in broilers challenged with *Salmonella enterica* serovar Enteritidis.

A total of 360 one-day-old broiler chicks were randomly distributed to four experimental groups a 2 X 2 factorial set up of treatments; Control, Control + Challenge, Probiotics (10 mg of *B. Subtilis* strain HU58/kg of feed; HU58™ plus 100 mg of *B. licheniformis* SC307; Prepro™/kg of feed; Microbiome LABS, Saint Augustine, FL), and Probiotics + Challenge. Each treatment was replicated in 6 pens (n=6) with 15 chicks per pen. At 21 d of age, all birds in Challenge groups were inoculated orally with 250 µl of 1 X 10^9 CFU *S.* Enteritidis.

At 21 d post-*Salmonella* challenge, chickens challenged with *Salmonella* had the 11% lower ($P < 0.05$) BW gain compared to the control non- challenge groups. Chickens that were supplemented with probiotics in the *Salmonella* challenged groups had only 5.1% reduction in BW compared to the control group. At 5, 12, and 21 d post-*Salmonella* infection, chickens challenged with *Salmonella* had 1.99, 1.93 and 1.71 log Salmonella CFU/g of cecal contents while chickens supplemented with probiotics and challenged with *Salmonella* had 0.73, 1.59, and 1.32 log lower *Salmonella* CFU/g of cecal contents respectively. Chickens supplemented with probiotics and challenged with *Salmonella* had higher ($P < 0.05$) anti-*Salmonella* IgA compared to the control birds with *Salmonella* infection. At 21d post-*Salmonella* infection, chickens supplemented with probiotics and challenged with *Salmonella* had comparable villi height compared to the control non-challenge group, while control birds infected with *Salmonella* had the shortest villi height ($P < 0.05$). Increased villi height and crypt depth can improve nutrient digestibility and absorption and may explain the improved production performances in probiotic supplemented birds. It can be concluded that *B. subtilis* and *B. licheniformis* probiotic can be a tool to decrease *Salmonella* loads in the broiler intestine and *B. subtilis* and *B. licheniformis* supplementation can be expected to decrease broiler carcass contamination with *Salmonella*.

Keywords: *Bacillus* spp. probiotic, supplementation, *Salmonella* challenge, immune response, broiler chickens

MI2

Efficacy of Synbiotics to promote gut integrity and reduce *Salmonella* colonization in broilers

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The aim of this experiment was to study the efficacy of Synbiotics (a combination of *Saccharomyces cerevisiae, Bacillus subtilis, B. licheniformis, Lactobacillus acidiophilus, L. plantarum, L. lactis, Streptococcus thermophillus* and Fructooligosaccharides) to promote gut integrity and reduce *Salmonella* colonization. Two hundred and forty, one-day-old, female chicks were divided into 4 groups of 60 each. Each group was divided into 3 replicates of 20 each. Birds in-group 1-3 were served as treatment groups. Birds in-group 4 was a positive control group. Birds in-group 1 were provided Synbiotics 2 ml/L drinking water (DW) skip a day from 1-42 days of age (6 hr/day). Birds in-group 2 were provided synbiotics 2 ml/L DW for 3 consecutive days (6 hr/day) in each week. Birds in-group 3 were provided Synbiotics 1 ml/L DW every day from 1-42 days of age (6 hr/day). Birds in-group 4 were provided fresh water ad lib. At 21 and 42 day-old, three birds in each replicates were euthanized to collect intestine for histopathology analysis, and also collect rectal content to test for butyric acid activity. Body weight and feed conversion ratio was calculated at 21 and 42 day-old. At 21 day-old, all birds were challenged with 1ml of *Salmonella* Enteritidis Nalidixic acid resistance strain (108 cfu/ml). At 21 and 28 day-old, birds were cloacal swabbed for testing for *Salmonella* colonization. The results revealed that at 21 and 42-day-old birds in treatment groups showed better villi height and crypt depth than those birds in positive control group especially in the duodenum. At 21-day-old, birds in group 1 revealed significantly higher duodenum villi height and crypt depth (910.75±416.39; 77.78±48.70) than those of birds in group 4 (709.05±93.86; 39.52±9.74) ($P<0.05$). In addition, at 21-day-old, butyric acid activity of birds in treatment groups 1 (4.28±2.50), 2 (3.04±2.70) and 3 (4.07±2.20), showed significantly higher than birds in group 4 (1.91±1.51) ($P<0.05$). At 42-day-old, average body weight (kg) and FCR of birds in-group 1 (2.08±0.34; 2.27±0.09), 2 (2.17±0.15; 2.14±0.03), 3 (2.04±0.36; 2.23±0.06) showed better than birds in-group 4 (1.99±0.42; 2.37±0.18). At 21 day-old (before challenge), no *S.* Enteritis Nalidixic acid resistant strain was found in any group. However, at 28 day-old, *S.* Enteritis Nalidixic acid resistant strains were found lower in treatment group 1 (78%), 2 (78%), 3 (68%) than the positive control group 4 (100%). In conclusion, Synbiotics promoted the chicken performance and gut morphology and butyric acid activities.

Keywords: butyric acid activity, gut integrity, synbiotics, *Salmonella* reduction

MI3
Five years dynamic of *Salmonella enterica* in commercial poultry farms with and without probiotics application

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Probiotics application in commercial livestock serves for several needs such as improve production performance, promote animal health and support food safety in animal production. Numerous studies indicated the effect of probiotics on *Salmonella* reduction in chicken but most of publications imply only in experimental study scale. The insufficient information on real farm situation poses *Salmonella* control by probiotics use in commercial farms still obvious. To answer this question, we analyzed the data collection harbored from an ISO17025: 2005 service laboratory in Thailand, scope on probiotics application and *Salmonella* occurrence.

Data were analyzed from the collection of samples submitted from 4 commercial chicken breeder farms. Each farm submitted samples continuously for at least 5 consecutive years. In order to compare effect probiotics on *Salmonella* occurrence, 2 sets of data were generated in comparison of farms probiotics usage status; set 1) Early stage of probiotics application (EPA) farm and Continuous probiotics application (CPA1) farm (2007 - 2011), set 2) Continuous probiotics application (CPA-2) farm and non-probiotics application (NPA) farm (2014 - 2018).

A total of 902 samples were included in data set 1 and 4,222 samples in data set 2. To reduce the bias of data, environmental samples were excluded, only fecal samples (CPA 1, CPA-2 and EPA) and boot swab samples (NPA) were processed in the analysis. In data set 1, the percentage of *Salmonella* positive sample in EPA farm gradually decreased after the onset of the probiotics (average 34.18% to 18.82%). While the average percentage of positive sample during the identical 5 years in CPA-1 farm was 1.01% and the variation of positive sample is between minimum 0% to maximum 2.46%. In data set 2, the 5-years average occurrence in CPA-2 farm was 3.70% (minimum 2.40%, maximum 5.68%) and 5.33% (minimum 2.75%, maximum 8.66%) in NPA farm. Serovar information of samples also included in the analysis. The most present serovar was changed year by year and different between farms.

In conclusion, the information from data set 1 and 2 suggested that application of probiotics possible to reduce the *Salmonella* occurrence in commercial breeder farm and the continuous application of probiotics could stabilize the farm *Salmonella* status.

However, according to the data was obtained from the commercial laboratory service, multiple limitations are noted. The data in this study could not represent the chicken population in the farm since it was obtained from customer submission samples. The variation between the type of sample from each farm was depended on farm's *Salmonella* monitoring and management. This information may be affected by other *Salmonella* control procedures such as vaccination and farm management during the period of analysis.bioactives against sub-acute ruminal acidosis (SARA) and mastitis.

Keywords: probiotics, *Salmonella enterica*, commercial poultry farm

MI4
Fermented feed stuff increased orexin level associated with increased food intake and weight gain in weaning pigs

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Weaning exposes pigs to various stress factors, leading to growth retardation due to low feed intake, as well as disorders of gastrointestinal function. Fermentation of feed can be beneficial from a health standpoint, especially in the early stages of the pig lives. This study was conducted to investigate the effects of fermented feed stuff on the performance and gastrointestinal hormones involved in feed intake and growth in weaning pigs. A total of 320 Duroc × Landrace × Yorkshire weaning pigs (28 ± 2 d of age with body weight (BW) of 7.38 ± 0.24 kg) were divided into unfermented diet group (UFD) and fermented diet group (FD). Each group consisted of 8 replicates (pens), with 20 piglets per pen. The pigs from the UFD group were fed a basal diet, while the piglets from the FD group were fed a basal diet with 5% feed stuff, which was replaced by the fermented feed stuff for 21 days. Blood samples and tissue samples from the stomach, jejunum and ileum were obtained from six pigs from each group on day 10 of the trial for further analysis.

Pigs fed the fermented diet had higher average daily feed intake (ADFI) and average daily gain (ADG) during the first week, last 2-weeks, and over the entire 3-week period compared with pigs fed unfermented diet. Moreover, feed conversion was only improved by fermentation during the last 2-weeks (P<0.05). Pigs fed fermented diet had a higher serum orexin level and up-regulation in the expression of the prepro-orexin (PPOX) gene in the gastric fundus, jejunum and ileum mucosa (P<0.01), and the expression of IGF-1 (P<0.05) and IGFR (P<0.01) gene in jejunum was compared with pigs fed an unfermented diet. Results indicated that dietary supplementation with fermented feed stuff improved growth performance of weaning pigs by increased orexin, IGF-1 and IGFR levels.

Keywords: Fermented feed stuff, piglet, growth performance, orexin, IGF-1

MI5
Feeding pellets inoculated with *B. Amyloliquefaciens* strain H57 improves production parameters in sheep

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The live export of sheep is important to the Australian economy. There is a continuing need to improve the productivity and health of these sheep, and probiotics may be beneficial in this[1]. Previous reports showed that feed intake, feed conversion, live weight (LW) and body condition score (BCS) all improved when sheep were fed a diet containing probiotic *Bacillus amyloliquefaciens* strain H57 ('H57')[2]. Our study investigated the production responses when H57 is included in high-fibre pellets commonly fed to young sheep during live export.

Merino wethers (<1 year old, 35.1 kg LW & BCS 2.2) were fed high-fibre, 'export' pellets (control sheep, n=18), or the pellets inoculated with H57 (H57 sheep, n=18), for 28 days, followed by 14 days where all were fed the control pellets only. Pellet intake (PI) was recorded daily, with LW and BCS measured weekly, and feed conversion ratio (FCR) calculated weekly as PI/LW change. Samples of blood and rumen fluid were collected for analysis every 14 days. Data were analysed by a one-way ANOVA with repeated measures and significance at $P< 0.05$.

After 28 days in treatment, H57 sheep gained 25% more LW than control sheep (6.0 vs 4.8 kg). Compared to control sheep, H57 sheep were also of markedly higher BCS (2.94 vs 2.44), and although they consumed 6.9% more pellets (1429 vs 1337 g/day), they had a better FCR (6.8:1 vs 8.4:1). The H57 sheep also had higher concentrations of acetate and total volatile fatty acids (VFA) in rumen fluid than control sheep. All blood metabolites, leptin and insulin levels were not different and within the expected range for healthy sheep. Some of the responses persisted in H57 sheep even after the treatment, and over the 14 days when they were fed pellets without H57.

The marked differences in the production parameters measured are consistent with other studies[1]. While the energy-yielding products of rumen fermentation (VFA and acetate) were improved in H57 sheep, positive responses may have also occurred in the intestines of these sheep, all resulting in increases in tissue deposition measured as changes in LW and BCS.

In conclusion, young Merino wethers fed high-fibre, 'export' pellets inoculated with H57 ate more pellets, grew faster and converted the pellets into LW and body condition more efficiently than peers fed the same pellets without H57. The marked improvement in these production parameters could be expected to bode well for young Merino sheep prior to and during live shipment. The mechanisms by which these production responses occurred remain to be elucidated.

Keywords: probiotic, H57, *Bacillus*, sheep, export

MI6
Effect of microbial-derived and acid based feed additives on the antibiotic resistome in broilers

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Use of antibiotics results in the emergence of antibiotic resistance, which is a cause of global concern for human and animal health. The extent to which antibiotic resistance is associated with the use of chemical and biological agents used for the expressed purpose to control, deter, inhibit or kill harmful microorganisms is poorly understood, according to FAO, 2018. Three studies conducted in Thailand, USA and Austria evaluate the effect of antibiotics, probiotics and an acid-based feed additive on the prevalence of antibiotic-resistant bacterial population in broilers.

Study Thailand: Shotgun metagenomics was used to evaluate the effect of an antibiotic compound as well as a microbial derived product on the broiler caecal microbiota (microbiome), virulence factor abundance (virulome) as well as the antibiotic resistance genes (resistome). Taxonomical analysis revealed the positive influence of the product on the chicken gut microbiota, indicated by significantly increasing the abundance of the healthy microbiota, such as short chain fatty acid (SCFA) producing bacteria and by reducing the population of potentially pathogenic microorganisms (*Escherichia coli*, *Salmonella enterica*, *Campylobacter jejuni*, *Eimeria* spp., etc. This resulted in a significantly lower abundance of bacterial antibiotic resistance genes and virulence factors in the caecal microbiome of the chickens.

Study USA: The aim of this study was to evaluate the effect of a microbial derived product tested in the study in Thailand, an organic acid-based feed additive and ampicillin on the prevalence of antibiotic-resistant *E. coli* in the ceca of broilers. Administration of ampicillin in broilers for five days led to a significant increase in the abundance of *E. coli* strains resistant to ampicillin, amoxicillin-clavulanic acid, cefoxitin, and ceftriaxone. The effects of the microbial derived and acid-based feed supplementation on the prevalence of resistant *E. coli* are demonstrated by the significantly lower ceftriaxone minimal inhibitory concentration (MIC) values for this group than for the antibiotic group. Additionally, the group received microbial derived product exhibited lower MIC values than the ampicillin group.

Study Austria: The aim of this study was to evaluate the effect of an acids-based feed additive, as well as fluoroquinolone antibiotics, on the prevalence of antibiotic resistant *E. coli*. Treatment of broilers with enrofloxacin significanlty increased the number of *E. coli* resistant to ciprofloxacin, streptomycin, sulfamethoxazole and tetracycline; it also decreased the number of *E. coli* resistant to cefotaxime and extended spectrum beta-lactamase-(ESBL) producing *E. coli* in the ceca of broilers. The supplementation of feed with organic acids based product did not contribute to an increase of antibiotic resistant *E. coli*. The oposite was observed: significant decrease in *E. coli* resistant to ampicillin and tetracycline compared to the control and antibiotic group. The reason for such a decrease needs to be investigated further.
In summary, the findings from this experiment provided more evidence on the potential of microbial derived and organic acid-based feed additives as safe antibiotics' alternative in poultry farming.

Keywords: resistance, antibiotics, probiotics, acidifiers, microbials

MI7
Evaluation of a water applied biopromoter and feed administered MOS as antibiotic alternatives in Breeders and Broilers

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As the poultry industry discontinues the use of antibiotics, effective use of alternative strategies such as prebiotics are being investigated. The primary problems historically addressed by antibiotic use in the poultry industry are key parameters used to determine the effect of antibiotic alternatives. A measure of efficacy in broilers or meat-producing birds is growth promotion; where as in longer-lived, broiler breeder birds, disease prevention and egg production are important. A key benefit of antibiotics was the broad spectrum use in birds of different ages and purposes to improve bird production. In this study, we evaluate the effect of a multi-pronged prebiotic approach using a water additive biopromoter composed of inactivated fermented *Bacillus subtilis* fragments and yeast cell wall extracts, in addition to, a feed additive mannan oligosaccharide (MOS) composed of betaglucans from yeast cell wall extracts on a mineral carrier. Both of these prebiotics have demonstrated immunomodulatory capabilities, and we hypothesize that the combination will improve the production of both broilers and breeders as the use of antibiotics becomes less of an option.

A series of two trials were done to evaluate the effect of the biopromoter and MOS in both broilers and broiler breeders. A randomized controlled broiler study consisted of 215 broilers per treatment group raised in floor pens. The broiler trial compared birds administered a commercial diet, a diet with 0.5 kg/ton antibiotic (BMD) or a diet of MOS at 2kg/ton and biopromoter in the water at 0.2 mL/bird on days 3 and 17 of life. The broiler breeder study compared two 20,000 bird commercial houses of broiler breeders with a known farm history of colibacillosis. On this farm, one house was a control and received no treatment, and the second house received MOS at 2 kg/ton continuously during weeks 25-34 of life and 0.2 mL/bird biopromoter in the water weeks 24 and 26 of life.

In the broiler trial, MOS and biopromoter treated birds (2,388 g) had a 42-day body weight greater than untreated (2,243 g) or BMD treated (2,295 g) birds. Feed conversion ratio was also improved in broilers treated with MOS and biopromoter at 1.585 when compared to both untreated and BMD treated birds, 1.744 and 1.704, respectively.

Broiler breeders not administered the prebiotic combination experienced a spike in mortality and decreased production diagnostically associated with colibacillosis. MOS and biopromoter treated broiler breeders remained healthy and had an average egg production rate 23% higher than the control flock. Peak egg production was 84.48% in the treated flock and 67.03% in the control flock. The improvement in health, egg production and peak resulted in the treated flock producing a total of 899,230 eggs, which was 1.8X the total number of eggs produced by the control flock (497,425 eggs).

In conclusion, using a feed administered MOS product and a water applied biopromoter improved the beneficial production parameters of both broiler and broiler breeder production.

Keywords: mannan oligosaccharide, *Bacillus subtilis*, prebiotics, broilers, broiler breeders

MI8
Supplemental *Bacillus subtilis* DSM 32315 modulates intestinal structure, microbial composition and improve the performance in broiler chickens

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Probiotics offer one alternative to antibiotic growth promoters as they have been shown to improve the development and maintenance of a stable gut microbiome in poultry, which leads to reduced enteric disease and improved growth performance. Therefore, the objective of this study was to delineate the effects of *Bacillus subtilis* DSM 32315 (*BS* DSM32315) on intestinal morphology, microbial composition and performance in broiler chickens. A total of 240 Arbor Acre (AA) male birds were randomly assigned to 2 dietary treatments with 10 pens of 12 birds per pen. The dietary treatments included a basal diet (Control), a basal diet supplemented with 500g/MT of *BS* DSM32315, 1.0x10^6 CFU/g feed). Corn-soybean meal based basal diets were formulated on the recommendation which is normally used in china for starter (day 1-14), grower (day 15-28) and finisher (day 29-42) phases. Water and pelleted feed were provided ad libitum. Supplemental *BS* DSM32315 significantly increased (P<0.05) body weight, average daily gain, and feed intake of broilers at 28 and 42 d of age. Intestinal lesion scores were significantly reduced (P<0.05) in birds fed *BS* DSM32315, while there was tendency for higher (P=0.077) expression of Ileal tumor necrosis factor (TNF)-α. In the cecum, PCA plot defined groups from control and treatment groups occupied distinct positions. Birds supplemented with *BS* DSM32315 had higher abundance of *Firmicutes* and lower abundance of *Bacteroidetes* which also increased the abundances of *Christensenellaceae* and *Caulobacteraceae*, and simultaneously reduced the abundances of potentially harmful bacteria such as *Vampirovibrio*, *Escherichia/Shigella* and *Parabacteroides*. The villus height (VH) and VH to crypt depth ratio of ileum was signifcantly higher (P<0.05) in treatment group relative to control. *Clostridiales* and *Bacteroidales* accounted for the largest proportion in the community, which were respectively increased and decreased in treatment group as compared to control. Within *Clostridiales*, the majority belonged to the *Ruminococcaceae* and *Lachnospiraceae* families. Functional comparison based on KEGG orthologue groups demonstrated a decreasing trend (P<0.10) in the enrichment of the pathways for enzyme families, metabolism of cofactors and vitamins in birds fed *BS* DSM32315. Overall, supplementation of supplemental *B. subtilis* DSM 32315 altered microbial composition and intestinal morphology, thus improving the growth performance.

Keywords: *Bacillus subtilis* DSM32315, intestinal morphology, gut microbiome, performance

MI9
Bacillus subtilis DSM 32315 alters immunity, nutrient transporters and cecal microbiome of broiler chickens under necrotic enteritis challenge

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Poultry production programmes require elimination or reduction of the use of in-feed antibiotics growth promoters (AGP). This has led to an increase in the occurrence of necrotic enteritis, making it an economically significant poultry disease requiring an alternative nutritional interventions. Probiotics offer one alternative to AGP because they can elicit specific actions that promotes the development and maintenance of a stable gut microbiome, leading to a reduction in enteric disease and consequently improved performance. This study examined the effects of *Bacillus subtilis* DSM32315 (*BS* DSM32315) probiotic and antibiotic enramycin in broiler chickens challenged with pathogenic strains of *C. perfringens* on cecal microbial populations, functional diversity, nutrients transporters and mRNA expression of cytokines. Day-old Arbor Acre broiler chickens (n=360) were randomly assigned to three dietary groups; control, basal diet fed-group only; antibiotic, basal diet + enramycin 5mg/kg; and probiotic group, Basal diet + *BS* DSM32315, $1x10^6$ CFU/g of feed. Antibiotic and probiotic groups were challenged with *C. perfringens* at d 1 and from d 14 to d 21. Birds supplemented with enramycin and *BS* DSM32315 significantly ($P<0.05$) increased the species richness and the abundance of bacteroidetes by 6.8% at 35 days. Absolute qPCR method showed that at 14, 21 and 35 days of age, the bacterial abundance of *B. bifidum*, *Enterobacter*, and *L. salivarius* were significantly higher ($P<0.05$) in enramycin and *BS* DSM32315 group, while relative abundance of *E. coli* was significantly ($P<0.05$) higher in control group. The expression of anti-inflammatory cytokine of IL-10 and S-IgA were upregulated, while expression of pro-inflammatory cytokines of IL-6, TNF-α, and IFN-γ were downregulated. In addition, nutrient transporters of PepT1, LAT2 and CAT2 were upregulated in supplemented group and GLUT2, SGLT1, rBAT, carbohydrates and vitamins metabolism cofactor are enriched in *BS* DSM32315 fed-group. On the other hand, control group exhibited up-regulation in IL-6, TNF-α, and IFN-γ. Thus, it indicated that supplementation of *Bacillus subtilis* DMS 32315 reduced the effects of enteritis and enhanced the gut-microbial community and immune parameters in broiler chickens.

Keywords: Antibiotic growth promoter, *Bacillus subtilis* DSM32315, *Clostridium perfringens*, gut microbiome, immune response

MI10
Effect of *Bacillus*-based probiotics on improving the intestinal health and performance under enteritis challenge in broiler chickens

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This study investigated the effects of *Bacillus*-based probiotics on performance and intestinal health in broiler challenged with *Clostridium perfringens*-induced necrotic enteritis. One-day-old Arbor Acre (*n*=480) were randomly assigned to four treatments with 10 cages of 12 birds. Dietary treatments were; basal diet negative control (NC), with no probiotics nor antibiotics formulated to contain 2,930 and 3,060 kcal/kg with 24.1 and 16.0% CP, for starter and finisher diet, respectively; basal diet + enramycin (5 mg/kg), an antibiotic growth promoter (AGP); basal diet + *Bacillus subtilis* DSM 32315 (1×10^6 CFU per g of feed, BS) and basal diet + *Bacillus licheniformis* (1×10^6 CFU per g of feed, BL). Growth performance, intestinal morphology, intestinal lesion scores and short-chain fatty acids (SCFAs) were assessed. Average daily weight gain was significantly (P=0.01) higher in BS and AGP-fed groups. Feed conversion ratio was lowest in BS fed group compared to other dietary treatments (P=0.06). Similarly, mortality was lower in all probiotic fed groups compared to AGP-fed group (P=0.001). Intestinal lesion scores was not different among dietary treatments at d 21 (P=0.10) while it was significantly lower (P = 0.03) in birds fed AGP at d 35. In the duodenum and jejunum villus height to crypt depth (VH: CD) was higher compared with NC and BS. Probiotics-fed groups showed higher total SCFAs, acetic and butyric acid concentrations at d21 post-challenge than other groups. The present study indicated that Bacillus-based probiotics can ameliorate the enteritis conditions caused due to *Clostridium perfringens* challenge and the effects are similar to AGP-fed birds.

Keywords: Antibiotic growth promoter, *Bacillus licheniformis*, *Bacillus subtilis*, broiler, necrotic enteritis

MI11
Innate immunomodulation with BCG in porcine monocytes enhances responsiveness to heterologous agonists

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Immunomodulation engages the host's own immune system to fight against disease. The innate immune system recognizes and responds to a large range of foreign agonists, while the adaptive immune system recognizes very specific antigens. The vaccine strain of Mycobacterium bovis (*Bacillus Calmette-Guerin*; BCG) induces an alternative phenotype in monocytes that enhance their ability to respond to a range of heterologous agonists. This alternative phenotype is a form of innate memory and is characterized by long lasting epigenetic and metabolic changes in innate cells, such as monocytes and NK cells, that enhance the responsiveness of these cells to future heterologous agonists. As a method to improve animal health and reduce the use of antibiotics, we interrogated the ability of BCG to alter the innate memory phenotype in pig monocytes. Primary porcine monocytes were stimulated with either live or inactivated BCG (Danish strain). After 24h the supernatants were collected and the cells maintained in culture for 5d. Cells were restimulated with the heterologous agonist lipopolysaccharide (LPS; TLR4 agonist) or Pam3CSK4 (synthetic triacylated lipopeptide; TLR 2 agonist). Cells and supernatants were collected after restimulation for gene expression and cytokine production analysis. Enhanced innate memory (trained immunity) is characterized by increased cytokine production relative to non-stimulated controls. Priming with either live or inactivated BCG enhanced IL-1β and TNFα cytokine production when restimulated with either LPS or Pam3CSK4. Monocytes primed with BCG, but not restimulated with a heterologous agonist, did not produce measurable amounts of cytokine, indicating that the increased cytokine production observed with LPS or Pam3CSK4 restimulation was not due to residual cytokine production from the primary BCG stimulation. No significant differences were observed in IL1B or TNFA gene expression 6h after restimulation with LPS, but live BCG priming upregulated expression of caspase-1 and NLRP3 to LPS restimulation. Collectively, BCG can alter the monocyte innate memory phenotype and enhance responses to heterologous agonists. Innate memory may serve as a mechanism to enhance immune responses and reduce the use of antibiotics.

Keywords: innate training, immunomodulation, pig, monocyte, heterologous protection

MI12
Phages for the Replacement of Antibiotics, and Reduction of *Salmonella*, in Poultry Farms in Kenya

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Globally, the poultry industry has increased by 5% every year for the past three decades, showing a higher growth rate than the pig (3%) and beef (1.5%) sectors. In Kenya, poultry farming represents about 30% of the total agriculture contribution to the Gross Domestic Product (GDP), with an estimated 75% of rural families keeping chicken. Infectious diseases associated with poultry farming and egg production pose high risks to the poultry industry, as well as to the farmers' and consumers' health. The most responsible species for enteric disease in humans transmitted by poultry products is *Salmonella enterica*. Moreover, pullorum disease and fowl typhoid, caused by *S. enterica* serovars Pullorum (*S.* Pullorum) and Gallinarum (*S.* Gallinarum) respectively, have been listed by the FAO as the most important bacterial diseases affecting chicken health and productivity in Kenya. Current methods of controlling or preventing *Salmonella* infections in poultry farms include the use of antibiotics. Furthermore, according to a recent FAO report, an estimated 75% of antibiotics administered to poultry are released in the environment and contribute to the emergence of antimicrobial resistance (AMR). Alternative strategies are being sought to curb the problem associated with antimicrobial resistance. As such, there is a growing interest to explore the use of bacterial viruses or bacteriophages (phages).

Due to the lack of knowledge and of prior reports on phage therapy in Kenya, the technology needs to be introduced and tested as viable, safe and effective. Therefore, the goals of the project are to optimize control measures to reduce antibiotic use as well as AMR *Salmonella* strains in Kenyan poultry farms by using available *Salmonella*-killing bacteriophages from the Félix d'Hérelle Reference Center for Bacterial Viruses (www.phage.ulaval.ca) in Canada, as well as by using newly isolated phages from Kenya which have the capacity to kill Kenyan *Salmonella* strains. To that end, several naturally occurring phages with lytic activity against a range of *Salmonella* strains were isolated from chicken feces and water samples collected from various Kenyan poultry farms. Preliminary data suggest that phages able to infect and kill a strain of *S.* Pullorum could be successfully isolated. Currently, we are in the process of characterizing these newly isolated phages and the best candidates will be tested in a chicken model of pullorum disease in the near future.

Keywords: Bacteriophages, *Salmonella*, Pullorum disease, Poultry, Kenya

MI13
Transmissible antibiotic resistance genes present in *Escherichia coli* from USA and Thailand poultry

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Conventional poultry production has used sub-therapeutic levels of antibiotics in the diet to prevent disease and stimulate growth for many decades. Health concerns over the potential of antibiotic resistant bacteria in the food supply has resulted in consumer demand and regulatory changes limiting the use of antibiotics, thereby making alternatives to antibiotics part of the mainstream in most poultry markets today. One of the expected benefits of using antibiotic alternatives is a reduction in antibiotic resistance genes in the environment. In 2014, <5% of broilers in the USA were "Raised without Antibiotics" and this increased to >40% by 2018. To determine the prevalence and type of antibiotic resistance genes present in the USA poultry industry before the concerted push to raise poultry without antibiotics, we sequenced the genomes of 111 *Escherichia coli* strains from broilers and turkeys collected in 2014 and 2015 and analyzed them for acquired antibiotic resistance genes. Transmissible resistance genes to aminoglycosides, tetracyclines, sulfonamides, and beta-lactams were the most prevalent. A multiplex PCR was developed to detect seven of the antibiotic resistance genes. This assay was used to determine the presence of antibiotic resistance genes in poultry *E. coli* isolates from the USA and Thailand. The average number of antibiotic resistance genes detected per isolate was 1.7 in both countries with a maximum of five of the seven genes in any one isolate, but different genes were predominant in each county. Over 30% of the USA isolates possessed aminoglycoside resistance gene *aac3VIa*, compared to only 1% of the Thailand isolates. Beta-lactam resistance gene, *blaTEM1,* and tetracycline resistance gene, *tetA*, were the most prevalent in Thailand isolates at 70% and 60% compared to 25% and 27% in the USA isolates. Over time there was a significant decrease in the number of antibiotic genes per isolate in the USA. The average number was 2.2 genes in 2015 and 2016 which dropped significantly to 1.2, 1.3 and 1.4 in 2017, 2018 and 2019 respectively. This multiplex assay will be used for continuous monitoring of transmissible antibiotic resistance genes in avian *E. coli* across countries and time.

Keywords: transmissible antibiotic resistance genes, *Escherichia coli*

MI14
Potential of enzymatically hydrolyzed yeast (AVIATORTM) binding to enterotoxigenic *Escherichia coli in vitro*

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The use of *Saccharomyces cerevisiae* or enzymatically hydrolyzed yeast (EHY) as a nutritional feed supplement is commonly applied in many livestock productions. It improves animal production, promote health and also reduce the need for antibiotic use. The major component of EHY is mannan oligosaccharide which can improve gut health and act as a high-affinity ligand offering competitive binding site options for Gram negative bacteria.

The objective of this study is to evaluate the efficiency of AVIATOR™ bind to enterotoxigenic *E. coli*. The qualitative and quantitative EHY assays were performed *in vitro* with four enterotoxigenic *E. coli* strains. The qualitative assay was performed using overnight *E. coli* culture in TSB broth mix with various EHY concentrations and observed for the agglutination. The EHY-*E. coli* complex was agglutinated and easily observed in various concentration of EHY as clumping cell. Furthermore, the quantitative assay was also performed by counting the unbound *E. coli* after EHY reaction. The result showed that EHY has ability to reduce the number of *E. coli* approximately 3-4 log cells from original cell number. In conclusion, EHY showed the high potential agglutinate and reduce the number of enterotoxigenic *E. coli in vitro*. This positive phenomenon of EHY will associated with pathogen removal from the gastrointestinal tracts without attachment and colonization *in vivo*.

Keywords: Aviator, *Escherichia coli*, ETEC, enzymatically hydrolyzed yeast, agglutination

MI15
Potential of enzymatically hydrolyzed yeast (Aviator™) strongly agglutinate with *Salmonella* Typhimurium and *S.* Enteritidis

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The use of *Saccharomyces cerevisiae* or enzymatically hydrolyzed yeast (EHY) as a nutritional feed supplement is commonly applied in many livestock productions. It improves animal production, promote health and also reduce the need for antibiotic use. The major component of EHY is mannan oligosaccharide which can improve gut health and act as a high-affinity ligand offering competitive binding site options for Gram negative bacteria.

The objective of this study is to evaluate the efficiency of AVIATOR™ bind to *S.* Typhimurium (ST) and *S.* Enteritidis (SE) *in vitro*. The qualitative and quantitative EHY assays were performed *in vitro* with *S.* Typhimurium and *S.* Enteritidis strains. The qualitative assay was performed using overnight ST and SE culture in TSB broth mix with various EHY concentrations and observed for the agglutination. The EHY-*Salmonella* complex was agglutinated and easily observed in various concentration of EHY as clumping cell. Furthermore, the quantitative assay was also performed by counting the unbound *Salmonella* after EHY reaction. The result showed that EHY has ability to reduce the number of *Salmonella* approximately 3-4 log cells from original cell number. In conclusion, EHY exhibited the high agglutination and reduce the number of SE and ST *in vitro*. This positive phenomenon of EHY will associated with pathogen removal from the gastrointestinal tracts without attachment and colonization *in vivo*.

Keywords: Agglutination, Pig, Probiotic, *Salmonella*, Yeast

MI16
Evaluating the ability of probiotics to inhibit *Clostridium perfringens* cause diarrhea in pigs

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Using antibiotics in animal feed caused resistance in bacteria and was be forbidden in some countries. Thereby probiotics have evolved the alternatives to antibiotics in the pig industry. It is well documented that some probiotics produce bacteriocins that have the ability against pathogens in gastrointestinal tracts. There are many studies reported on the ability of probiotics in the inhibition growth of Gram-negative bacteria. However, Gram-positive pathogens are also one of the main causes of many diseases in pigs. Hence, our studies concerned the identification of probiotics against Gram-positive pathogens to enrich the application of probiotics and improve currently probiotic products. In this study, we carried out screening test whether our candidate probiotics are able to inhibit the growth of *C. perfringens in vitro*.
Twenty strains of probiotics either *Lactobacillus* spp. or *Bacillus* spp. and ten strains of pathogenic *C. perfringens* were isolated from healthy pigs and diarrheagenic piglets, respectively. The ability of probiotics to inhibit *C. perfringens* was tested by using the direct spot agar on nutrient agar in anaerobic conditions. The inhibition zone (clear zone) was measured as representative of probiotics inhibitory effect on pathogens. We found out 10 candidate strains of probiotics can inhibit *C. perfringens* with inhibiting zone diameter ranging from 6-18 mm. These strains will be used in the next experiments for selecting the best strains and the optimal growth condition on bacteriocins production.
Though this is a preliminary study with a small number of samples, this study showed potential probiotics to protect pigs against *C. perfringens*.

Keywords: Probiotic, *Clostridium perfringens, Lactobacillus* spp., *Bacillus* spp.

MI17
The inhibitory effect of *Bacillus* spp. to against the pathogenic *Escherichia coli* isolate from pig in Thailand

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E. coli infection is one of the important causes of diarrhea in piglets, resulting to economic loss due to increase piglet mortality, morbidity and decrease growth rate. The antibiotic is a common remedy for control *E. coli* spread in farm whether for prophylaxis, metaphylaxis or therapeutic purpose. Because of antibiotic using is popular in pig farm, the antibiotic resistance should be concerned. Thus, there is a need to study alternative antimicrobial agents for reduce or replace the using of conventional antibiotic. Probiotics are the interesting topic for developing the alternative antimicrobial agent since its well documented ability to produce the antimicrobial compound. The aim of this study was to investigate the inhibitory effect of *Bacillus* spp. against the growth of pathogenic *E. coli* on nutrient agar.

This study used *Bacillus* spp. to test the ability to inhibit growth of pathogenic *E. coli* isolate from nursery pig in Thailand by direct spot test. Enrofloxacin and gentamicin were used as control. The result showed the *Bacillus* spp. showed its ability to inhibit growth of pathogenic *E. coli* by showed the inhibition zone (clear zone) on nutrient agar comparable with the results from the control (enrofloxacin and gentamicin), however, with a small difference in diameter of inhibition zone.

In conclusion, the present study clearly showed the ability of *Bacillus* spp. to inhibit growth of pathogenic *E. coli*, therefore this *Bacillus* spp. could be a candidate for develop the alternative antimicrobial agent for the replacement of antibiotics using in pig farm. However, further study is needed for acid, bile tolerance properties and effect of its cell free supernatant (CFS).

Keywords: Probiotic, *Bacillus* spp., *Lactobacillus* spp., Enterotoxigenic *Escherichia coli*, ETEC

MI18
Effect of selected yeast fraction on the growth of *Clostridium perfringens*: Quantitative determination of growth inhibition and adsorption capacity

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To provide *in vitro* evidences on the antimicrobial effect of yeast cell wall (YCW), the effectiveness of YCW fractions in inhibiting the growth of several *C. perfringens* strains was quantitatively determined. The bacterium was grown in the presence of different YCW fractions at different concentration levels. The effect of YCW fractions on the growth parameters was analyzed. One product out of four materials was selected as the best candidate for *C. perfringens* inhibition. The selected product, at an optimal dosage of 1.25 mg/mL, increased the lag phase duration, and reduced the maximum growth rate and the final cell count in a significant manner with respect to the control. The adsorption of the pathogen to YCW was studied using the isotherm adsorption approach. The effect of YCW dosage, incubation time, and bacterial concentration on the adsorption was evaluated. The study proved that the product adsorbed C. perfringens cells in a dose and time dependent manner. Equilibrium isotherms showed that the cell adsorption onto the product was fast, stable over the time, and occurred with high affinity and capacity. The selected product sequestered up to 104 cells of *C. perfringens* per mg. To the best of our knowledge, this is the first report showing the in vitro efficacy of yeast fraction products to inhibit the growth of *C. perfringens,* and to reduce the culturable cells by an adsorption process. The *in vitro* approach proposed herein is as a powerful tool to study the adsorption of aerobic or anaerobic pathogens by eubiotics.

Keywords: yeast cell wall, antimicrobial, *Clostridium*, adsorption, feed additives

MI19
Evaluation and selection of Lactic acid bacteria based on inhibition capability against *Streptococcus suis* for probiotics product

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Streptococcus suis (*S. suis*) serotype 2 is one of the most important pathogens in pigs, which causes septicemia, meningitis, arthritis and pneumonia pigs. Moreover, this bacterium is considered an emerging zoonotic agent. Recently, increased antibiotic resistance of *S. suis* has been reported worldwide, and raises issues regarding food safety. There are several potential alternative methods to replace the use of antibiotics. One of them is to promote pig health by directly supplement probiotic bacteria and another is to use their antimicrobial substance, to inhibit the growth of pathogens. The objective of this study was to evaluate the potential of cell-free supernatant of selected lactic acid producing probiotic strains to inhibits the growth of 12 isolated *S. suis* (i.e. SS-01 to SS-12), in which isolated from infected nursery pig and its pathogenicity was confirmed by PCR technique. All the probiotic bacterial strains used in this study (*Lactobacillus plantarum*, *Lactobacillus acidophilus* and *Pediococcus pentosaceus*) were selected mainly on the *in vitro* of inhibition ability on Enterotoxigenic *E. coli*, Enterohemorrhagic *E. coli* and the adhesion ability on swine intestinal mucus as shown in our previous study. The ability of different probiotic species and stains to inhibits *S. suis* serotype 2 was evaluated by zone of activity on agar well diffusion. The zone of activity was classified into four groups: no inhibition as follows: 0-7 mm; low inhibition: 8-14 mm.; medium inhibition: 15-21 mm., and high inhibition: >21 mm. The different lactic acid probiotics showed variety of capability to inhibit the growth of *S. suis* serotype 2. *Lactobacillus plantarum* KMP-F23-1 showed both medium and high inhibition zone of activity for SS-08, SS-03, SS-10 (i.e. 18.25, 26.00, and 21.75 mm., respectively) when compared with other lactic acid producing probiotics. *Lactobacillus plantarum* CU20 also showed medium and high inhibition zone of activity for SS-06, SS-07, SS-08, and SS-10 strains (i.e. 17.50, 17.50, 17.75, and 21.25 mm., respectively). *Lactobacillus acidophilus* KMP-TC001 showed high inhibition zone of activity with a diameter of 21.50 for SS-10. For *Pedicococcus* spp., *Pediococcus pentosaceus* PdAvPd02 showed high inhibition zone of activity for SS-10 (i.e. 20.00 mm.). According to inhibition zone of activity, we, therefore, included only *Lactobacillus* and *Pedicococcus* that showed medium to high inhibition zone of activity to become probiotics candidate in probiotic product, targeting this multi-strains probiotic product is able to minimize the growth of *S. suis* in pigs. In conclusion, different lactic acid producing probiotics such as *Lactobacillus* spp. and Pediococcus spp. showed variety of inhibition zone of activity for *S. suis*, thus we recommended that multi-strains of probiotics should be included in particular product in order to conquer the *S. suis* problem in pig industry.

Keywords: Probiotics, lactic acid bacteria, *Streptococcus suis*, inhibition, pig

MI20
Probiotic strain modulates gut microbiota and control the inflammatory response in chickens

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A probiotic strain (*bacillus subtilis* DSM 29784, BS29784) is capable of inducing beneficial effects on growth performance and could be therefore a reliable alternative to antibiotic growth promoters. The underlying mechanisms of probiotics, however, are often not fully understood. An *in vivo* investigation of microbiota profile aimed to observe a positive effect of BS29784 on butyrate producer bacterium such as Ruminococcus or *Lachnoclostridium*. An *in vitro* approach, using Caco-2 cells line, showed a decrease of pro-inflammatory compounds (IL8, iNOS) following the supplementation of BS29784. This was mainly explained by an activation of the NFkB pathway. Finally, we also demonstrated the positive correlation of tight junction gene expression with TransEpithelial Electrical Resistance, a sensitive indicator of barrier tissue integrity. In the present study, we used a two-step approach to identify major metabolites produced by BS29784 known to have beneficial effects on broiler performance and health. The first step consisted in cultures of the BS29784 grown in Luria-Bertani and Tryptic Soy Broth culture media. After 4h, 10h and 24h, the supernatant of cultures was analysed with UPLC/MS to identify the metabolites produced *in vitro*. The second step was an *in vivo* study, in which 1-day old broiler chicks were continuously administered BS29784 via the diet. At d13, intestinal samples from different locations were collected and analysed for a targeted metabolite analysis. A DNA extraction was performed on the intestinal samples to determine the relative abundance of *Bacillus* species in different intestinal locations (via qPCR). Nicotinic acid and hypoxanthine were the two main metabolites that were increased in the supernatant of BS29784 cultures. An increase in their concentrations was also measured in ileum and jejunum samples of 13-day old chickens to which the strain was administered. The wound healing assay confirmed the beneficial effect of these two metabolites on barrier function.

Keywords: *B. subtilis*, chicken, microbiota, inflammation, metabolite

MI21
Performance of broilers fed AGP free diets supplemented with a direct-fed microbial under hot climate

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This trial was done to determine broiler live performance to 42 days, under a hot climate in India. 2080 VenCobb 400 broilers were allocated in 4 groups with 26 birds per floor pen and 20 replicates per group: negative control group (NC), positive control group (PC), direct-fed microbial group (DFM) at Log 5 CFU/g of feed and Bacitracine group (BMD) at 500 g/T of feed. The DFM product was composed with 3 species of sporulated *Bacillus*: *Bacillus amyloliquefaciens, bacillus licheniformis* and *bacillus pumilus*. The room temperature reached a peak of 31.64 to 33.29°C from 4 to 6 weeks old. PC, DFM and BMD groups were fed with a challenging feed formula: 5 to 10% of DDGS, 8 to 10% of mustard cake extract and 5 % of medium quality meat and bone meal (which is still usually used in India). NC group was with a classical and non-challenging corn and soybean-based diet.

The body weight at 42 days was 2.707 kg in NC group, but only 2.144 kg in PC group, due to the feed challenge. It reached 2.205 kg in BMD group and 2.254 kg in DFM group, significantly higher than PC group (p=0.001).

The broilers were vaccinated against Newcastle disease during the trial at 7 and 21 days. The humoral vaccine response was evaluated by HI-test at 42 days. The response was 5.05 Log2 in NC group, 4.75 Log2 in PC group, 4.10 Log2 in BMD group and 5.85 Log 2 in DFM group, significantly higher with DFM (p=0.001). The cell-mediated immunity was also evaluated by basophilic hypersensitivity test. The response was respectively 70.05, 72.65, 65.65 and 95.20 significantly stronger with DFM (p=0.004).

The ileal microbial count in CFU/g was evaluated at 42 days. The quantity of *E. coli* was respectively 3.942, 3,849, 3.968 and 3.510, significantly lower with DFM (p=0.019). The quantity of *Clostridium perfringens* was respectively 3.515, 3.748, 3.510 and 3.158, also significantly lower with DFM (p=0.015).
This direct-fed microbial enabled to improve the growth performances under a natural heat stress, helped to improve the vaccine response and reduce the pressure of potentially pathogenic bacteria like *E. coli* and *Clostridium* in the gut.

Keywords: broiler, direct-fed microbial, hot climate, feed challenge, performances

MI22
Different from antibiotics: Improving gut health additives by understanding their mode of action

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Over the last two decades, several types of gut health supporting additives have been shown to have at least the potential to improve gut health and animal performance and as such, to be applied as alternatives to antibiotics. Initial research on these products was largely focused on their direct 'antimicrobial' effect, as it was tempting to hypothesize that any successful alternative for antibiotics would need to have a similar (antimicrobial) effect. However, it becomes clearer that gut health additives have working mechanisms that are very different from those of antimicrobials.

I will argue that direct antibacterial effects most likely do not underlie the main working mechanisms of additives, and that improvement of the development and application of these additives will depend on the investigation of their mode of action at the microbial and cell-biological level. This will be exemplified with recent research data on different classes of feed supplements, including butyrates, probiotics and phytogenics.

Butyrate, for instance, is a molecule that is well known for its ability to elicit numerous effects in the digestive tract. While most publication describe positive effects of butyrate supplementation, I will present data demonstrating that the effects of supplementing livestock animals with commercial butyrate products are heavily dependent on the enteric location where butyrate is delivered in the digestive tract. For example, in at least some conditions, elevated butyrate concentrations in the fore- and midgut of broilers may induce negative effects on caecal microbiota diversity and/or inflammation, as opposed to increased butyrate concentrations in the hindgut.

In addition, monoglycerides of short-chain fatty acids, the metabolites of probiotics and the phytochemicals in botanical products are unlikely to have substantial specific bacteriostatic effects, as exemplified by EU-funded study evaluating their effectiveness against *Campylobacter*. More likely, they exert their function by modulating effects that can be trigger at low intestinal concentration, such as the inhibition of bacterial quorum sensing.

Lastly, results will be shown from a trials demonstrating that further improvement in gut health and economic profitability of the producer can be achieved, by implementing research based programs, combining different classes of additives.

Keywords: butyrate, phytogenics, probiotics, working mechanism

MI23
Antimicrobial Effect of *Bacillus* Probiotics against Foodborne Pathogens

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Foodborne illness is still a major health problem worldwide, according to a report released from the Center for Disease Control and Prevention (CDC). Nowadays, antimicrobial resistance is a major threat to human and animal health which is also related to the multidrug resistance of foodborne pathogens in food-producing animals such as poultry and pigs. The aim of this study was to evaluate the antimicrobial activity of 3 species of *Bacillus*; *B. subtilis* 16AvBa10, *B. licheniformis* KMP9 and *B. amyloliquefaciens* 16AvBa18 against 3 species of foodborne pathogen including *E. coli* ATCC 25922, *S.* aureus ATCC 6538 and *Salmonella* for reference strains (*S.*Typhimurium DMST 15674, *S.* Enteritidis DMST 15676) and field isolated (*S.* Typhimurium 001, *S.* Enteritidis 022, *S.* Virchow 001, *S.* Hadar 002) by delayed antagonism in solid nutrient medium method. It was found that the 3 species of *Bacillus* were able to inhibit the growth of all foodborne pathogens. And the ability of *Bacillus* on pathogenic inhibition varies among the difference species and strains. *B. licheniformis* KMP9 showed the highest effectiveness against field isolated *Salmonella* for ST 001, SE 002 and *S.* Virchow 001 was 20.7 $\pm$ 3.7 mm, 22.8 $\pm$ 1.7 mm, 27.4 $\pm$ 2.5 mm, respectively while *B. subtilis* 16AvBa10 showed the highest antibacterial ability on both reference strain of S. Typhimurium DMST 15674, *S.* Enteritidis DMST 15676 and *S.* Hadar 002 was i.e. 17.5 $\pm$ 3.3 mm, 8.8 $\pm$ 1.7 mm and 26 $\pm$ 5.7 mm, respectively. For the other foodborne pathogens such as *E. coli* ATCC 25922 and *S. aureus* ATCC 6538, the *B. licheniformis* showed a highest inhibition zone of 28.0 $\pm$ 3.5 mm and 30.8 $\pm$ 1.2 mm, respectively. However, *B. amyloliquefaciens* 16AvBa18 also showed the potential on antibacterial activity of *E. coli* and *S. aureus* and *Salmonella* many serovars too. Although Lactic acid bacteria always inhibited gram negative bacteria such as *Salmonella* and *E. coli*, some species of *Bacillus* showed the ability on it too. These studies suggested that the *Bacillus* are one alternative to use for foodborne pathogen control in Livestock.

Keywords: *Bacillus* spp., Antibacterial activity, Foodborne pathogen

MI24
Characterization of virulent bacteriophages infected multidrug-resistant *Aeromonas hydrophila*

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In the context of protein deficiency due to the rapid increase of the world's population and the decrease in production, freshwater fishes provide a high source and quality of nutrition with affordable price for consumers. Nevertheless, the disease outbreak in fish, particularly Motile Aeromonas Septicemia Disease (MAS) caused by multidrug-resistant (MDR) *Aeromonas hydrophila* was widespread and gave rise to high economic losses for farmers. The MDR *A. hydrophila* can not be treated with antibiotics using in aquaculture. The alternative methods should be applied for the eradication of MDR *A. hydrophila* such as herbs and bacteriophages. Therefore, we aimed to isolate and characterize *A. hydrophila* specific bacteriophages. The MDR *A. hydrophila* BT09 isolated from diseased striped catfish (*Pangasianodon hypophthalmus*) was screened and used for bacteriophage isolation. Fifty-four bacteriophage strains were isolated from 120 water samples and three strains namely pAh5.5BT, pAh6.2BT and pAh6.2TG were selected based on the criteria of plaque diameter, un-adsorption rate, and lytic cycle time. The results showed that the selected bacteriophages exhibited the largest plaque diameter of 1.19±0.27 mm, 1.17±0.09 mm, 1.50±0.02 mm and the lowest percentage of free phage with 16.3%, 13.2%, and 35.5%, respectively. Besides, all of them determined the shortest lytic cycle of 15 minutes. The TEM images showed that all of the bacteriophages had an icosahedral head, long contractile tail, particularly with the collar and belonged to the *Myoviridae* family. The selected bacteriophages exhibited wide host range with and high specificity with the isolated *A. hydrophila*. Single strain or combination of the bacteriophages offered the widest host range with 81.8%. The bacteriophages can be used as a potential alternative to antibiotics to control MAS in striped catfish and other freshwater fish species.

Keywords: *Aeromonas hydrophila*, antibiotic resistance, bacteriophage, Motile *Aeromonas*, Septicemia

MI25
Control of Mycotoxins in Farm Animals: a Key Step in Antibiotic Free Production

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Mycotoxins are toxic secondary metabolites produced by certain fungi such as *Penicillium*, *Fusarium* and *Aspergillus*. Mycotoxin producing fungi damage crops, causing severe economic losses to food and feed production. Moreover, several mycotoxins impair immunity and organ functions, causing loss of productivity, diseases and, in extreme cases, death in animals that consume contaminated feed. Several studies have highlighted the significance of interactions between mycotoxins and intestinal diseases such as clostridial necrotic enteritis and coccidiosis in poultry. In swine, mycotoxins are known to cause a severe immune suppression and increase the severity of pathogens such as *Salmonella* spp. and *Pasteurella multocida*.

In farm animals, mycotoxins can impair gut health via three main routes:

1. They promote the opening of tight junctions in the intestine, by actively suppressing the production of tight junction proteins such as claudins and occludins.
2. The promote villi fusion and atrophy. Furthermore, some mycotoxins such as deoxynivalenol and fumonisins are capable of inhibiting the production of intestinal transporter proteins such as GLUT2 and SGLT1.
3. They promote immunosuppression, by directly triggering apoptosis of immune cells; or immune overstimulation by modulating the expression of several cytokines. Immune overstimulation is not beneficial to production animals, due to the high metabolic cost and because it is an extra stress factor.

When it comes to estimating the mycotoxin contamination risk, it is important to keep in mind that mycotoxins always co-occur and often lead to synergistic interactions, where the toxicity of one mycotoxin is increased by the presence of others. As reported by several studies, the likelihood of synergistic interactions are higher at subclinical levels. Furthermore, grains do not only contain mycotoxins, but they might be contaminated by different types of bacteria and other toxic substances, such as pesticides and heavy metals, that increase the overall risk. As shown by several studies, co-occurrence of mycotoxins often results in greater immunosuppression, reduced feed intake, decreased weight gain and nutrient utilization. In this context, it is important to mention that mycotoxin risk thresholds that issued by international agencies, such as EFSA and FDA, only take into account data based on *in vitro* studies on single mycotoxin contamination, thus presenting huge limitations when it comes to estimating the real toxicity of mycotoxins in the field.

Because of the broad variety of negative effects on the gut and immune system, control of mycotoxins should be the first step in antibiotic free production.

Keywords: mycotoxins, immune suppression, gastrointestinal health

MI26
Improved growth performance and reduced mortality in broiler chickens supplemented with two novel strains of *Bacillus subtilis*

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The use of probiotics can be a natural and sustainable alternative to the practice of including sub-therapeutic levels of antibiotics in livestock and poultry feed to improve intestinal health. Probiotics have the potential to reduce presence of certain deleterious or pathogenic microorganism, reducing efficient growth in food animals. A randomized complete block design was used to evaluate the effects of two novel *Bacillus subtilis* strains, Correlink™ ABS-747 and ABS-1781 on broiler chicken performance. Cobb 500 males were housed for 42 d in a single environmentally controlled room with 78 floor pens (26 pens/treatment) at 50 birds/pen. Diets were administered in 4 feeding phases 0-11 (ST), 11-25 (GR), and 25-35 d (FR) and 35-43 (WD). An antibiotic-free basal diet was mixed for each phase and *Bacillus subtilis* was added to each basal diet to make treatment diets, which were identical in ingredient and nutrient composition. Study treatments were: Control, 747 (Control + 1.5×10^5 CFU of ABS-747/g feed), and 1781 (Control + 1.5×10^5 CFU of ABS-1781/g feed). During each study phase, birds had *ad libitum* access to feed and water. Body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR), unadjusted and adjusted for mortality (MA), and production efficiency index (PEI) were determined for each feeding phase and overall. Data were analyzed with SAS (v. 9.4, SAS Institute, Cary, NC) using pen as the experimental unit, treatment as a fixed effect, and block as a random effect. No differences (P=0.11 to P=0.90) among treatments were found for BW, ADG, MA_ADG, ADFI, MA_ADFI, FCR, MA_FCR, and PEI during ST, GR, or FR phases. Mortality was reduced with the inclusion of ABS-747 (4.2%) and ABS-1781 (4.1%) during GR (P<0.06) compared to Control (5.1%). Inclusion of ABS-1781 reduced (P<0.01) mortality during FR compared to Control (3.1 vs 3.8, respectively). Inclusion of ABS-747 and ABS-1781 during WD improved (P<0.0001) ADG, MA_ADG, FCR, MA_FCR, PEI and mortality compared to Control. During the 42-d trial, and compared to Control, birds supplemented with ABS-747 and ABS-1781 improved final BW by 4.7% (2.07, 2.16, and 2.17 kg, respectively; P<0.0001), ADG by 4.8% (48.3, 50.6, and 50.7 g/d, respectively; P<0.0001), MA_ADG by 4.9% (49.2, 51.3, and 51.9 g/d, respectively; P<0.0001), FCR by 8.9 points (1.881, 1.778, and 1.806, respectively; P<0.0001), MA_FCR by 8.6 points (1.787, 1.703, 1.699, respectively; P=0.0003), and PEI by 8.2% (248, 273, and 264, respectively; P=0.0006). Inclusion of ABS-1781 reduced (P=0.03) 42-d mortality by 13% from 2.7% in Control to 2.4%. Overall, broiler chickens supplemented with Correlink™ ABS-747 and ABS-1781 demonstrated superior performance to birds fed the control diets.

Keywords: *Bacillus subtilis*, probiotic, broiler, growth performance

MI27
Occurrence of antimicrobial resistance in different swine farm management systems using TaqMan array cards

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Antimicrobial agents contribute to the emergence of antimicrobial resistance (AMR) by selection of resistant bacteria. For veterinary medicine, most antimicrobial agents are used for treatment and/or as feed additives for prophylaxis. Recently, antimicrobial agent-free farms have increased, but whether AMR is reduced in these farms is unclear. We previously developed a TaqMan array card (TAC) which included 79 sequence specific PCR targets for detection of antimicrobial resistance associated genes for ten important antimicrobial classes used in human and veterinary medicine. In this study, TAC was used to detect AMR directly in stool samples to determine the occurrence of AMR across different swine farm management systems.

We studied in three different fattening pig farm categories including 1) extensive-antimicrobial-use - both treatment and feed additive 2) limited-antimicrobial-use - treatment only, and 3) no-antimicrobial-use. Eighty pig farms located in Thailand were enrolled and five stool samples were randomly collected from healthy 20-25 weeks old fattening pigs from each farm, yielding 400 stool samples. Two hundred milligrams of stool underwent DNA extraction and 20 µl was used as DNA template. Resistance gene copy number was normalized with bacterial 16S copy number of each sample for comparable across sample.

The number of resistance genes in extensive-antimicrobial-use farms (28.5 ± 4.3) was higher than limited-antimicrobial-use farms (23.3 ± 3.7) and no-antimicrobial-use farms (22.5 ± 3.3; $p<0.05$, one-way ANOVA). The prevalence of resistance genes in extensive-antimicrobial-use farms was higher than the other 2 groups for 20 resistance genes, including *bla*$_{SHV}$, *bla*$_{CTX\text{-}M1\ group}$, *bla*$_{CTX\text{-}M9\ group}$, *bla*$_{GES}$, *bla*$_{VEB}$, *bla*$_{CMY2\text{-}LAT}$, 23S rRNA 2075G-*Campylobacter* spp., *mphA*, *qnrB1*, *gyrA*83L-*E.coli*, *aacC2*, *aac(6')-lb*, *armA*, *rmtB*, *mcr-1*, *mcr-2*, *dfrA5-14*, *dfrA17*, *catA1*, and *floR* ($p<0.05$, Chi-square test). We then generated a logistic regression model to predict antimicrobial exposure by using resistance genes as predictor. The prediction model showed 75% and 83% sensitivity, 87% and 83% specificity, 81% and 83% accuracy for training and tested data set respectively.

Our study indicates that the conventional system of extensive-antimicrobial-use influences the occurrence of AMR. TAC is a rapid high throughput tool which may be useful for large scale surveillance. The prediction model may use for monitoring of farm management systems.

Keywords: Antimicrobial resistance, AMR, TaqMan array card, Swine

MI28
MRSA in the nasal microbiome in neonatal pigs – a pilot study for developing competitive exclusion

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Livestock Associated Methicillin-resistant *Staphylococcus aureus* (MRSA) is a good colonizer in pigs and occupationally exposed people become LA-MRSA positive. As this is considered to be a public health risk, measures to reduce LA-MRSA colonization in pigs should be explored, such as competitive exclusion. We studied the porcine nasal microbiome shortly after birth and the association with colonization of *S. aureus* and MRSA. Nasal swab samples (n=104) were obtained from eight neonatal pigs, from two litters, directly after birth until six weeks that were processed for Illumina MiSeq using the V3-V4 region of the 16SRNA and *tuf* gene for *staphylococcal* spp. identification. *S. aureus* and MRSA were quantified by real-time PCR (CFUeq) and culture. Error-free, non-chimeric Sequence Variants (SV) were identified using dada2 pipeline followed by its compositional analysis using CoDaSeq (Github: ggloor/CoDaSeq). We accounted for compositional effect of sequencing data by CLR (Centered Log Ratio) transformation and repeated measurements of animals via rmcorr and mixed effect model. RSV/TSV present in less than 10% of samples and relative abundance below 0.001 were filtered-out. Contaminant RSV/TVS were identified by Frequency based algorithm from decontam r package. A total of 2764 16S RSVs and 1239 *tuf* TSVs were obtained. With 16S sequencing, species belonging to the genera *Corynebacterium*, *Streptococcus* and *Acinetobacter* were correlated with a low number of MRSA (CFUeq) and cultured *S. aureus*. Notably, *tuf* sequencing could resolute up to 22 different *Staphylococcal* species, of which *S. microti*, *S. haemolyticus* and *S. hyicus* were the most abundant. Only, *S. microti, S. simulans, Macrococcus canis* were negatively correlated with a low number of MRSA (CFUeq) and cultured *S. aureus*. The identified bacterial species in this study can be further explored for development of competitive exclusion to control LA-MRSA in pigs.

Keywords: microbiome, MRSA, competition, colonisation

MI29
Antioxidant potential of *Pediococcus pentosacceus* strains isolated from porcine milk

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Oxidative stress occurs throughout the life of mammals. Early weaning stress syndrome of piglets is a bottleneck problem restricting the development of intensive pig breeding, which often leads to severe oxidative stress in piglets and intestinal barrier dysfunction. Porcine milk not only provides rich nutrition and immunoglobulin, but also contains diversified microorganisms which are an important but often overlooked source of probiotics for piglets. Here, eighty isolates of *Pediococcus pentosacceus* from porcine milk were screened to evaluate antibacterial activity *in vitro* and the property to protect hosts from oxidative injury *in vivo* by drosophila paraquat resistance assays. The 21 suckling piglets were orally administrated with *P. pentosacceus* Q82 at different doses and all animals were sampling on the seventh day after weaning.

Our results reveal that *P. pentosacceus* Q82 was susceptible to 19 antibiotics and against several enteropathogens including *Staphylococcus aureus, Salmonella* and ETEC. Compared with the control, the mRNA expression of CAT in ileum and liver were significantly increased and the concentration of GSH-PX and CAT in liver were significantly also increased. In the nucleus, the expression of Nrf2 is increased in the rectum, lactobacillus was higher in the treatment group by 16S RNA sequence. This study aims to decipher systematically the probiotic effect of *P. pentosacceus* from porcine milk and sheds new light on new probiotics source and rapid drosophila screening probiotics model for prevention and relieving severe oxidative stress of piglets.

Keywords: *Pediococcus pentosacceus*, oxidative stress, Drosophila melanogaster, porcine milk, Nrf2

MI30
Developing a global dynamic dashboard as a one-stop shop for AMR related research and development in One Health Sectors

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Antimicrobial resistance (AMR) poses a worldwide threat for both human and animal health. Global efforts to address AMR have grown steadily in the last couple of years, but require more cross-sectoral coordination. The increasing emergence and spread of AMR have been discussed in many countries and at a range of international fora including the UN General Assembly, the World Health Assembly, the G7, the G20, the OIE and the FAO, resulting in high political interest and commitment. In July 2017, the G20 called for a new international research and development (R&D) Collaboration Hub to maximise the impact of existing and new basic and clinical antimicrobial research initiatives, which led to the establishment of the Global AMR R&D Hub.

The Hub, launched in May 2018, supports global priority setting and evidence-based decision-making on allocation of resources for AMR R&D through the identification of gaps, overlaps and potential for cross-sectoral collaboration and leverage in AMR R&D. Currently the global partnership consists of 16 countries, the European Commission and two philanthropic foundations and four observers (WHO, OECD, FAO, OIE).

One of the key activities for the Global AMR R&D Hub is the development of a dynamic dashboard (the dashboard) that will present close to real-time data on all AMR R&D investments globally. The dashboard will present high level (categorized) information on research projects throughout the research and innovation value chain on therapeutics, preventives, diagnostics, surveillance, policy and interventions across all One Health sectors. Data is being collected in a staged approach, beginning with new products against human bacterial infections. The first stage of the dashboard will be launched in the first quarter of 2020.

Following the launch, the dashboard will expand to present AMR R&D investments into animal health followed by plant and environmental health. To ensure the applicability and utility of the dashboard to the animal health sector, the Global AMR R&D Hub has begun additional work and extensive consultation in order to:

- implement any lessons learnt from collecting human AMR R&D investments when searching for and categorizing animal health-related projects and investments
- develop standard key words that will be used to search databases containing public and charitable funding information to ensure relevant animal health projects and investments are captured
- identify additional sources of information on animal health AMR R&D projects and investments
- define categories that will ensure there is meaningful representation of animal heath projects and investments on the dashboard, and
- collaborate with similar initiatives to ensure there is no duplication of effort.

Addressing AMR requires global action with active participation from all world regions and all One Health sectors. Through the development of the dashboard, the Global AMR R&D Hub will be the global knowledge centre on all AMR R&D activities and investments. The dashboard will support global priority setting and decision making and lead to more efficient use of international resources through the identification of gaps, overlaps and potential for cross sectoral collaboration and leveraging in AMR R&D.

Keywords: dynamic dashboard, One Health, research projects, funding

MI31
Improving research coordination to focus efforts to reduce AMR in animal production

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STAR-IDAZ International Research Consortium on Animal health (IRC) is a global initiative to coordinate research programs at international level to contribute to the development of new and improved animal health strategies for at least 30 priority diseases/infections/issues, including antimicrobial resistance and the development of innovative alternatives to antibiotics. The Consortium is supported by the OIE, that also hosts its scientific secretariat. Target deliverables include candidate vaccines, diagnostics, therapeutics, other animal health products and procedures, and key scientific information/tools to support risk analysis and disease control.

STAR-IDAZ IRC is establishing a Working Group (WG) of experts to identify research gaps for the development of alternatives to antibiotics, focusing in particular on mechanisms of immunomodulation, compartmentalization of resources, and influence of the microbiome. The WG will also investigate the mode of action of antibiotics in growth promotion. STAR-IDAZ IRC members will use the identified research gaps to guide future research funded by their organizations.

The aim is that new research will improve understanding of the relationship between control of sub-clinical infection and growth on the role of the microbiome in maintenance of health and on the identity and mode of action of putative new antimicrobial products– targeting the pathogen and/or the host. This will ultimately help in the development of new non antibiotic-based antimicrobial products and approaches for controlling infections and enhancing productivity, while maximizing the life of existing and new therapeutics.

Keywords: AMR, animal, STAR-IDAZ, IRT, production

MI32
Prevalence and Antimicrobial Resistance of *Salmonella* Isolated from Racehorses and Horsemen in Northeastern Thailand

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Food-borne disease caused by *Salmonella* spp. is one of important public health problems. The antimicrobial resistance causes failure of regular therapy and increases the cost of treatment. The objectives of this study were to determine prevalence and antimicrobial resistance of *Salmonella* spp. isolated from racehorses and horsemen in Northeastern Thailand. A total of 63 samples from racehorses and horsemen were collected (30 and 33 samples, respectively) at farms in Northeastern Thailand. The samplings were collected during April – December 2018. All samples were examined for *Salmonella* spp. isolates and identification by ISO 6597:2002. The prevalence of antimicrobial resistance patterns was assessed using disk diffusion technique among 7 antimicrobials. *Salmonella* spp. contaminated to racehorses and horsemen were 4.86% and 3.03%, respectively. The identified serovars from racehorses were *S.* Abony (23%) and *S.* Inganda (15%); from horsemen was *S.* Tumodi II. Penicillin was high resistance of *Salmonella* spp. isolated from racehorses. The prevention and control of *Salmonella* spp. transmitted between racehorses and horsemen were very important such as hygiene, sanitation and standard farm management.

MI33
Use of *Clostridium perfringens*-specific bacteriophage to control necrotic enteritis in broiler chickens

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The present study was aimed to determine the dietary supplementation of *Clostridium perfringens*-specific bacteriophage (CPBA) in broiler chickens infected with experimental necrotic enteritis (NE). Total of seven-hundred-eighty day-old feather-sexed male broiler chicks were individually weighed and randomly allocated into 5 treatments (12 replicates with 13 birds per replicate): 1) non-challenged control group (NC); 2) NE-challenged control group (PC); 3) PC with CPBA supplementation at 10^5 cfu/kg (low CPBA); 4) PC with CPBA supplementation at 10^6 cfu/kg (medium CPBA); and 5) PC with CPBA supplementation at 10^7 cfu/kg (high CPBA).

The chickens were orally gavaged with 10-dose coccidiosis vaccine on d 9 and *C. perfringens* on d 14, 15, and 16 to experimentally induce NE. Each pen was used as an experimental unit and all data were analyzed by one-way ANOVA using General linear model (GLM) procedure of SAS 9.4. Body weight gain at 21 d were lower in the PC group compared with the NC group (P<0.001). Dietary CPBA increased body weight gain (P=0.001) compared with the PC group. Mortality due to NE infection was highest in the PC group (P=0.002) and compared with PC group overall mortality was decreased (P=0.038) with the medium and the high CPBA treatments.

Jejunal NE lesion scores were lower in the all CPBA groups regardless of CPBA doses (P<0.001) compared with the PC group at 2 days post *C. perfringens* challenge. The concentration of acetate was increased in the PC group compared with NC group (P=0.034). Also the concentration of Lactate was highest in PC group compared with NC and CPBA groups.

In conclusion, *C. perfringens*-specific CPBA supplementation ameliorated growth performance of broiler chickens with NE infection and can be considered as effective feed to lessen production additive and gut health losses due to NE infection in broiler chickens.

Keywords: necrotic enteritis, growth performance, gut health, *Clostridium perfringens*, broiler chickens

MI34
Antimicrobial resistance in *Salmonella enterica* isolated from meat-type ducks in Nakornpathom province Thailand

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The emergence of antimicrobial resistance (AMR) in *Salmonella enterica* isolated from meat-type ducks has become important public health issues because duck meat is widely consumed in many countries. As seen in other food animals, study of AMR in ducks will elucidate root cause of AMR and provide data for alternative to antibiotics (ATA) development in the future. Nakorn Pathom province located in Central region of Thailand and there is the high density of duck population. Due to intensive duck-raising system in this area, antimicrobial agents have been used to treatment and control the spread of infectious bacterial pathogens. It is possible that extensive or indiscriminate use of antimicrobial in duck farms might be led to increased level of antimicrobial resistance (AMR) in *Salmonella* in this area. Therefore, the objective of this project was to characterize of antimicrobial resistance in *Salmonella* isolates from meat-type ducks in Nakorn Pathom province Thailand. A total of 705 fecal samples were obtained from 3 meat-type duck farms. Fecal samples from the same flock at different ages (one-day old duckling, 40-42 days old, 60-70 days old) were collected at each farm. All fecal samples were subjected for isolation and identification of *Salmonella spp*. All *Salmonella*-positive samples were tested for serotyped by the Kauffmann-White scheme. Antimicrobial susceptibility was determined in all *Salmonella* isolates. The presence of resistance genes in AMR *Salmonella* isolates were detected based on their resistance phenotype using PCR. A total of 89 of 705 (12.6%) were positive for *Salmonella*. One hundred and thirty-two *Salmonella* isolates were identified. Serovar Altona was the predominant serotype in this study (36.4%). Most of the isolates showed highly resistant to sulfamethoxazolem (100%), tetreacycline (24.2%), streptomycin (21.2%), spectinomycin (18.2%), ampicillin (15.9%). None of the isolates was resistance to cefoperazone, gentamicin, ciprofloxacin. The bla_{TEM}, *aadA2*, *strA, dfrA12* genes were detected in *Salmonella* resistant strains to ampicillin (52.4%), streptomycin (57.1%), streptomycin (21.4%), trimethoprim (25%), respectively. The plasmid-borne *qnr* genes in fluoroquinolone-susceptible *Salmonella* strains were *qnrB* (3.8%) and *qnrS* (1.5%). Overall, meat-type ducks production is an important sector in poultry industry. The highlight of our study revealed that ducks are potential source of AMR in food-producing animals but there is still a lack of knowledge of AMR in duck population. Therefore, the data from the research will provide more complete picture of trend and situation of AMR in poultry production in Thailand.

Keywords: Antimicrobial resistance, *Salmonella,* ducks

MI35
Organic produce as a potential alternative source to reduce the spread of antimicrobial resistance bacteria

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Antimicrobial resistance (AMR) is now a serious public health issue worldwide. In response to reduction of antimicrobial use, organic agricultural products have been a new trend for consumers due to the reputation of being environmental-friendly and the limited use of artificial chemical substances, especially antimicrobials. However, the main concern of organic products is the contamination of antimicrobial resistant organisms derived from animal manure used in organic farms. The objectives of this study are to evaluate the concentrations of fecal coliforms and *Escherichia coli*, to determine the association between environmental factors affecting on bacterial loads in conventional and organic vegetables and fruits sold in fresh markets and supermarket, and to determine antimicrobial resistant pattern of *E. coli*.

Seven types of samples consisting of sweet basil, spring onion, coriander, cabbage, lettuce, cucumber and tomato were selected since they are commonly consumed raw or minimally cooked in Thai cuisine. A total of 335 conventional produce were collected from six fresh markets (n=168) and 24 supermarkets (n=167), while organic samples (n=168) were collected from 26 supermarkets. Enumeration method was used to determine the level of fecal coliforms and *E. coli* according to the United States Food and Drug Administration (U.S.-FDA).

The average concentrations of fecal coliforms in conventional samples from fresh markets and supermarkets, and organic samples from supermarkets were $3.52x10^4$, 507, and 51 MPN/g. The average concentrations of *E. coli* in conventional from fresh markets and supermarkets and organic samples from supermarkets were $1.88x10^4$, 44.6, and 26 MPN/g. Season, types of markets and types of vegetables were statistically associated with the concentrations of *E. coli* based on negative binomial regression model. The highest indicator bacterial contamination was observed in sweet basil followed by lettuce, coriander, spring onion, cabbage, cucumber and tomato, respectively.

The concentrations of *E. coli* in organic samples were statistically significant lower compared to conventional samples. Thus, the contamination of antimicrobial resistant organisms might be lesser. High concentrations of *E. coli* observed in fresh markets may occur due to cross contamination from direct contact and less processing before selling. Furthermore, the elevated concentrations of *E. coli* are observed in rainy season maybe because rain can facilitate *E. coli* contamination in produce by splashing the bacteria and aerosol formation. Antimicrobial susceptibility test of *E. coli* will be carried out and the results are expected to support organic produce as an option of antimicrobial resistance reduction.

Keywords: *E. coli*, organic produce, vegetables and fruits

MI36
Typing of resistance plasmid *Escherichia coli* for future development of conjugative inhibitors

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Plasmid is a mobile genetic element that could carry multiple resistance determinants. Horizontal transfer of resistance plasmid occurs between intra- and inter-species and is the major route for emergence and spread of antimicrobial resistance (AMR). Inhibition of R-plasmid transfer is a choice to reduce dissemination of AMR. Research for development of new molecules as conjugate inhibitor(s) should be encouraged. However, data on epidemiology and evolution of R-plasmid is still limited. Commensal *Escherichia coli* resides intestinal tracts of humans and animals and their resistance phenotype and genotype reflect antimicrobial use, while *E. coli* is ubiquitous. Therefore, this study aimed to perform plasmid replicon typing to investigate epidemiology and evolution of R-plasmid in *E. coli* along the food chain. It is to produce molecules used for plasmid curing in the future.

A total of 1,338 *E. coli* were obtained from rectal swab from clinically healthy pigs (n=839), pork (n=396), and humans (n=103) during 2008-2017. These samples come from thirteen provinces across Thailand including Chiang Rai, Nongkhai, Mukdaharn, Udonthani, Nakornratchsrima, Buriram, Suphan Buri, Aung Thong, Kanchana Buri, Ratchaburi, Chachoengsao, Sakaew and Chon Buri. Plasmid replicons were typed by using multiplex PCR for 18 incompatibility (Inc.) groups including A/C, B/O, F, FIA, FIB, FIC, FIIA, HI1, HI2, I1, K, L/M, N, P, T, W, X, and Y.

The result shows that sixteen of eighteen Inc. groups were found except Inc L/M and T. The highest three Inc. groups, i.e. Inc. F, FIB and K were identified in every region of Thailand and possessed more than 60% of each sample type i.e. human, 64.55%; pork, 65.85% and pig, 63.39%. Inc. W, X and HI2 were detected only in *E. coli* isolated from pigs. From geographic data, four Inc. types (i.e. F, FIB, K and N) were identified across Thailand. The Inc. HI1 and I1 groups found in the isolates from every part of Thailand, except the Central part.

The results indicated that plasmids in a variety of Inc. groups can be identified in *E. coli* in Thailand. Particular plasmid patterns were related to particular sources of the isolates (i.e. humans, animals and food). Transferability of plasmid to another bacterium is now under progress. The plasmids should be characterized in the further. The similar research should be conducted in other bacterial species and along food chain. The observations also suggest that novel molecules for curing and inhibiting plasmid should be developed. The molecules must be additionally tested in *in vivo* due to the existence of variable plasmid in clinical strains.

MI37
Multidrug efflux systems as potential targets for new drug development in *F. columnare* isolated from Asian sea bass (*Lates calcarifer*)

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Flavobacterium columnare is a Gram-negative long rod bacterium that causes columnaris disease. This disease leads to economic loss in freshwater fish production in Thailand including Asian sea bass (*Lates calcacrifer*). Antibiotics in many classes have been used for therapeutic objectives such as quinolones, tetracyclines and sulfonamides. Imprudent usage of antibiotics has been evident. However, antimicrobial resistance (AMR) strains have been increasingly reported in *F. columnare,* especially resistance to quinolones. Besides, comprehensive study of quinolone resistance genes and mechanisms is still very limited. This study aimed to investigate quinolone resistance-associated genes of *F. columnare* using genome analysis. The quinolone resistant *F. columnare* isolates exhibited the highest MIC values to oxolinic acid and enrofloxacin (>64 µg/mL and 1 µg/mL, respectively) were submitted to whole genome sequencing and resistome analysis were performed. The complete genome of *F. columnare* consists 3.1 Mb and 2,941 protein coding sequence were predicted. Resistome analysis revealed that 169 genes were predicted as AMR genes and 45 genes were responsible for quinolone resistance. Most of them belong to efflux pump gene family including resistance-nodulation-cell division (RND), major facilitator superfamily (MFS), ATP-binding cassette (ABC) and multidrug and toxic compound extrusion (MATE) transporter efflux pump. Genes relevant to RND efflux pump family, which have been recognized as a major mechanism involved in multidrug resistance phenotype were found at the highest number. This is the first comprehensive study of quinolone-associated genes in *F. columnare* in Thailand and the results also highlight multidrug efflux mechanisms as potential targets for novel drug development against *F. columnare*.

Keywords: antimicrobials, Asian sea bass, characterization, quinolones, *Flavobacterium columnare*

MI38
Resistome analysis of *Aeromonas veronii* NK02 isolated from Nile tilapia (*Oreochromis niloticus)* by focusing in aminoglycoside resistance associated genes

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Thailand is one of the major producers and exporters of freshwater fish in the in the world, of which the most important species is Tilapia (*Oreochromis niloticus*). The production is threatening by bacterial diseases especially *Aeromonas veronii*. The fish farmers usually use antibiotics for treatment of the infections, of which gentamicin is a commonly used antibiotic. Non-prudent use of antibiotics may lead to resistance in aquaculture. This study aimed to determine the susceptibility of *A. veronii* to gentamicin and analyze the resistome based on whole genome sequencing. *A. veronii* NK02, the clinical isolate that is common is used. The *gyrB* gene was first sequence and the MICs of five antimicrobials including amoxicilin sulfamethoxazole/trimethoprim, gentamicin, enrofloxacin, and oxytetracycline were determined by broth microdilution method. The NK02 was resistant to amoxicillin and gentamicin with the MIC value over than 256 µg/ml. The whole genome of *A. veronii* NK02 was sequenced with illumine Hiseq platform. Subsequently, the genome assemble data was submitted to CARD and Resfinder database for resistome analysis, respectively. The results showed the presence of ten antimicrobial resistance genes (ARGs) associated with resistance to aminoglycoside, beta-lactams, quinolone, sulfonamide and tetracycline. In comparison to ARGs database, two of ARGS, *aac(3)-IIb* and *aac(6')-Ib-cr*, were detected. The genome of *A. veronii* NK02 was blasted against the existing database by Blast2GO. The results showed that the nucleotide identity of genes was higher than 85% and related to the phenotypic resistance. Additionally, these genes can be also detected in genome assembly of *A. veronii* isolated from freshwater fish from India, Spain and Korea retrieved from NCBI genome database. However, the nucleotide identity to aminoglycoside resistance genes from those strains was lower than *A. veronii* NK02 (≤60%). However, the percentage of identity to aminoglycoside associated genes is not associated with the MIC value. In conclusion, the aminoglycoside associated genes found in *A. veronii* NK02 may acquire from other resistant bacteria circulated in the aquatic system or selective pressure from non-prudent use of antibiotics. Thus, alternative to antibiotics should be developed for treatment of *A. veronii* either in aquaculture to avoid selection of ARGS and other antimicrobial resistance gene.

MI39
Antimicrobials Susceptibility of *Vibrio* spp. infected Marine Asian sea bass (*Lates calcarifer*) Cultured in Krabi, Thailand

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Asian sea bass (*Lates calcarifer*) is one of the cultured marine fish that has been widely cultivated in South and Southeast Asia, including Thailand. The fish is known for its rapid growth rate, and high tolerance to environmental conditions. One of the major problems in raising this fish is a disease challenge caused by *Vibrio* spp. and antibiotics have been used as a tool to prevent the outbreak. This could result in emergence and spread of antimicrobial resistance, particularly in the aquatic environment. This study was conducted to examine antimicrobial susceptibility of *Vibrio* spp. from infected marine cultured Asian sea bass in Ko Lanta, Krabi Province, Thailand. It is a case study with purposive sampling from the farm post-outbreak of scale drop, fin and tail rot disease which associated with Vibriosis. A total of 40 *Vibrio* isolates were collected from the skin lesions, liver, spleen, and kidney of 12 Asian sea bass. Phenotype identification showed three species of *Vibrio*, namely *V. campbellii* (KR05), *V. parahaemolyticus* (KR08; 20; 21; 30) and *V. harveyi* (KR16; 26; 31; 37; 38; 39; 40). Five antibiotics, enrofloxacin, norfloxacin, oxytetracycline, sulfamethoxazole/trimethoprim and oxolinic acid were tested by Kirby Bauer disk diffusion susceptibility test. Nine strains of *Vibrio* spp. showed inhibition zone ≥ 20 mm and three strains (KR05; KR08; KR26) exhibited undetermined zone of inhibition. The observations showed that most (75%) *Vibrio* spp. strains still are still susceptible to antibiotics tested. However, in order to prevent the emergence and spread of antimicrobial resistance associated with fish farming, prudent use of antibiotics in aquaculture needs to be encouraged. Alternative to antibiotics should be researched for disease prevention and treatment of diseases, especially caused by *Vibrio* spp.in fish farming, not limited to marine cultured Asian sea bass.

MI40
Sex pilus specific bacteriophage to drive bacterial population towards antibiotic sensitivity

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We used plasmid-mediate pilus-specific phages to demonstrate that they not only infect and lyse bacteria harbouring self-transmissible plasmids but also select for mutants which had lost the plasmids and were therefore resistant to the phage, but also a smaller number of mutants with mutations in the plasmid *tra* region so that pili were not produced. The percentage of plasmid-minus mutants increased with prolonger passage in the presence of phage. In groups of young chickens infected with *Salmonella* possessing a highly self-transmissible AMR plasmid pilus phage reduced colonisation and spread between chickens but also selected for massive plasmid loss. These phages also increased plasmid loss in more repressed plasmids but this was less marked. The phages also eliminated conjugation ability.

MI41
Mutations of Streptomycin Resistance Genes in *Mycobacterium tuberculosis* Thai Isolates

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Drug-resistant TB is an alarming issue in Thailand which is one of the 22 high TB burden countries and molecular characterization of anti-TB drugs resistance for this region is necessary. Streptomycin (SM) is recommended by World Health Organization (WHO) as a part of the standard regimens for the retreatment of multidrug-resistant tuberculosis (MDR-TB). To date, the information on SM resistance (SMR) gene mutations correlated to the SMR of *Mycobacterium tuberculosis* (*Mtb*) Thai clinical isolates is limited.

In this study, by using PCR amplification and DNA sequencing analysis, SMR associated mutations of *rpsL*, *rrs*, *gidB* and *whiB7* genes were examined in 101 *Mycobacterium tuberculosis* clinical isolates with various drug susceptibility profiles from Thailand. Their mutation patterns, the frequency related to SMR and the subsequent utility for diagnostic value were determined.

The *rpsL* mutations, Lys43Arg, Lys88Arg and Lys88Thr, and the *gidB* mutations, Trp45Ter and Gly6Asp, were found to be correlated with SMR. The Lys43Arg was the most predominant *rpsL* mutations (94.1%) and found in 69.6% of the SM resistant isolates. Among them, mixed *rpsL* WT and Lys43Arg sequences were found in a SM mono-resistant isolate. The *rpsL* mutations had the highest sensitivity (73.9%) and specificity (96.4%) for the detection of SMR in *Mtb* Thai isolates. Surprisingly, *rrs* mutations associated to SMR were absent in this study. The combination of *rpsL* and *gidB* mutations exhibited 76.1% sensitivity and 96.4% specificity for the identification of SMR. *whiB7* was not responsible for the resistance in SM resistant isolates lacking *rpsL* and *rrs* mutations. Our study suggested that the majority of SMR in *Mtb* Thai isolates were responsible by *rpsL* and *gidB* polymorphisms.

MI42
Antagonistic activity of *Bacillus* Probiotics against Enterotoxigenic *Escherichia coli* (ETEC) and colistin resistant *E. coli* from Pigs in Thailand

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In Thailand, the livestock development department has announced the acts for antibiotics controlling in animal feed since 2018. Many medicated feed including colistin has been controlled and will be used for treatment only. Probiotics are an alternative to reduce antibiotic usage in animals. The aim of this study was to evaluate the antagonistic activity of 21 strains of 4 *Bacillus* species including *B. subtilis,* 13 strains; *B. licheniformis,* 3 strains; *B. pumilus,* 1 strain and *B. amyloliquefaciens,* 4 strains against 8 strains of pathogenic *Escherichia coli* including *E. coli* ATCC 25922, 5 strains of enterotoxigenic *E. coli* (ETEC) and 2 strains of colistin resistant *E. coli* containing *mcr-1* by delayed antagonism in solid nutrient medium method. It was found that 19 strains of 3 *Bacillus* species including *B. subtilis, B. licheniformis and B. amyloliquefaciens* were able to inhibit the growth of *E. coli* reference strain and 7 strains of enterotoxigenic *E. coli*. The inhibitory ability of *Bacillus* on varies among the difference species and strains. *B. subtilis* KMPN008 showed the highest effectiveness against *E. coli* reference strain and 2 colistin resistant *E. coli* strains of which the inhibitory zone was 25.7 $\pm$ 3.1 mm, 27.0 $\pm$ 4.1 mm and 35.8 $\pm$ 4.7 mm, respectively. B. *amyloliquefaciens* 16AvBa17 showed the highest antibacterial ability on both enterotoxigenic *E. coli* 02 and 03. The other 3 strains of enterotoxigenic *E. coli* (i.e. strains 01, 04 and 05) were inhibited by *B. subtilis* 3 strains. The inhibitory zone of KMP- BCI-1, KMP CU 4 and KMP-BCI-2 was 29.8 $\pm$ 8.3 mm, 36.2 $\pm$ 4.8 mm, and 35.8 $\pm$ 3.8 mm, respectively. These studies suggested that use of multiple species and strains of *Bacillus* will generate better inhibitory effects on enterotoxigenic *E. coli* from pigs.

Keywords: *Bacillus* spp., Antibacterial activity, Enterotoxigenic *E. coli*

SESSION 3
Innovative drugs, chemicals and enzymes

ORAL PRESENTATIONS

Strategies to reduce antibiotics in swine production in China

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Antibiotics have been widely used in a prophylactic way in piglet diets to promote growth performance and reduce diarrhea incidence. However, the resistance of pathogens to antibiotics and the risk of residues of antibiotics in animal products induced a growing interest in the use of alternatives to in-feed antibiotics. According to a recent announcement from Chinese government, the antibiotic growth promoter (AGP) except Chinese herbal extracts will be forbidden from production starting from January 1st, from production in animal feed from July 1st and totally out of feed products in circulation from end of next year (2020). Since the in-feed antibiotics are mainly used for relieving the weanling stress and promoting the growth of piglets, different efforts for manipulation of weanling piglet's diets from scientific and industrial community have been inspired by following this new legislation, which including development and extension of antibiotics alternatives, low protein diets, well-chosen feed ingredients, as well as its pre-digestion or pre-processing. On the other hand, environmental control and management measures have also been adopted to improve the health and growth of the pigs, especially the piglets during weanling and nursery stages. Generally speaking, antibiotics alternatives are the first to be extensively investigated and adopted, including the commonly used acidifier, essential oils, medium-chain fatty acids, zinc oxide, probiotics, prebiotics, oligosaccharides, plant extracts and the newly developed antimicrobial peptides, which are mainly functioning through regulation of the intestinal micro-environments including epithelium barriers and commensal microbiota. At the same time, by considering the immature status of intestinal development and enzyme secretion in weanling piglets, selection of feed ingredients which can be easily digested, or with higher digestibility and lower anti-nutritional factors, a diet with lower protein level but more balanced amino acids provision, or a diet supplemented with beneficial fiber, are now been extensively tested and widely applied in the weanling piglets diet. To decrease the burden of intestine for digestion, the pre-digestion or pre-treatment of individual feed ingredient or whole diet by using enzymes, fermentation, their combination or heat treatment are becoming more and more popular, which are considered to produce more nutrients with lower molecular weights, increase digestibility and promote absorption, as well as eliminate the anti-nutritional factors and reduce pathogenic bacteria in the feed. To the last but not least, providing a warm and clean environment has been approved to be efficient for controlling the diarrhea incidence in weanling piglets, and the timely and reasonable manure management has also been suggested to be a contribution of clean space for swine growth. In summary, the strategies mentioned in this review are the current efforts in China aiming to promote the reduction of in-feed antibiotics in swine production and the green development of husbandry industry. We hope this review will be also helpful for the full-chain antibiotics reduction including antibiotics in feed and for therapeutic purpose, which ultimately achieves the goal of no antibiotic residues in meat products.

Keywords: Antibiotics, Alternatives, Dietary manipulation, Management, China

Non-antibiotic treatments for honey bee diseases in the era of omics

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Honey bees provide pollination service to 35% of the world's food crops. The microsporidia parasite, *Nosema ceranae*, affects honey bee health in many ways and has been implicated in the steep global population decline of honey bees. Fumagillin-B is the only antibiotic approved for control of *Nosema* disease in honey bees and has been extensively used in North America for more than 50 years. However, Fumagillin-B has been linked to the problems of resistance development and off-target effects. Further, the product is, in fact, coming off the market due to supply issues. This formidable challenge has spurred scientists to look for alternative treatment options for *Nosema* disease in honey bees. Using genomic, comparative genomics, and transcriptomic approaches, we identified virulent factors that are essential for invasion and replication by *N. ceranae* in honey bees and genes and pathways implicated in host-*Nosema* interactions. We also identified the factors and processes involved in the battle between *Nosema* and the honey bee host, providing us with a list of potential targets for innovative therapeutics to break down the life cycle of the parasite. RNA interference (RNAi) is a remarkable process in which RNA molecules suppress gene expression by neutralizing specific targeted mRNA molecules and has provided unique opportunities in combating diseases caused by pathogens in a wide range of organisms. We explored the potential of RNAi as a therapy for controlling *Nosema* disease in honey bees through two different angles. First, we explored the possibility of silencing the expression of a *N. ceranae* virulence gene encoding polar tube protein 3 (PTP3) that is involved in the host cell invasion as a therapeutic strategy for controlling *Nosema* disease. Our studies showed that the oral ingestion of a dsRNA corresponding to the sequences of PTP3 could effectively suppress the expression of the PTP3 gene in *N. ceranae* infected bees and reduce the *Nosema* load. Secondly, we employed the RNAi strategy to reduce the expression of a honey bee gene, namely naked cuticle (*nkd*), which is a negative regulator of host immune function. Our studies found that *nkd* mRNA levels in adult bees were upregulated by *N. ceranae* infection and that RNAi-mediated knockdown of *nkd* transcripts could efficiently silence the *nkd* expression in *Nosema*-infected bees. We also found that the oral ingestion of dsRNA specific to *nkd* could lead to the upregulation of expression of several genes encoding Antimicrobial peptides (AMPs) such as *Abaecin, Apidaecin*, and *Defensin-1* that are regulated by the Toll pathway. Further, the oral ingestion of a dsRNA specific to *nkd* led to the significant reduction of *Nosema* spore loads and the extension of honey bee lifespan, clearly demonstrating that silencing the host *nkd* gene can activate honey bee immune responses, suppress the reproduction of *N. ceranae*, and improve the overall health of honey bees. The results of our studies strongly suggest that RNAi-based therapeutics hold great promise for the effective treatment of honey bee diseases and will have positive implications for bee disease management practices in the future.

Keywords: Honey bee, pollinator, Nosema, RNA interference (RNAi), therapy

In vitro and *in vivo* characterization of a Gly-substituted DLP4 cationic peptide against *Staphylococcus aureus* CVCC 546

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Staphylococcus aureus is notorious for its ability to become resistant to antibiotics, generating interest in novel antimicrobial strategies. The clinical development of antimicrobial peptides (AMPs) is currently under evaluation. However, most of these AMPs have modest direct antibiotic activity and toxicity. Thus novel peptide D13 designed and optimized from DLP4 with enhanced antibacterial properties was develped. *In vitro* and *in vivo* efficacies were evaluated.

Homologous sequence alignment, circular dichroism analysis, DNA gel retardation, flow cytometry, scanning electron micsroscopy (SEM), transmission electron microscopy (TEM), K^+ leakage and *In vivo* efficacy was tested against *Staphylococcus aureus* CVCC 546 in a mouse thigh infection model.

Base on homologous sequence alignment, a variant D13 from DLP4 with enhanced antibacterial properties was designed. The defensin D13 exerted potent antimicrobial activity against *S. aureus in vitro*, with the minimum inhibitory concentrations (MICs) in the range of 2 to 8 μg/mL. D13 exhibited lower hemolysis toward murine erythrocytes and cytotoxicity against RAW 264.7 cells than DLP4 (0.37% VS 0.42% of hemolysis and 80.9% VS 59.7% of survial, respectively) at the concentration of 128 μg/mL. D13 could distroy the cell membrane of *S. aureus* CVCC 546, resulting in an increase in the K^+ leakage. Gel retardation and CD spectra analyses demonstrated that D13 bound specifically to DNA and disrupted the DNA conformation. After treatment with 4×MIC D13 for 2 h, wrinkled and collapsed *S. aureus* cells were observed in SEM and TEM. Additonally, D13 was highly efficacious in a thigh model of infection with *S. aureus* CVCC 546, causing a 1.8 log10 reduction of CFU in thighs, and a downregulation of TNF-α, IL-6 and IL-10 levels.

The multiple modes of action of D13 against *S. aureus* may minimize resistance development of the target microorganisms. The result suggests that D13 could be a novel promising antimicrobial candidate to treat infectious deseases caused by *S. aureus* in livestock.

Keywords: D13, *Staphylococcus aureus*, antimicrobial peptides, mechanism

Afterlife of bacterial cell debris: Peptidoglycan in the gastrointestinal tract

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An ever-growing body of evidence suggests, that the microbiota in the gastrointestinal tract plays a key role in regulating host metabolism and health. While most attention is directed towards the living bacteria in the gastrointestinal tract, there is little focus on dead and decomposing bacteria. This is surprising, as biomass from dead and decomposing bacteria in the gastrointestinal tract can surpass that of living bacteria. A more holistic view of the microbiota is thus required, particularly including dead and decomposing bacteria.

A major part of the bacterial biomass is peptidoglycan, a complex and sturdy polymer forming the bacterial cell wall. It is built from long glycan strands held together by short peptide bridges. The glycan strand is composed of the *β*-(1,4)-linked sugars *N*-acetylglucosamine and *N*-acetylmuramic acid, the latter of which is uniquely found in peptidoglycans. The peptide bridge is composed both of common L-amino acids and extremely rare D-amino acids. A further structural peculiarity of peptidoglycan is, that the peptide bonds between amino acids in the peptide bridge are not only formed via their backbone but also their side chains, a feature not found in proteins.

Peptidoglycan fragments are continuously released into the gastrointestinal tract as bacterial cells divide, die and decompose. Together with the live microbiota, they could potentially affect the gastrointestinal functionality of the host. To help expand our knowledge about the interplay between diet, microbes and the host, innovative tools to study bacterial waste biomarkers as well as bacterial viability are required.

We have adapted a method used previously to estimate bacterial biomass in soil samples to study bacterial waste biomarkers by quantifying the peptidoglycan building block muramic acid in soluble and insoluble fractions of intestinal content. A novel microbial muramidase has recently been described in literature, which is capable to selectively hydrolyze peptidoglycan from bacterial waste while leaving the live microbiota untouched, thereby improving animal performance. Using our novel method to study bacterial waste biomarkers in digesta samples from animals fed with this novel microbial muramidase, we could show that the enzyme leads to an increase in the amount of soluble peptidoglycan debris by hydrolyzing large, insoluble bacterial waste. We believe that these large and insoluble bacterial waste fragments impair nutrient uptake and when selectively degraded leading to an improved gastrointestinal functionality.

Keywords: Gastrointestinal functionality, bacterial cell debris, peptidoglycan, muramidase, biomarker

Fighting Antimicrobial resistance by optimizing gastrointestinal functionality: A holistic approach

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The occurrence of antimicrobial resistance (AMR) is an issue of global concern and it is catalyzing consumer-driven and regulatory pressure to eliminate antibiotic use in animal production. By 2050 AMR is expected to be a leading cause of death in humans.

Modern animal production has consistently been intensified in order to improve productivity. Increasing stocking density increases the risk of infectious diseases outbreaks. In the past, such diseases were largely controlled by antibiotic growth promoters (AGPs). Even in the absence of clinical diseases, these compounds exerted a beneficial effect on farm animals, since they were capable to control dysbacteriosis and enteropathogens. Since the ban of AGPs in Europe in 2006, the incidence of intestinal problems and dysbiosis has steadily increased. Until recently, 'health solutions' such as AGPs, have provided the 'silver bullet' for gastrointestinal functionality in animal nutrition and health, however, the current industry approach conceals fundamental gaps in the understanding of gastrointestinal functionality and its interactions with husbandry practices including nutrition.

Antibiotic Free (ABF) production systems are a reality in several regions of the world and they are often combined with changes in housing condition, feeding management and health programs. These changes, in combination with strategic use of feed additives, are necessary to optimize the management of the myriad of factors that may influence gastrointestinal functionality.

In farm animals, effective gastrointestinal functionality is vital in determining their health, welfare and productive performance. Several definitions of gut health can be found in the literature; however, they often lack of a precise and unifying meaning or etiology. A new definition of gastrointestinal functionality has been recently offered: "gastrointestinal functionality is a steady state with the gastrointestinal tract and its microbiome are in a healthy equilibrium to perform normal physiological functions which play a critical role in overall health and well-being". This definition pivots around six key components, namely: the diet, effective intestinal barrier, host-microbiome interaction, effective digestion and absorption, effective immune status, and neuroendocrine function of the gut. Each of these components are intimately intertwined by multifaceted physiological mechanisms and pathways. Optimization of gastrointestinal functionality is crucial to increase nutrient digestibility and thus maximizing value from feed, to sustain host physiological functions such as innate and adaptive immunity and thus increasing resilience to environmental challenges, and finally, to maintain eubiotic conditions.

While it is clear that antibiotics must be used responsibly to ensure the health and welfare of animals, we advocate for the replacement of AGPs and reduction in the prophylactic use of antibiotics with alternative nutritional solutions and innovations combined with farm and health management. Future ABF production and its sustainability depends on the development a larger understanding and practical application of concepts related to gastrointestinal functionality that imply complete holistic management of the production system. Maintaining effective gastrointestinal functionality by integrating key husbandry and management practices in a holistic approach, is the most promising concept to promote farm animals' productivity, health and welfare, and to reduce globally the use of antibiotics in animal farming and decrease AMR.

Keywords: gastrointestinal functionality, antimicrobial resistance, nutrition, feed additives, holistic

Organic acids as antibiotic alternatives in monogastric animals

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Young animals have a high susceptibility to various stressors, including bacterial pathogens, oxidative stress and inflammation, leading to reduced growth performance, high mortality and morbidity rates and compromised animal welfare. The empirical benefits of using antibiotics to address animal health issues in animal agriculture (using therapeutic doses) and increasing the overall productivity of animals (using sub-therapeutic doses) are well established. This practice may lead to the spread of antimicrobial-resistant bacterial pathogens in both animals and humans, posing a significant public health threat, which is furthering the need to reduce the use of antibiotics within the animal production chain. Therefore, it is critical to develop cost-effective antibiotic alternatives for ensuring the long-term sustainability of animal production. Organic acids have been known for their ability to prevent food spoilage and extend the shelf-life of many perishable commodities. This capability brought attention to their possible usage in combating bacterial diseases and fighting infections. It has been demonstrated that organic acids have good potential as antibiotic alternatives in feeds for monogastric animal production. The potency of these acids is dependent on the physiological status of the targeted microorganisms and the physicochemical characteristics of the surrounding environment. The combination of different organic acids and other compounds (synergistic effect) such as essential oils seem to be a promising approach to improve the efficacy of organic acids in applications. High-throughput systems technologies have been developed recently, which will allow us to dissect the mechanisms underlying the functions of organic acids and facilitate the use of organic acids in monogastric animal production. This presentation summarizes the efficacy, feasibility and potential mechanisms of the application of organic acids as antibiotic alternatives in monogastric animal production.

Keywords: Organic acids, Medium chain fatty acids, Infection, Antibiotic alternatives, Monogastric animals

25-OH-D3: An indispensable tool to managing antibiotic free feeding programs for commercial broilers

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Antibiotic free (ABF) or raised without antibiotics (RWA) programs are accompanied by a dramatic increase in the incidence of enteritis in commercial broiler flocks. Enteritis is commonly a result of clinical/subclinical coccidiosis or clostridiosis intercurrent with coccidiosis to further cause necrotic enteritis. Researches suggested that maintaining a healthy immune system is one of the keys for success in an RWA program.

25-OH-D_3 (a metabolite of vitamin D_3) has been implicated to support anti-inflammatory reaction. Locally-produced 1a, 25-$(OH)_2$-D_3 (destined for autocrine/paracrine functions of vitamin D) from 25-OH-D_3 by 1a-hydroxylase in immune cells including monocytes, and macrophages inhibits pro-inflammatory cytokine expression and promotes anti-inflammatory cytokine production. This is mediated by inhibition of the nuclear factor *kappa*-B (NFkB), a transcription factor with a critical role in inflammatory response. As such, 25-OH-D_3 may become indispensable in managing the inflammatory nature of enteritis.

Coccidiosis and necrotic enteritis models were applied to 0-to-21-day-old broilers. The birds were challenged with either *Eimeria maxima* or *Eimeria maxima* plus *Clostridium perfringens*. Birds were fed 25-OH-D_3 dosed at either 34.5 (LD) or 69 (HD) mcg per kg diet.

HD birds exhibited higher serum 25-OH-D_3 level than LD birds regardless if they were challenged with *E. maxima* only or *E. maxima* plus *C. perfringens*. At day 21, seven days post *E. maxima* challenge, HD birds had 13 points mortality-adjusted feed conversion ratio (adj. FCRm) advantage over LD birds. In addition, HD birds had lower oocyst shedding (105,211/g) compared to LD birds (150,725/g). At day 21, seven days post *E. maxima* challenge and a day post three consecutive days of *C. perfringens* challenge, HD birds had 4 points advantage of adj. FCRm over LD birds. They also had higher IL-10 (an anti-inflammatory cytokine) gene expression than LD birds. Both HD and LD birds produced similar necrotic enteritis lesions and *E. maxima* lesions.

In conclusion, 25-OH-D_3 could be a convenient tool in managing RWA programs for broilers under the experimental model of disease-challenged conditions reported herein by ameliorating the inflammatory response associated with enteritis.

Keywords: 25-OH-D3, Antibiotic Free, Coccidiosis, Clostridiosis, Enteritis

Alternatives to Veterinary Antimicrobials (AVANT): A new EU project focused on diarrhoea in pigs

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AVANT is a 5-year EU Innovation Action project that will start in January 2020. The aim is to develop and test innovative alternatives to antimicrobials for management of post-weaning diarrhoea in pigs. This is one of the disease conditions for which most antimicrobials are used in livestock worldwide, and effective alternatives to colistin and zinc oxide are missing for treatment of infections caused by enterotoxigenic *Escherichia coli* (ETEC). The AVANT portfolio includes 7 interventions for which pre-clinical studies will be performed to test safety/efficacy and optimize product formulation and administration on an industrial scale. These interventions include a symbiotic product, in-farm faecal transplantation, anti-EHEC phage- and polymer-based products, immunostimulants and various feeding strategies targeting sows at farrowing or piglets at weaning. The most promising interventions will be selected for farm trials to assess their clinical efficacy. Moreover, the effects of these interventions on reduction of antimicrobial use will be determined at the study farms and the potential reduction in antimicrobial use attributable to the adoption of these alternatives at the EU level will be predicted by modelling. The inclusion of a variety of interventions with different modes of action offers the advantage of comparing the efficacy of different intervention measures under controlled conditions and provides the opportunity of integrating multiple interventions with synergistic effects. Moreover, this approach contributes to minimize the risks of the project if a single intervention will perform poorly in the pre-clinical phase.

The strategic objective of AVANT is to significantly reduce antimicrobial consumption by demonstrating field interventions that target the animal species and disease condition accounting for most antimicrobial use in the EU. The introduction of innovative interventions that reduce antimicrobial consumption, disease incidence and production losses is expected to have a great commercial and societal impact by revolutionizing the concept of disease control in the animal health industry and by minimizing the risks of AMR transmission, with positive consequences on animal welfare, public health and economy. In order to accomplish this ambitious goal, AVANT has assembled a highly inter-sectorial consortium with a balanced participation by the public (n=6) and private sector (n=8) that covers complementary types of knowledge (scientific and practical) and meets the high degree of cross-disciplinarily ('dry' vs 'wet' sciences) that is required to demonstrate innovative and sustainable alternatives to antimicrobials. The AVANT consortium comprises 4 leading industries in the animal health sector, 4 highly specialized SMEs, 5 prestigious universities and the Federation of Veterinarians of Europe. Collaboration between these sectors will ensure access to the knowledge, tools and infrastructures necessary to advance our

interventions rapidly and promote project activities and results to the relevant audiences effectively. Our 'multi-actor' consortium ensures that the innovative solutions developed by AVANT cover real needs and are applicable on real farms.

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Keywords: post-weaning diarrhoea, pigs, EU project, inter-sectorial consortium

SESSION 3
Innovative drugs, chemicals and enzymes

POSTER PRESENTATIONS

IN1
Evaluation on the effects of β-mannanase on intestinal health in broilers, based on 31 trials

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β-Mannans, a type of polysaccharide fibers found in most vegetable feed ingredients, are capable of provoking a wasteful innate immune response, which causes intestinal inflammation[1], and affects numerous metabolic functions[2] and performance[3]. Degrading the β-mannans with an enzyme can reverse the adverse effects[1-3].

The evaluation includes 31 trials conducted under field (28) and field-like (3) conditions, during the last six years to evaluate the effects of a β-1,4-mannanase on broiler performance and intestinal health. Data integrity and relevance of the trials was ensured by screening against a set of pre-established inclusion criteria. All lesions were scored according to a robust scoring system, developed by Elanco[4]. 3580 apparently healthy birds were euthanized and necropsied during the trials for collection of lesion scores related to bird health and welfare, and the scores of 23 conditions related to intestinal health were combined in an intestinal integrity index (I2). Statistically significant improvements were, among others, demonstrated on the I2 index (Control (C)=92.6 and β-mannanase (T)=93.6; P<0.0001), Cellular sloughing (Incidence: C=18.4% and T=14.3%; P<0.01), Excessive intestinal fluid (Incidence: C=17.6% and T=14.8%; P<0.05), Gross *E. acervulina* lesions (incidence: C=30.2% and T=24.7%; P<0.01), Litter Eater (Incidence: C=17.6% and T=14.1%; P<0.01), Feed passage (Incidence: C=12.3% and T=10.1%; P<0.05), and pododermatitis (Incidence of severe lesions: C=18.5% and T=13.8%; P<0.001).

The evaluation demonstrated that the use of a β-1,4-mannanase feed enzyme to degrade β-mannans may improve intestinal health and reduce the incidence and severity of pododermatitis.

Keywords: β-Mannananase, intestinal health, broilers

IN2
The Efficacy of Sodium Humate to Control Diarrhoea and Support Performance of Fattening pigs

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Sodium Humate (HNa) is the sodium salt of humic acid derived from the decomposition of organic matter from plants and animals. HNa can inhibit bacterial and fungal growth and decrease levels of mycotoxin contamination as well as food poisoning. This study was conducted in 10 WOA fattening pigs in a commercial farm in Chonburi province of Thailand.

According to the study, 42 pigs at average weight 29.46 kg were divided into 3 equal groups (14 pigs/group): C-control with common feed, TA-Treatment A with HNa 1 kg/ton feed, and TB-Treatment B with HNa 2 kg/ton feed. Pigs were fed for 21 days. The daily clinical observation was done once a day. Individual weighting was conducted after 21 days feeding. Statistical analysis was by a t-test and significant difference at $P<0.05$.

During the study, no pig showed serious diarrhea to be detected by the observer. Final weight (kg/pig) after 21 days of feeding was 42.00±2.35, 42.50±3.91, and 43.96±3.96 for C, TA, and TB, respectively, $P>0.05$. Feed intake (kg/pig/day) was 1.22^{a}, 1.22^{a}, and 1.24^{b} for C, TA, and TB, respectively, $P<0.05$. ADG (g/day) was 597.14±112.06, 620.95±186.08, 690.68±188.51 for C, TA, and TB, respectively, $P>0.05$.

From this study, HNa at 1 and 2 kg/ton feed for 21 days of feeding showed the positive efficacy to the performance of fattening pigs. Pigs fed with HNa 2 kg/ton feed showed a significantly higher feed intake. In conclusion, HNa can be a possible alternative to antibiotics. Further study in the higher number of pigs will be conducted for reliable statistical analysis.

Keywords: Sodium Humate, efficacy, diarrhoea, performance, fattening

IN3
Inhibition of *Staphylococcus aureus* Biofilm Formation and Its Persisters by novel fungal defensin P2

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There is an urgent need to discover new active drugs to combat methicillin-resistant *Staphylococcus aureus*, which is a serious threat to humans and animals and incompletely eliminated by antibiotics due to its intracellular accumulation in host cells, aggregation of biofilms and persisters. The novel antimicrobial peptide P2 from fungus *Pyronema omphalodes* with multiple antimicrobial mechanisms can be used as candidates.

Confocal laser scanning microscopy (CLSM), crystal violet staining methods, qRT-PCR, intracellular antibacterial activity, cytokines regulation in vivo were used in this study.

S. aureus ATCC43300 and *S. aureus* E48 had the ability to produce biofilm, which were identified by the Congo red and crystal violet staining. The genes related to biofilm formation of the two strains were analyzed by PCR. P2 is a new fungal defensin which is screened from recently sequenced fungal proteome.The results showed that P2 inhibited the initial formation of biofilms, and P2 at 8×MIC achieved 99% inhibition rate and it also eliminated mature biofilms (elimination rate: 64.7%-81.4%). The above effect on mature biofilms was further confirmed by laser confocal microscopy. At the same time, the persister bacteria (approximately 99%) in biofilm were efficiently killed by P2 of 16×MIC within 24 h, which was superior to plectasin. P2 may inhibit/disrupt biofilm formation by regulating *SarA* and *icaD* genes expression. P2 exhibited the potent activity against intracellular MDR *S. aureus* (bacterial reduction in 80–97%) in RAW264.7 macrophages, which was better than vancomycin. P2 regulated the cytokine in mice challenged with *S. aureus* E48. Finally, we observed in vivo that 5 mg/kg of P2 inhibited the bacterial translocation and alleviated multiple-organ injuries (liver, spleen, kidney and lung), and improved the survival of *S. aureus*-infected mice (100%), superior to vancomycin (30 mg/kg). Moreover, P2 also had a good inhibitory effect on the biofilm in mice.

The novel fungal defensin antibacterial peptides P2 has a potent efficacy to *S. aureus* biofilms and persisters, which is superior to vancomycin and plectasin. P2 has some protective effect on mice model with *S. aureus* E48-induced peritonitis and biofilm. These data suggested that P2 may be a candidate for novel antimicrobial agents against MDR staphylococcal infections.

Key words: P2, *Staphylococcus aureus*, intracellular activity, anti-biofilm ability

IN4
Clearing the lipopolysaccharide after killing multiple-drug resistant *Escherichia coli* by chimeric peptides-A6 and G6

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Antibiotics rapidly kill pathogenic Gram-negative bacteria, but simultaneously accelerate lipopolysaccharide (LPS) release from the cell envelopes, which leading to downstream cascade of inflammatory and sepsis. It is known that some antimicrobial peptides (AMPs) have potent high antibacterial activity against Gram-negative bacteria. However, antibiotics and most AMPs cannot adequately clean LPS due to weak LPS-neutralizing capacity. Here, to clear "battlefield" - LPS after killing bacteria, the LPS-targeted "smart" chimeric peptides (SCPs)-A6 and G6 were generated by connecting LBP14 (targeting LPS) and N6 (killing pathogen) via rigid and flexible linkers, respectively, and their properties, functions and mechanisms were firstly determined *in vitro* and *in vivo*. Results showed that both linkers retained the independent original biological activities from each parent component. Both A6 and G6 exerted low toxicity and no bacterial resistance, and more rapidly killed multiple-drug resistant *Escherichia coli* and more effectively neutralized LPS toxicity than treatment from N6 alone. At a dose of 0.125 μmol/kg, SCPs-A6 and G6 enhanced the mouse survival (100%), superior to N6 (60%) and polymyxin B (40%, 5 μmol/kg), alleviated lung injuries by blocking mitogen-activated protein kinase and nuclear factor kappa - β p65 activation. It uniquely showed that SCPs-A6 and G6 may be promising dual-function candidates as novel antibacterial and anti-endotoxin agents to treat bacteria and sepsis.

Keywords: lipopolysaccharide, *Escherichia coli*, chimeric antimicrobial peptides

IN5
Effects of supplemental dietary gamma-aminobutyric acid on growth performance and stress indicators in broiler chickens raised at different stocking densities

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Stocking density has critical implications for the welfare, health, and productivity of commercial broilers. Gamma-aminobutyric acid (GABA) is a primary inhibitory neurotransmitter and widely used in the animal industry as a safe feed additive to improve growth performance and to alleviate physiological stress. However, the direct role of dietary GABA in alleviating the stress of high stocking density and interaction between two of them have never been investigated in broiler chickens. Therefore, this study was conducted to test whether dietary GABA could improve growth performance and alleviate physiological stress in broiler chickens raised at high stocking density.

A total of 900 one-day-old male broiler chicks (Ross 308) were assigned to 4 treatments with 10 replicates of 15 or 30 birds in a completely randomized design. A 2 × 2 factorial treatment arrangement was used with stocking density and dietary GABA as the main factors. Experimental diets were formulated to mix the basal diet with or without 100 mg/kg of GABA and fed to birds kept at 7.5 birds/m^2 (low stocking density; LSD) or 15 birds/m^2 (high stocking density; HSD).

Body weight gain was decreased in chickens raised at HSD compared with LSD-raised chickens during all phases (P<0.05). Feed intake was decreased in HSD-raised chickens compared with LSD-raised chickens during the starter and whole phases (P<0.01). Feed conversion ratio was decreased during the starter phase, however, it increased during the finisher phase in HSD-raised chickens. Relative liver weight was increased in chickens fed the GABA-supplemented diet compared with chickens fed the basal diet on day 21 (P=0.021) and was increased in chickens raised at HSD compared with chickens raised at LSD on day 35 (P=0.013). Blood heterophil to lymphocyte ratio was decreased in chickens fed the GABA-supplemented diet compared with chickens fed the basal diet (P=0.037). Also, the concentration of corticosterone in serum samples tended to decrease in chickens fed the GABA-supplemented diet compared with chickens fed the basal diet (P=0.088).

The present results demonstrate that increasing stocking density impaired growth performance. Dietary GABA did not affect growth performance, but it lowered the heterophil to lymphocyte ratio in blood and the concentration of corticosterone in serum regardless of stocking densities. Our finding suggests that dietary GABA is effective in mitigating stress responses, but the effect is independent to stocking density.

Keywords: gamma-aminobutyric acid, stocking density, growth performance, stress indicators, broiler chickens

IN6
High-performance plasma biomarker for Penicillin-G resistance in a model of *Staphylococcus aureus* bacteremia

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Rapid determination of antimicrobial susceptibility/resistance is the key factor in selecting an appropriate antimicrobial treatment and eradicating infections promptly. Conventional antimicrobial susceptibility tests (ASTs) are not precise enough and are very time consuming. In addition, resistant bacteria generally release an enzyme to convert the active antimicrobial agent to an inactive metabolite, and this motivated us to develop a liquid chromatography-mass spectrometry (LC-MS/MS) method in our previous study for the rapid determination of the resistance level as well as the selection of the correct antimicrobial treatment. In this study, we extended our previous exploration to determine the resistance of *Staphylococcus aureus* to penicillin-G in an animal-infection model by means of the LC-MS/MS rapid method. The method was successfully applied to the rapid determination of resistance in a *S. aureus* bacteraemia model. This newly developed method is able to determine the extent of antimicrobial resistance qualitatively and quantitatively within 1 h, and can be used to replace conventional AST methods which take 3 days to determine resistance.

Keywords: Mass spectrometry, antibacterial susceptibility test, chicken infection model, antibacterial resistance, Rapid diagnosis

IN7
In vitro Synergistic Potentials of Novel Antibacterial Combination Therapies against Pathogenic Bacteria

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Bacteria have remarkable abilities to acquire resistance against antibiotics by several mechanisms. New strategies are needed to block the development of resistance and to prolong the life of traditional antibiotics. This study aimed to increase the efficacy of existing antibiotics by combining them with the opportunistic phenolic compound gallic acid (GA) and its derivatives. Fractional inhibitory concentration (FIC) indexes of phenolic compound-antibiotic combinations against *Salmonella enterica* serovar Typhimurium, *Escherichia coli* and *Staphylococcus aureus* were determined. Based on the FIC indexes and clinical importance, 3 combinations were selected to evaluate their effects on the virulence factors of these bacteria. The *in vitro* cytotoxicity of GA and hamamelitannin in the *Rattus norvegicus* (IEC-6) cell line were evaluated. Phenolic compounds were demonstrated to yield considerable antibacterial effects as the MICs of epigallocatechin, GA and hamamelitannin found against different strains were (32–1024), (128–1024) and (512–≥2048) µg/mL, respectively. The FIC indexes of the combined antibacterials against these strains were 0.281–1.016. The ultrastructural morphology and time-kill assays showed that the GA-ceftiofur combination, and hamamelitannin-erythromycin and GA-ampicillin combinations more efficiently inhibited the growth of *S.* Typhimurium and *E. coli*, respectively, compared to the individual antibiotics. Biofilm viability and the swimming and swarming motilities of *S.* Typhimurium in the presence of GA-ceftiofur and *E. coli* in the presence of the hamamelitannin-erythromycin and GA-ampicillin combinations were more competently inhibited than individual antimicrobials. The 50% inhibitory concentrations of GA and hamamelitannin in IEC-6 cells were 564.55 µM and 988.54 µM, respectively. The phenolic compounds increase the efficacy of existing antibiotics might be by disrupting virulence factors. We can conclude that these antibacterial combinations are safe and can be potential medications to treat *S.* Typhimurium, *E. coli* and *S. aureus* infections in animals and humans. Further study to confirm this effect in *in vivo* system and to determine the precise mechanism of action should be undertaken to establish these combinations as medications.

Keywords: Antimicrobials, Antimicrobial resistance, Phenolic compound, Biofilm, Fractional inhibitory concentration indexes

IN8
Eimeria challenged study with natural coccidiosis prophylaxis on alternatives to anticoccidals

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One of the biggest challenges faced by the global poultry industry is coccidiosis. However widespread use of anticoccidials resulted in the reduction of sensitivity or even resistance worldwide. In view of this situation, more and more phytogenic molecules are introduced to anticoccidials market trying to offer alternative solutions.

The active ingredient of the product in this study is 3,4,5-Trihydroxybenzoicacid (THB) which had proven effect against *Eimeria* spp. both in vitro and in vivo. The mechanism is to reduce the activity of sporozoites as well as to inhibit invasion and proliferation in host cells.

The aim of this study is to evaluate the effect of natural base product, COZANTE™ on the performance of broiler challenged with *Eimeria tenella*, *E. necatrix*, *E. acervulina* and *E. maxima* comparing with ionophores (Salinomycin) and chemicals (Diclazuril and Narasin-Nicarbazin).

The result of the study was COZANTE™ 120 g/MT and 150 g/MT worked as well as ionophores and chemicals on decreasing lesion scores caused by *Eimeria tenella*, *E. necatrix*, *E. acervulina* and *E. maxima*. COZANTE™ 120 g/MT and 150 g/MT worked as well as Diclazuril and Narasin-Nicarbazin on decreasing OPG (oocysts per gram of feces) number and significantly better than Salinomycin. We also found lower mortality with all COZANTE™ groups than ionophores and chemicals. There was no significant difference on body weight ($p>0.05$) between all groups. There was no significant difference on FCR ($p>0.05$); but the significant difference between COZANTE™ 120 g/MT and positive control (1.7: 1.9).

Keywords: coccidiosis, *Eimeria*, Cozante, Thb, OPG

IN9
Effects of tylan removal and increasing dietary roughage concentration on liver abscess disease

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Liver abscess formation is a multifaceted disease of great importance to feedlot cattle health management with the potential to cause substantial economic losses to U.S. beef industry stakeholders. In 2011, reduction in industry profitability due to unrealized liver value was estimated to be $15.8 million. Tylosin is used to reduce liver abscess rates in US feedlots, but limited data are available to suggest whether increased roughage concentration in high energy diets is a viable strategy for managing this disease. Therefore, a study was conducted to determine the impact of typical finishing rations with and without tylosin and the titration of roughage concentration in finishing diets without tylosin on liver abscess prevalence and severity (score) at harvest.

A randomized complete block design with 4 treatments and 12 pen replications per treatment was used with 65-70 steers/pen (3,340 steers total; 796 lb. average arrival weight). Treatments included 7.1% corn stalks (DM basis) and 9.6 g/ton tylosin (7.1 TYL), 7.1% stalks without tylosin (7.1 NT), 13.1% stalks without tylosin (13.1 NT), and 19.1% stalks without tylosin (19.1 NT). Rumensin (monensin [fed entire study]) and Optaflexx (ractopamine [fed last 29 days of study]) were included in all treatments. Mean feeding period was 161 days.

Total liver abscess disease was reduced ($P = 0.006$) in the 7.1 TYL group (13.03%), compared with 7.1 NT (19.18%). The A+ (1.99%) and A+/ adhered (0.87%) were reduced ($P < 0.04$) in the 7.1 TYL group, compared with 7.1 NT (3.75% and 3.27%, respectively). When comparing 7.1 TYL to 13.1 NT and 19.1 NT, no difference in total disease or A-, A, or total A+ scores was observed ($P \geq 0.13$). The 7.1 TYL treatment had fewer ($P = 0.008$) A+/ adhered livers, compared with 13.1 NT (2.93%) and 19.1 NT (2.21%). In 7.1 NT, 13.1 NT, and 19.1 NT treatments, increased roughage content decreased ($P \leq 0.03$) total disease (19.8%, 11.88%, and 14.4%) and A+ scores (8.83%, 5.57%, and 6.01%). Increasing roughage to 13.1 NT and 19.1 NT treatments resulted in linear increased feed consumption (0.9 and 1.3 lbs/day, respectively), less total live weight gained (3 and 14 lbs, respectively), reduction in carcass weight (6 or 21 lb., respectively), and had increased feed: gain (0.31 and 0.59, respectively), compared with 7.1 TYL.

In conclusion, tylosin was effective for the reduction of total liver abscess occurrence and severity. While increased corn stalks were able to compensate for the effects of tylosin removal on liver abscess occurrence, dietary roughage was not effective for controlling the severe liver abscesses that negatively affect packing plants. In addition, cattle fed increased corn stalks were unable to compensate for the lower dietary energy intake, which reduced the total amount and efficiency of beef produced.

Keywords: liver, abscess, tylosin, roughage, cattle

IN10
Effects of tylan defined feeding duration and dietary roughage type on liver abscess disease

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The pursuit of a reduction in liver abscess prevalence in feedlot cattle is of great importance to health management and beef industry stakeholder profitability. Disease effects are multifaceted, but primarily materialize in liver condemnation, lost feedlot performance, and reduced carcass weight and quality grade. The impact is further magnified by the increased prevalence of severe disease (A-plus liver score) at harvest that is associated with carcass trim and condemnation. Tylan Premix (tylosin) is labelled for reduction of incidence of liver abscesses associated with *Fusobacterium necrophorum* and *Arcanobacterium pyogenes*. To further understand liver abscess disease, a study was conducted to determine the impact of a defined tylosin duration feeding period and roughage type on liver abscess disease prevalence and severity (score) at harvest.

Five treatments with 8 replicates were used in a 2 x 2 + 1 incomplete factorial design utilizing 9,396 steers. Treatments included corn silage without tylosin fed (SILNOTY), corn silage with tylosin fed continuously (SILALLTY), corn silage with tylosin fed continuously until the last 30 days prior to harvest (SILBAATY), corn stalks with tylosin fed continuously (STKALLTY), and corn stalks with tylosin fed continuously until the last 30 days prior to harvest (STKBAATY). Roughage was formulated at an equal forage neutral detergent fiber (NDF) level in each diet. With the exception of 234 heads/pen in Block 6, 235 heads/pen was enrolled in the remaining blocks. Tylosin treatment (10 g/ton [100% dry matter basis]) was initiated after finishing ration adaptation. Mean feeding period was 152 days.

Tylosin and roughage source both impacted liver abscess formation. Total liver abscess prevalence and A-plus score were significantly reduced ($P<0.001$) by 47.6% and 62.4%, respectively, by feeding tylosin continuously (SILALLTY), compared with not feeding tylosin (SILNOTY). Both total liver abscess prevalence and A-plus score significantly increased ($P < 0.05$) for cattle fed stalks as a roughage source, compared with SILALLTY and SILBAATY. A trend ($P = 0.06$) was observed for increased total liver abscess prevalence in SILBAA (19.7%), compared to SILALLTY (17.2%). No significant difference in total liver abscess prevalence was detected for STKBAA (21.0%), compared with STKALLTY (21.9%).

In conclusion, these results demonstrate the importance of Tylan Premix as a therapeutic feed additive for reducing the incidence of liver abscesses. Removal of tylosin in the silage and stalks diets during the defined feeding duration period did not have a substantial impact on liver abscess prevalence or severity. Steers fed stalks had an increase in total liver abscess disease and A-plus score, compared with steers fed silage and tylosin. These results do not support the use of corn stalks as a roughage source at a similar forage NDF level as silage for the reduction of liver abscess disease prevalence or severity.

Keywords: liver, abscess, tylosin, roughage, cattle

IN11
Inhibitory effect of SCFA and MCFA on contaminats of liquid pig feed and intestinal bacteria

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Microbial contaminants present in liquid pig feed seem to be susceptible to short chain fatty acids (SCFA) and medium chain fatty acids (MCFA). Adding these acids triggers growth inhibition and this would be valuable to optimise production, storage and hygiene of liquid pig feed. Similarly, intestinal microbes found in pigs seem to be susceptible to SCFA and MCFA but may depend on bacterial strain and other conditions. The objective of this *invitro* study was to determine the growth inhibitory effect of several SCFA and SCFA+MCFA combinations on 6 bacterial and 2 yeast strains. The SCFA and/or MCFA products (1 l/t) were added to a liquid bacterial growth media after which the media was inoculated with 9% v/v overnight cultured microbial inocula. Growth media pH and temperature were adjusted for tested strains as follows: *Escherichia coli*, *Salmonella enterica*, *Staphylococcus aureus*: TSGY, pH 5, 37°C; *Clostridium perfringens*: TSGY, pH 6.2, 37°C, anaerobic; *Streptococcus suis*: TSB with 2% serum, pH 7, 37°C microaerophilic; *Campylobacter jejuni*: BHI, pH 6.5, 37°C, microaerophilic; *Candida humilis* and *Saccharomyces cerevisiae*: YM, pH 5, 25°C. Growth inhibition was determined measuring culture optical density at 600nm at multiple time events over a 24h period, except for *Campylobacter jejuni* where another method was used at 24h. Growth inhibition (%) per product was statistically analysed relative to the negative control using a two-tailed Student t-test. All products inhibited the growth of tested bacterial strains dependent on time after inoculation, except for the growth of *Campylobacter jejuni*. The SCFA+MCFA combination showed superior growth inhibition, whereas lactic acid had the lowest efficacy. Only the SCFA+MCFA combination was able to inhibit the growth of yeast cells effectively. *E. coli* showed a time-dependent susceptibility to SCFA and MCFA. In conclusion, SCFA and SCFA+MCFA inhibited growth of several bacterial strains. The SCFA+MCFA also inhibited yeast cell growth.

Keywords: SCFA, MCFA, bacteria growth inhibition, yeast growth inhibition, SCFA and MCFA combination

IN12
Effects of dietary fiber in weaning pig diets on growth performances, nutrient digestibility and intestinal health

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This study aimed to determine the effect of dietary fiber level for weaning pig on growth performances, nutrient digestibility and intestinal health from intestinal morphology and bacterial count parameters. Total of 30 weaning pigs at 21±3 days of age were kept in the individual cage and fed randomly with 3 experimental diets consisting of three different levels of total dietary fiber (DF) at 130, 140 and 150 g/ kg with the same ratio of insoluble fiber per soluble fiber at 4.0. All diets were formulated with nutritional balance using the source of insoluble and soluble dietary fiber from grounded rice hull and pectin. Pigs were fed *ad libitum* with experimental diets for 28 days after weaning. Growth performances were determined for the weekly and overall period. Fecal characteristic was determined daily for calculated of diarrhea incidence. At d29 after fed with experimental diet, four pigs per treatment were randomized and euthanized for the collection of tissue samples in different segments of the small intestine (duodenum, jejunum and ileum). The sample of gut digesta from ileum, caecum and colon were collected for the microbial count. The intestinal morphology was determined by a light microscope at 40X magnificent. For digestibility study, twelve weaned pigs were housed in an individual cage and fed 7 days with an experimental diet for fecal collection. The nutrient digestibility of experimental diet using indirect method and 0.5% chromic oxide was mixed in the diet as an indicator. The FCR of weaning pig at the first week was improved (P=0.023), however, there was not significantly different (P>0.05) on growth performances of weaning pig evaluated overall period (28 days). The diarrhea incidence numerically decreased in a high level of dietary fiber. The nutrient digestibility coefficient of energy and protein in weaning pig were not different among treatment diet while digestibility coefficient of crude fat and fiber tended to increase (P =0.082 and P= 0.074) when the increasing of DF level. The dietary fiber was not affected to villus height, crypt depth and villus height per crypt depth ratio (VH: CD) of duodenum and jejunum. The increasing of DF level in diet tended to increase the villus height and enhance VH: CD especially at DF level 140 g/kg diet. The bacterial count in gut content was not affected by dietary fiber level. In conclusion, a dietary level at 140 g/kg in weaning pig diet by adding grounded rice hull could improve feed efficiency and decreases diarrhea incidence of the pigs at the first week after weaning without any effect on nutrient digestibility, intestinal morphology and some bacterial count in gut content.

Keywords: weaning pig, dietary fiber, digestibility, intestinal health

IN13
The use of the dry-off facilitator velactis (cabergoline) in selective dry cow therapy

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The dry period is a crucial resting period for the dairy cow that is close to calving and in transition to a new lactation. One important objective for drying off dairy cows is to minimize the risk of intramammary infections (IMI) and ensure a healthy production in the next lactation.

The level of milk production before drying off and the incidence of milk leakage during the week after the last milking are known to be risk factors for new IMI during the early dry period. The reason why it is recommended to reduce the level of milk production at dry-off is that, the higher the milk production at the moment of dry-off, the higher the risk of new IMI. As mentioned above, for every 5 kg increase in milk production at dry-off above 12.5 kg, the odds of a cow having an IMI at calving increases by 77% (Rajala-Schultz *et al*., 2005). The reduction of milk leakage resulting from decreased milk production could also decrease the incidence of new IMIs.

Velactis (cabergoline) is a dry-off facilitator that offers a novel way to reduce milk production at the time of drying off. It facilitates the drying off procedure when used together with the abrupt cessation of milking. Velactis contains cabergoline, a prolactin release inhibitor which acts on the hypothalamus and suppresses prolactin production in the pituitary gland. Prolactin is stimulates milk production by the alveolar cells of the mammary gland, thus causing milk to accumulate in the udder. By reducing prolactin, Velactis acts to decrease milk yield in dairy cows at dry-off. As a result, there is also a reduction in the risk of milk leakage, new IMI and discomfort at dry-off and during the dry period. Studies show that one intramuscular injection of Velactis at dry-off significantly decreases milk leakage the first day after dry-off by 81% and consequently decreased new IMI across the dry period and immediately post calving by 21% (Hop et al, 2019). In a recent study in commercial dairy herds in the UK (Bradley *et al*., 2019), Velactis use was associated with a lower incidence of clinical mastitis in the following lactation. Uninfected quarters from cows receiving both Velactis and an internal teat sealant (ITS) at drying off were at a significantly lower risk of developing clinical mastitis in the first 100 days-in-milk in the subsequent lactation compared with quarters in cows receiving ITS alone or antibiotic alone. When used in combination with an ITS, Velactis offered a clear alternative to antibiotic DCT in uninfected cows. Moreover, the decrease in the number of cases of clinical mastitis will result in reduction in the use of antibiotics for treatment of cows with mastitis.

In summary, dry-off facilitators like Velactis are a useful aid for drying off modern dairy cows in the current era of minimising antibiotic dry cow therapy whilst having a positive effect on udder health and animal welfare.

Keywords: cabergoline, Velactis, mastitis, dry-off, antibiotic

IN14
A paper-based microfluidic device (DON-Chip) for rapid and low-cost deoxynivalenol quantification in food, feed and feed ingredients

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Mycotoxin contamination causes over 5 billion dollars of economic loss per year in the North American food and feed industry. A rapid, low-cost, portable and reliable method for on-site detection of deoxynivalenol (DON), a representative mycotoxin predominantly occurring in grains, would be helpful to control mycotoxin contamination. In this study, a paper-based microfluidic chip capable of measuring DON (DON-Chip) in food, feed and feed ingredients was developed. The DON-Chip incorporated a colorimetric competitive immunoassay into a paper microfluidic device and used gold nanoparticles as a signal indicator. Furthermore, a novel ratiometric analysis method was used to improve detection resolvability. Detection of DON in aqueous extracts from solid food, feed or feed ingredients was successfully validated with a detection range of 0.01-20 ppm (using dilution factors from 10-10^4). Compared with conventional methods, the DON-Chip greatly reduces the cost and time of mycotoxin detection in the food and feed industry.

Keywords: paper-based microfluidic device, deoxynivalenol quantification, competitive immunoassay, low-cost, on-site detection

SESSION 4
Phytochemicals

ORAL PRESENTATIONS

Sensing and reacting: micronutrients and phytochemicals in gut health

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The gastrointestinal tract is confronted with a cornucopia of diverse chemicals, pathogens and physicochemical states that it must analyze and react to appropriately to optimize nutrition and to defend against harm. It presents the largest and most vulnerable body surface that faces the outside world. Integrated responses to these challenges require the gut to sense its environment. This it does through a range of detection systems for specific chemical entities, pathogenic organisms and their products, and physico-chemical properties of its contents. Receptors for nutrients and micronutrients include taste receptors, free fatty acid receptors, peptide, micronutrient and phytochemical receptors, many of which are located on enteroendocrine cells. Hormones released by enteroendocrine cells act locally, on other organs such as the pancreas, and via the nervous system to optimise digestion. Pathogen detection is both through antigen presentation to T cells and through pattern recognition receptors (PRRs). Activation of PRRs triggers local tissue defence, for example, by causing release of antimicrobials from Paneth cells. Toxic chemicals, including pharmaceuticals, are sensed and then avoided, expelled or metabolized. Bacterial products are also detected. Sensory information is communicated to four major effector systems: the enteroendocrine hormonal signalling system; the innervation of the gut, both intrinsic and extrinsic; the gut immune system; and the local tissue defence system.

Phytochemicals are a special component of foods. They occur in low amounts, and add little directly to nutrition. Phytonutrients can contribute to improved nutrient conversion, reduced food spoilage, antimicrobial actions, improved palatability, enhanced gut health, including immune defense and mucosal growth promotion. They provide signals to the intestine that can have beneficial downstream effects.

They are thus valuable food additives that can promote gut health and improve animal productivity. Many of the phytochemicals act on specific receptors, and commonly these receptors belong to the TRP (transient receptor potential) class of receptors. For example, capsaicin from peppers acts on TRPV1 receptors and cinnamaldehyde acts on TRPA1 receptors. However, our knowledge of the receptors for phytochemicals is incomplete.

Major challenges include determination of the mechanisms of action of phytochemicals, quantitative evaluation of their effects, determining how they interact with the microbiota, and investigation of their benefits at specific life stages, for example in growers, in pregnancy, in early life, and under environmental threat.

Mechanisms of Baitouweng Decoction in the Treatment of Diarrhea Caused by *Escherichia coli*

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Baitouweng decoction (Pulsatilla decoction, PD), from Treatise on Febrile Diseases in the Eastern Han Dynasty, is a classical prescription in traditional Chinese medicine that has therapeutic effects on wetness-heat-induced diarrhea, Which is composed of four herbs Baitouweng (Pulsatillae Radix), Huang Lian (Coptis Rhizome), Huang Bai (Cortex Phellodendri), and Qin Pi (Cortex Fraxini). Although PD has a good therapeutic effect on diarrhea caused by Escherichia coli or its toxins, the experimental study of its antibacterial activity in vitro is not very good. So how does PD kill bacteria?

Escherichia coli (*E. coli*) is an intestinal infectious disease that is harmful to animal health and even causes death. Lipopolysaccharide (LPS) is a biologically active substance and exists in the outer membrane of pathogenic *E. coli*. In the infectious foci, LPS is released from dead *E. coli* to local microenvironments and transported to tissues and organs. LPS displays a potent ability to induce inflammatory responses. Microvascular endothelial cells (MVECs) are important sites for the exchange of substances inside and outside the blood vessels, forming a major defense barrier in the body. MVECs play an important role in maintaining normal physiological and immune functions of the body and maintaining homeostasis. The current study found that the target area for pathological changes of various diseases is MVECs. LPS induces MVECs injury, which leads to disorder of function and inflammation of the intestine.

Neutrophils, which are polymorphonuclear leukocytes, form into the blood circulation after being matured in the bone marrow. They are the innate immune cell of the body, participate in the body's immune response and enhance the defense of the body. Neutrophils are powerful effector cells for clearing bacterial infection and controlling inflammation. Neutrophils function as the first line of defense against bacterial infection. Once they recognize the signals of infection, neutrophils rapidly transmigrate to the infected tissue through vascular endothelial cells and exert its role in resisting pathogens and controlling inflammation. Many studies have shown that impaired MVECs are key factors in inducing many diseases and their complications in the body. The bactericidal function of PMNs is closely related to the state of MVECs. MVECs play an important role in fighting with bacterial infections in PMNs through multiple pathways. Our previous studies found that the bacterial toxin (LPS) released by *Escherichia coli* can damage the microvascular endothelial cells, thus weakening the function of trans endothelial neutrophils to kill bacteria. PD and its active ingredients have the function of inactivating toxins and protecting microvascular endothelial cells from bacterial toxins. Therefore, neutrophils can release granulase after crossing microvascular endothelial cells to kill bacteria.

Keywords: Pulsatilla decoction, *Escherichia coli*, microvascular endothelial cells, neutrophils, kill bacteria

An anthocyanin-rich purple potato extracts reduce high fat diet and lipopolysaccharide (LPS) induced obesity and low-grade gut inflammation

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The gut epithelium acts as a physical and chemical barrier against pathogenic invasion and toxic metabolites. However, this barrier function can be compromised by high-fat diet and gut inflammation. This study aims to determine health benefits of anthocyanin-rich purple potato (PP) in low-grade inflammation obese mouse model. A dose-dependent inhibitory effect of PP supplementation was found to prevent the elevation of pro-inflammatory mediators (e.g. TNF-α, IL-1β, IL-6 and MCP-1) and changes of plasma lipid profile (e.g. total cholestrol, HDL and LDL) caused by HFD and low-grade inflammation. In addition, PP supplementation ameliorated the inflammation-induced loss of tight junction proteins such as ZO-1 and Clds and pro-inflammatory cytokine expression, while restored the expression of colonic anti-inflammatory cytokines IL-10 and microbial recognition receptors. As such, PP supplementation contributes to maintaining the intestinal epithelial barrier function and restore normal host defense function. Lastly, the PP supplementation at high dose was shown to shape gut microbiome by promoting probiotic growth that further leads to modification of the fecal metabolic profile in mice experiencing low-grade inflammation. These findings suggest that the purple potato derived phenolics is a promising anti-inflammatory agent; and thus, increasing consumption of deep color root vegetable contributes to chronic disease prevention through promoting gut health, due to gut playing a crucial role in maintenance of overall health.

Keywords: anthocyanin, gut health, inflammation, gut barrier, high-fat

Dietary resistant potato starch alters immunological status and microbial populations in swine to limit *Salmonella*

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Recent limitations to antibiotic use in livestock species in the U.S. have heightened research efforts to identify alternatives to maintain swine health and production. Prebiotics as feed additives, such as resistant starches, have been shown to support the growth and functions of beneficial members of the intestinal microbiota to increase microbial butyrate production, which is known to play an influential role in maintaining colonic homeostasis and moderating host immune responses. To investigate the potential benefit of resistant starches in swine production, pigs were fed either a standard diet or the same diet supplemented with 5 percent raw potato starch (RPS) for 21 days post-weaning. Pigs fed RPS had increased levels of butyrate in the cecum, a short-chain fatty acid known to affect host immune status. Mucosa-associated bacterial communities were significantly different between the two groups; for example, proteobacteria, commonly associated with intestinal inflammation, was reduced in the RPS-fed pigs. Changes in host responses indicative of enhanced mucosal defenses were observed; a network analysis of host and microbial changes in the cecum revealed that regulatory T-cells correlated positively with butyrate concentration, luminal IgA concentrations, expression of IL-6 and DEF1b, and beneficial mucosa-associated anaerobic bacteria. These positive effects on intestinal health prompted a follow-up study investigating the potential protective benefits against challenge with *Salmonella enterica* serovar I 4,[5],12:i:-, a serovar of increasing prevalence. Following 4 weeks of the RPS amended diet, pigs were challenged with the *Salmonella* strain and fecal shedding was monitored for 21 days, at which point *Salmonella* colonization of various tissues was assessed. Pigs fed RPS shed less *Salmonella* in their feces, and tended to have lower quantities of *Salmonella* in their intestinal tissues and cecal contents. Furthermore, a correlation was observed between butyrate concentration in the cecal contents and cumulative *Salmonella* fecal shedding over the 3 week period. Collectively, these data suggest a beneficial effect of RPS on the intestinal microbiota, host immune response, and colonic homeostasis that reduced colonization and shedding of an important human foodborne pathogen.

Keywords: *Salmonella*, resistant starch, immunomodulatory, intestine, dietary

Phytonutrients: The Next Generation

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Is food (and feed) medicine, as Hippocrates said? Perhaps not, but humans have recently (re) discovered that food not only provides nutrients for maintenance, it also contributes to their health. The animal production community has come to the same realization. Feed – critical to production performance – also affects animal health. In parallel, phytonutrients have developed as a relatively new category of feed ingredients capable of improving farm animal performance. This review will discuss some of the challenges of using phytonutrients in animal feed as growth promoting agents, in light of the recent progress of biological sciences.

The expectation of simple, silver-bullet solutions – widespread at all levels of the animal production industry – limits progress. Improving farm animal health and production is a complex problem that calls for complex solutions. When the mechanism triggered by an ingredient is understood, it opens the possibility of developing multiple-ingredient solutions that can robustly improve production when applied in the field. Today, the consensus is that phytonutrients are valuable feed ingredients *because* they kill pathogens in the gut of the animal. This is based on early observations that phytonutrients kill most pathogens *in vitro*, later substantiated by improved animal performance when used *in vivo*. This promoted the use of only the phytonutrients able to show antimicrobial effects *in vitro*, neglecting all others. In the last 10 years, research questioned this consensus and proposed a new one: a host-mediated response. This was enabled by the progressive appreciation that in a farm animal, production is a trade-off with other physiological processes. For example, farm animals are prone to high levels of inflammatory response which consumes nutrients that would otherwise be allocated to growth. This realization opened a new avenue for thinking in which, instead of killing pathogens, an ingredient could mitigate the response of the animal to its environment. Additionally, the appreciation of the gut as an intelligent sensory organ, and not only a tube for digestion, also paved the way for an alternative phytonutrient paradigm. Specific sensory receptors expressed by enteroendocrine cells detect low concentrations of dietary phytonutrients; once the signal is received and the information analyzed, the gut responds locally or conveys a signal to a distant organ. The response – for example improvement of digestion or lowered inflammatory response – materializes in improved performance or health status. Acknowledgement of such alternative mechanisms of phytonutrients will allow for the consideration of other phytonutrients aside from those that kill pathogens *in vitro*.

Regulatory positioning of key ingredients is undoubtedly a challenge of the next decade. Phytonutrients (as well as other ingredients such as probiotics) improve performance of farm animals because they help mitigate the response to the environment, especially via their immune effect. How can one develop such tools without being able to properly position them in the market because of regulatory limitations? The progress of science also needs to be integrated at the regulatory level to enable continued sparking of innovation.

Keywords: gut physiology, gut sensing, health, phytonutrients

Science-based use of plant extracts to improve animal health in post-antibiotic era: where are we?

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In recent years there are frequent pathogenic challenges all over the world that has caused immense economic loss in the animal production industry. Antimicrobial resistance can be attributed to some extent for these frequent challenges. Increasing concerns and reports about antimicrobial resistance in recent years have led to explore alternatives to antibiotics as growth promoter in animal production. Among different alternatives plant extracts have shown promising results in terms of zootechnical parameters, immune modulation and bringing down the negative effects of different pathogenic challenges. Recent scientific findings reveal that plant extracts in small concentrations can exerts their effect directly in the animals, leading to a host mediated response. This effect is beyond antimicrobial effect of plant extracts that was originally thought. With different molecular biology and nutrigenomics tools, now we have better in depth understanding of the mode of actions of different plant extracts at the level of genes, receptors and cell signaling pathways. This will help in developing new solutions to improve animal health and performance in near post antibiotic era.

In-vitro antibacterial activity of phytobiotic against *Eschericia coli* and *Mycoplasma gallisepticum*

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Avian Pathogenic *Eschericia coli* (APEC) and *Mycoplasma gallisepticum* are bacteria that commonly cause respiratory and digestive tract infections in poultry. Antibiotics such as enrofloxacin, tylosin, colistin have been quite extensively used in Indonesia to treat such diseases. However, since they are critically important to human medication, alternatives are needed. This study aimed to determine in-vitro antibacterial activity of 10 Indonesian plants known to have antimicrobial activities that were extracted with ethanol against *Eschericia coli* and *Mycoplasma gallisepticum*.

Sappan (*Caesalpinia sappan*) wood, guava (*Psidium guajava*) leaves, red ginger (*Zingiber officinale var. Rubrum*) rhizome, elephant ginger (*Zingiber officinale var. Rosc*) rhizome, nutmeg (*Myristica fragrans*) fruit, roselle (*Hibiscus sabdariffa*) calyx, and Indonesian bay (*Syzigium polyanthum*) leaf extracts were found to be effective against *E. coli* strain O78.K80.H12 (ATCC 43896). Sappan wood and guava leaves extracts showed the lowest minimum inhibitory concentration (MIC) of 256 µg/mL. Sappan wood and guava leaves extracts also showed additive effect against *E. coli*.

Only sappan wood and red ginger rhizome extracts showed to be effective against *Mycoplasma gallisepticum* strain S6 (ATCC 15392), with the lowest MIC of 400 and 800 µg/mL, respectively. Combination of sappan wood and red ginger rhizome inhibited the growth of *Mycoplasma gallisepticum* at concentrations of 200 : 400 µg/mL and 400 : 800 µg/mL.

This study showed that ethanol extracts of sappan wood, guava leaves, and red ginger rhizome were potential alternatives to antibiotics against *Eschericia coli* and *Mycoplasma gallisepticum*.

Keywords: *Mycoplasma gallisepticum, Eschericia coli, Caesalpinia sappan, Psidium guajava, Zingiber officinale*

In vitro and *in vivo* evaluation of therapeutic effects of neutrapath™ against *Salmonella* Typhimurium

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Antibiotic resistance in foodborne pathogens such as *Salmonella* is a major concern for public health safety. The poultry industry is compelled to seek alternative solutions to antibiotics in reducing the incidence of *Salmonella* colonization in broiler chickens at the farm level. NeutraPath is a formulated feed additive that features a proprietary blend of essential oils, medium-chain fatty acids and an activated toxin-adsorbing mineral. This formula has been shown to neutralize a variety of key virulence factors of pathogenic bacteria in addition to exerting direct bacteriostatic/bacteriocidal effects. This study was aimed to evaluate *in vitro* and *in vivo* effects of NeutraPath on *Salmonella enterica* sv. Typhimurium (ST) infection in broiler chickens.

An *in vitro* digestion model was used to simulate three gastrointestinal compartments with physiologically relevant pH and enzymatic conditions correspondent to that of crop, proventriculus and intestinal section respectively. In the *in vivo* trial, one-day old male broiler chicks were randomly allocated to one of three groups (n=30 chickens), i.e., Challenged control with non-treated feed and NeutraPath supplemented at 0.25% and 0.5% in feed. Chickens were orally gavaged with 10^6 CFU of live ST per chicken at 9-d old. Twenty-four hours post challenge, ceca-cecal tonsils were removed to evaluate *Salmonella* recovery and serum was collected for FITC-d determination. Differences between treatments were analyzed using one-way ANOVA.

In the *in vitro* trial, 0.25% NeutraPath significantly reduced total CFU of ST recovered in the proventriculus and intestinal compartments compared with control ($P<0.05$). NeutraPath treatment at 0.25% and 0.5% resulted in a 41.7% and 33.3% reduction in the prevalence of ST in ceca respectively compared to the challenged control ($P<0.05$ for both comparisons). Both dose levels also significantly reduced total ST CFU recovered in the ceca by 1.84 and 1.79 Log_{10} CFU/g compared to the challenged control ($P<0.05$ for both comparisons). Further, NeutraPath at both doses significantly reduced serum FITC-dextran levels ($P<0.05$).

Based on these *in vitro* and *in vivo* data, the NeutraPath treatment had the therapeutic potential to reduce ST colonization in broiler chickens and preserve the functional integrity of the intestinal barrier of chickens during ST challenge.

SESSION 4
Phytochemicals

POSTER PRESENTATIONS

PH1
Supplementation with encapsulated phytonutrients improves carcass characteristics in broilers

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Essential oils are aromatic volatile components of medicinal plants. Diverse bioactivity of these compounds makes them ideal candidates for use in animal health and nutrition. Indeed, essential oils are widely used as growth promoters to improve performance in poultry. In this study, a multiple-trial analysis was conducted to evaluate the efficacy and consistency of an essential oil blend (EOB; Phytomax, AVT Natural, India) in improving the carcass characteristics and performance of broilers.

Five independent research trials were conducted in a private poultry research farm in India. In each trial, performance and carcass characteristic of birds supplemented with EOB (250g/ton of feed) was compared with that of unsupplemented control (CT) group. A positive control (PC; Bacitracin, 500g/ton of feed) was included as a reference and compared to CT. Treatments were imposed during the entire growth phase from d 1-42 of life, with 6 to 10 replicates per treatment (20-25 birds/replicate; COBB430 and ROSS308 lines were used). Performance and mortality data were measured throughout the treatment period and summarized on d42. Carcass characteristics were evaluated in all experiments and blood samples were collected on d42 in experiment 5. Data were subjected to two-way ANOVA to test for the fixed effects of treatment, experiment and their interaction using GraphPad Prism 6 software. Dunnett's test was used to compare each of the treatment groups to the control group. Final body weight was increased with EOB (2,475 g; $P<0.09$) and PC (2,484 g; $P<0.02$) compared to CT (2,445 g), but there was no effect of treatment on FI (4,104, 4,112, & 4,125 g for CT, EOB, and PC respectively; $P>0.7$). Consequently, FCR was decreased with both EOB (1.67) and PC (1.67) compared to CT (1.70; $P<0.001$); however, a treatment by experiment interaction ($P<0.001$) revealed that improved efficiency was observed in only 3 of the 5 experiments. Mortality was decreased with both EOB (1.3%; $P\leq0.01$) and PC (1.6%; $P<0.05$) compared to CT (2.8%). Liver weight was increased with EOB (24.3 g/kg BW; $P<0.05$), but there was no difference between PC and CT (23.9 vs. 23.3 g/kg BW; $P>0.3$). Dressing weight and breast weight increased with EOB (783 & 281 g/kg BW; $P<0.005$) and PC (782 & 278 g/kg BW; $P<0.01$) compared to CT (767 & 269 g/kg BW). Serum cholesterol and abdominal fat decreased with EOB (136.8 mg/dL; $P<0.005$ & 13.3 g/kg BW; $P<0.1$) compared to CT (192.4 mg/dL & 14.3 g/kg BW), whereas PC had no effect on serum cholesterol (191.8 mg/dL; $P>0.5$) but decreased abdominal fat (12.9%; $P<0.02$) compared to CT. In conclusion, EOB positively influenced lipid metabolism and carcass quality with an improvement in zoo-technical parameters. Therefore, EOB can be used not only as a growth promoter but as a tool to improve meat quality in poultry.

Keywords: Phytogenics, Broilers, Caracass Quality, Meta-analysis, growth promote

PH2
IDENA, A long experience with new generation additives

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Since its creation in 1995, when the first cases of mad cows appeared in Europe, IDENA has focused all its R&D efforts on active ingredients from the botanical world to create a range of additives that substitute chemical molecules, growth factors, antibiotics and anticoccidians in animal feed.

A painstaking work in the sourcing of assets and scientific validation directly in farms to lead to targeted associations according to the different health and sanitary issues encountered in the livestock operations. This work also included the definition of the modes of action of these new molecules against coccidiosis parasites and pathogenic bacteria's but also, their activity on the beneficial bacteria in the digestive tract. For example, IDENA has developed applications to minimize the negative effects of coccidiosis in poultry, pigs and small ruminants.

This concept, which was first developed in the 2000s, provides the same or even better answers to conventional products. It is designed as a powder to be incorporated into feed and/or as a liquid to be introduced into drinking water or directly into the mouths of animals (piglets, calves and lambs).
In pig production, neonatal and weaning diarrhea is also a major concern at IDENA and receives highly effective solutions to accompany antibiotic reductions on farms.

Thus, applications have been studied and approved to manage and reduce the frequency of neonatal diarrhea in piglets. Other solutions are more focused on diarrhea around weaning.

All the alternative concepts designed by IDENA are combinations of several active ingredients working in synergy for a direct global effect on the pathogen, flora control and immunity. They benefit from a specific technology, ECHV, developed by IDENA, to protect and stabilize the assets.

IDENA is convinced that the replacement of antibiotics cannot be achieved by substituting one product for another. There are situations, sometimes very complicated, where even antibiotics no longer work. Improving livestock conditions and the sanitary environment is a priority to optimize the effectiveness of the programs implemented. Each farm is a special case that requires an adapted program.

Keywords: Alternative, botanic, coccidiosis, neonatal diarrhea, antibiotic reduction

PH3
Can a beneficial role of chitosan oligosaccharide supplementation make an alternative to antibiotic substitution in weaned pig?

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There is continued need for novel agents to improve intestinal function in weaned pigs. Some evidence suggests that chitosan oligosaccharide (COS) supplements, as a functional prebiotic, may enhance pigs' intestinal function for health benefit after weaning. Post-weaning stressors: nutritional, social and environmental stresses can affect feed intake, growth performance, and predisposition to diseases. Antibiotics are added to pig starter diets as growth promoters and to prevent infections. However, antibiotic addition to animal feed has been prohibited or limited in many countries due to antibiotic-resistance which threatens human health. The search for safe and environmentally friendly alternatives to antibiotic to promote health has become necessary in swine production. The objective of the present study was to test COS supplementation at 150 mg/kg with the molecular weight about 8 kDa and deacetylation degree (DD) about 90% as an effective alternative to antibiotic addition in weaned pigs' health and production. For the experiment, weanling pigs were divided into 3 groups (9 animals per group) and received either a basal diet, a supplemented diet with 150 mg/kg COS, or a supplemented diet with 110 mg/kg lincomycin for 56 days. Growth, feed conversion ratio, nutrient's ileal digestibility, small intestinal morphology and crypt cell proliferation were measured at 56 days of the experiment. The statistical significance (P<0.05) of the differences among the groups was determined by ANOVA in conjunction with Tukey's test. Compared with the control group, pigs supplemented diet with COS or with lincomycin significantly (P<0.05) showed: (i) consistently more digestible ileal contents (e.g. crude protein, crude fat, and calcium), (ii) increased absorption capacity (e.g. increased villus height/crypt depth ratio at the jejunum) and (iii) more active cell division (as indicated by Ki-67 marker of duodenal and jejunal crypt cells). In conclusion and implication, weaned pigs fed dietary supplementation at 150 mg/kg COS with 8 kDa and about 90% DD showed improvements in major nutrient digestibility and small intestinal morphology through cell proliferation similar to those of in-feed lincomycin. Therefore, supplementation of this COS characteristic may be an effective substitute for in-feed antibiotics during the post-weaning period.

Keywords: antibiotic, Chitosan oligosaccharide, dietary supplement, small intestine, weaned pig

PH4
A multi-hurdle approach using phytochemicals as natural alternatives to antibiotics for controlling *Campylobacter* in poultry

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Campylobacter is one of the leading causes of foodborne illness, resulting in an estimated 96 million cases of gastroenteritis and 21,000 deaths per year, globally. *Campylobacter* is prevalent in many food animals; however, the presence of *Campylobacter* on poultry products remains one of the leading causes of bacterial foodborne illness worldwide. *Campylobacter jejuni* naturally colonizes the ceca of chickens in high numbers, thereby leading to contamination of the carcass during slaughter. Therefore, a multi hurdle approach combining strategies to reduce *C. jejuni* cecal colonization in poultry gut, reducing survival in poultry products and the environmental persistence of this pathogen during poultry processing would potentially reduce the risk of human infections.

One of the missions of our laboratory has been to provide the poultry industry with efficient antibiotic alternatives for the control of *Campylobacter* in conventional and the organic poultry sectors. The use of phytochemicals as antimicrobial feed additives, food bio-preservatives and natural disinfectants is one such technology that is safe, effective and environmentally friendly. We have tested numerous phytochemicals for their anti-*Campylobacter* efficacy targeting three main areas (poultry production, post-harvest contamination, and limiting survival of pathogens in the processing environment) in a multi-hurdle approach from farm to fork. Our results indicate that plant-based, generally recognized as safe status (GRAS), compounds such as *trans*-cinnamaldehyde (obtained from cinnamon bark), eugenol (from clove oil), and carvacrol (from oil of thyme) are very effective in reducing *C. jejuni* in the poultry gut, on carcasses as well as inhibiting *C. jejuni* biofilms on common food processing surfaces. Using our work with eugenol as an example, in-water supplementation of 0.125% eugenol nanoemulsion consistently reduced *C. jejuni* colonization by at least 1.5 log CFU/g of cecal contents in 14-day old broiler chickens ($P<0.05$; 10 birds/treatment/trial). Eugenol was also effective in reducing the survival of *C. jejuni* on chicken skin and wings when applied as an antimicrobial wash or coating treatments. Washing the chicken skin with 0.5-2% eugenol for 1 min and antimicrobial coating of chicken wingettes reduced *C. jejuni* by 1-2 log CFU/sample ($P<0.05$). In addition, 0.25-1% eugenol was highly effective in inhibiting biofilm formation as well as inactivating a mature *C. jejuni* biofilm on common poultry processing surfaces. Follow up mechanistic studies (using real-time quantitative PCR and proteomics analysis) revealed that eugenol modulates key *C. jejuni* genes and proteins essential for intestinal colonization, persistence in the environment, and survival of the pathogen in meat products. (Funded by USDA-NIFA-OREI-2017-51300-26815).

Keywords: Campylobacter, Phytochemicals, Pre-harvest poultry, Post-harvest, Biofilm

PH5
Functional fermented proteins to replace medicinal zinc and reduce antibiotic treatments in pig production

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EP100i is a Lacto-fermented rapeseed protein designed to replace medicinal doses of zinc oxide and reduce antibiotic treatments in weaned pig production. In addition to a high digestibility of protein and fibre, the product modulates the bacterial composition of the gut flora and promotes beneficial gut bacteria. With this, improvements in the gut barrier and associated immune functions occur. As a result, high doses of zinc oxide, followed by antibiotic treatments, become unnecessary. This was tested in a large trial with weaned pigs fed individually with 1) a commercial control diet (negative control), 2) a control diet with 2500 ppm zinc oxide (positive control) and 3) with 10% EP100i included as protein in the feed formulation. Pigs were not given prescription antibiotics before and during the experiment unless fallen ill. Ill animals were treated separately and excluded from the experiment. The trial was carried out for 53 days starting at weaning. Each diet group had 6 replicas per diet group, with an average of 46 pigs per pen. Average daily gain, feed intake and animals excluded from the experiment, were recorded. Hindgut tissue and content, along with blood, was sampled from 10 slaughtered pigs from each diet group after 4 weeks (28 days) of diet supplementation. Microbiome, inflammation biomarkers and gut histopathology were analyzed to test the hypothesis. Overall, pigs from the control diet performed poorer in comparison with the groups with zinc oxide and EP100i. The EP100i and zinc oxide group both displayed similar gut modulation, growth performance and a higher number of animals completing the study. However, at the end of the experimental period, pigs from group EP100i were 1.6 kg heavier than those of the zinc oxide group. The hindgut of pigs from the control group was dominated by one bacterial group (*Prevotella* spp.). Pigs from the zinc oxide and EP100i group showed a significant increase in L*actobacillus* spp., Ruminococcaceae spp., and the Clostridiales group IV ($p<0.05$). Histomorphometry jejunum and colon was higher in the zinc oxide and EP100i groups than in the control group. However, histopathology and inflammation biomarkers were statistically significantly lower in pigs supplemented with EP100i than in the other groups ($p<0.05$). This suggested that feeding EP100i to piglets will result in a well-developed gut barrier function with a vast array of beneficial gut bacteria. Hence, pigs focus the energy into growing and becoming resilient to infections. We conclude that EP100i is an excellent alternative to the use of high doses of zinc oxide and antibiotic treatments in pig production

Keywords: Fermented, proteins, microbiome, inflammation, gut-development

PH6
Phytogenic feed additives as alternative to antibiotics in food animal production

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Phytogenic feed additives as alternative to antibiotics in food animal production

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Driven by regulatory actions and/or tied to customer demands for antibiotic-free meat, we have witnessed the ban on antibiotic growth promoters in key feed markets and observed a decline of the usage of antibiotics in animal feed. The ban on prolonged growth promoting and shotgun style prophylactic treatments is a major achievement in the battle against antibiotic resistance, a global threat to humanity.

Based on the available evidence, it is clear that phytogenic compounds represent a potential alternative to in-feed antibiotics. However, it is also known that there is no silver bullet and it is very challenging for phytogenic feed additives to replicate the action of antibiotics. A combination of additives and an integrative prevention program have proven to be a valid strategy to avoid bacterial resistance improving safety, efficacy and animal welfare.

Among the plethora of phytogenics, hops (*Humulus lupulus L.)* have caught our attention for their interesting properties. For several thousand years, hops have been utilized in folk medicine for their health-promoting effects. Hops are also widely applied in beer manufacturing due to their antimicrobial and preservative properties together with their bitterness. The phytochemicals in selected hop components have a chemical structure similar to ionophores. That is why they possess inhibitory activity against Gram+ bacteria and can be potentially used as a natural alternative to in-feed antibiotics.
Based on this knowledge, a new phytogenic feed additive, Anta®Phyt, containing selected hops phytochemicals and other plant ingredients was developed. Herewith, we report the results of the *in vitro* and *in vivo* characterization of its efficacy in broilers.

In vitro: Chicken ileal digesta were mixed with different concentrations of Anta®Phyt and monensin, as positive control. After incubation, lactic acid and volatile fatty acids were quantified. The application of Anta®Phyt significantly increased the production of acetic and propionic acid, while decreasing the production of lactic acid.

In vivo: Broilers were allocated to two groups. The treatment group received 400 g/t Anta®Phyt. Performance parameters were recorded. Dry matter content of faeces was determined. Faecal samples were analysed for specific rDNA sequences to monitor the intestinal microflora. The application of Anta®Phyt improved feed conversion by 2.1% and live weight gain by 3.6%, as compared to control. Moreover, it also decreased moisture content in the litter significantly by 7.21% and slightly reduced the microbial load of all analysed bacterial groups.

A shift from lactic acid production to propionic and acetic acid fermentation was observed. These short chain fatty acids contribute to the energy supply of the animal and stimulate gut epithelial proliferation. The comparable effects of monensin and Anta®Phyt, on the fermentation pattern observed *in vitro* and the slight reduction of microbial flora in the faeces *in vivo*, could be an indication for the antimicrobial activity of Anta®Phyt. Modulation of the intestinal flora may also explain the favourable effect on the litter quality. By preventing dysbiosis this plant based product is a potential natural alternative to antibiotics to improve broiler production.

Keywords: Phytogenic, feed additive, antibiotic alternative

PH7
Alternative to antibiotics effects of Quebracho tannin as an animal feed supplementation

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In pork production the early weaning is very efficiency way to increase sows reproductivity. And it is followed by the successful rearing of early weaned piglets. However, weaned piglets must rapidly adapt to dramatic changes in the social and physical environments, separation from maternal littermates, mixing with unfamiliar piglets, abrupt changes in diet from suckling the dam to ingesting solid feed from a feeder, and establishing a social hierarchy. Consequently, early weaning is very stressful for piglets. Diarrhea is the nemesis of the early weaned piglet. Enteropathogens infect the small intestine, which results in secretory or malabsorptive diarrhea. High death losses from diarrhea have dampened the enthusiasm for early weaning of artificially reared piglets. Usually, the nursing piglet is protected from enteropathogens by antibodies bathing the gut from the dam's colostrum and milk. Artificially reared early weaned piglets are protected from enteropathogens by feeding them diets containing additives, such as antibiotics. In addition, adding an antibiotic to the feed results in significant growth performance and improves food conversion rate.

The present study to assess the possibility of replacing antibiotics with one of phytochemicals QT, the treatment groups fed with dietaries addition of QT instead of antibiotics. This study used 21-day-old weaned piglets (Duroc × Landrace × Yorkshire; 6.51±0.17kg; 21±1d) divided to 3 groups. To assess the possibility is through analyzing the bodyweight changes, daily gain, hematology and biochemical index, blood amino acids level, diarrhea incidence rate, organs weight, and intestinal morphometric changes. The result showed big possibility of QT somehow could be replacing antibiotics.

Keywords: Tannin, Weaned piglet, Diarrhea, Blood hematology, blood biochemical index

PH8
Effect of oregano essential oil on SOD and GSH-Px activities and mRNA expression in the kidney and liver tissues of broilers

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In-feed antimicrobials have led to an antibiotic resistance fast escalating and dissemination of antibiotic resistance throughout the food chain. This experiment was conducted to evaluate the impact of oregano essential oil (OEO, contains 10% of essential oil from *Origanum vulgare ssp Hirtum* and 90% of inert carriers, named as Phytogen from Meritech) on activities and expression of mRNA and GSH-Px in kidney and liver tissues in the broilers. A total of 1000 one-day-old birds were randomly allotted to 5 diet treatments, with each treatment contained 4 replicates with 50 broilers per replicate: C (control with basal ration); A (AGP, C + Chlortetracycline 20 g/t +Virginiamycin 10 g/t feed); O1,O2 and O3 (Control plus 100, 150 and 200 g OEO/t diet, respectively). The results indicated that the supplementation of OEO (100,150 and 200g/t) into diets significantly improved SOD activity and up-regulated SOD mRNA expression level in the kidneys; OEO (150 g/t) of O2 group significantly increased renal GSH-P_X activity and OEO (100 and 150g/t) in O1 and O2 groups up-regulated GSH-P_X mRNA expression level in the kidneys of birds when comparing with C and A groups during the entire experiment. In addition, the hepatic levels of SOD of broilers in O1 and O2 groups were significantly increased comparing with the control and A groups. In conclusion, our results indicated that OEO contributed a better positive effect on anti-oxidative capacity in the kidney and the liver of broilers and this provided the useful insight to the development of potential alternative to antibiotic growth promoters for broiler feed.

Keywords: Broilers, Oregano essential oil, Glutathione peroxidase, Superoxide dismutase, mRNA expression

PH9
Oregano essential oil improved growth performance, meat quality and intestinal health of broilers

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The aim of this study was to evaluate the effects of oregano essential oil (OEO, contains 10% of essential oil from *Origanum vulgare ssp Hirtum* and 90% of inert carriers, named as Phytogen from Meritech) on growth performance, meat quality, intestinal microflora and morphology in broilers. A total of 400 Ross-308 one-day-old chicks were randomly divided into 4 diet treatments and the experimental period was 35 days. The treatment diets were: C (control with basal ration); A (C + Chlortetracycline 20 g/t +Virginiamycin10 g/t diet); O1 (C+ OEO 100g/t diet); O2 (C+ OEO 150g/t diet). The results showed that the supplementation of OEO or antibiotic in the diets significantly increased the average daily gain compared to the control group. Comparing to the control and antibiotic groups, OEO (150 g/t feed) in O2 group significantly improved feed conversion ratio of broilers at 35 days of age, and also improved meat quality through reducing shear force at 24 h after slaughter. The supplementation of OEO (150 g/t feed) significantly increased jejunum villus height, villus height-crypt depth ratio and muscle thickness compared with the antibiotic group. The rectal contents of *Lactobacillus* and *Bifidobacterium* counts of broilers fed diets containing OEO were significantly higher, and *Escherichia coli counts* was lower than those fed with antibiotic or control diet. In addition, OEO in O2 group significantly up-regulated the anti-apoptotic protein (along with decreased expression) of Bcl-2 in the jejunum compared with the antibiotic group. The results suggested that the dietary OEO supplementation improved broiler performance and meat quality through modulating intestinal bacteria and morphology. Therefore OEO could be used as an alternative for antibiotic growth promoter in the broiler diets.

Keywords: Broilers, Oregano essential oil, Growth performance, meat quality, intestinal health

PH10
Dietary oregano essential oil improved the growth performance and intestinal health in the weaned piglet

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Antibiotics play the important roles in the animal growth promotion and disease prevention but antibiotic growth promoters (AGPs) will be banned in China due to the public concern in 2020. The aim of the present study was to investigate the effects of supplementation of oregano essential oil (OEO, contains 10% of essential oil from *Origanum vulgare ssp Hirtum* and 90% of inert carriers, named as Phytogen from Meritech) on growth performance, intestinal morphology, rectal microflora, and serum immune function of piglets fed diets with or without AGPs. Sixty weaned piglets (initial body weight (BW) 7.12 ± 0.26 kg) were randomly allotted to 3 treatments, with each treatment contained 4 replicates with 5 piglets per replicate: C (control with basal ration); A (C with Chlortetracycline 50 g/t + Virginiamycin 20 g/t feed); O (C with OEO 250g/t feed). The results showed that supplementation of both OEO and antibiotics in the diets significantly increased the average daily gain of piglets comparing with the control group. Increased gain: feed was also detected in the OEO-fed piglets. Piglets fed OEO demonstrated the significantly higher jejunum villus height, villus height-crypt depth ratio, and muscle thickness compared with antibiotic and control groups. The rectal content *Lactobacillus* and *Bifidobacterium* counts of piglets fed diets containing OEO were significantly higher than those fed antibiotic or control diet. OEO supplementation predominantly decreased the the *Escherichia coli* counts of rectal and fecal content. Serum IgA and IL-10 concentration were enhanced in piglets fed the diet with supplementation OEO compared with the control and antibiotic groups. Furthermore, in-feed antibiotics increased expression of genes involved the immune functions in the jejunum. We could conclude that oregano essential oil can be used as a feed supplement to improve the gut health and immunity of piglets and may be a potential alternative to antibiotics in the weaned piglets.

Keywords: Weaned piglets, Oregano essential oil, Growth performance, Intestinal microflora, Immune level

PH11
Oxidized Derivatives of β-Carotene Support Immune Function and Help Optimize Growth Performance in Food Producing Animals

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β-carotene confers health benefits that occur independent of its provitamin-A activity. A nutritive antioxidant activity has been the proposed mechanism underlying these effects. However, the significance of the antioxidant role has not been clearly established, and efficacy trials with β-carotene have produced inconsistent results.

Our research shows that β-carotene very readily undergoes spontaneous oxidation in air and is transformed into a complex mixture consisting predominantly of copolymers with oxygen. The copolymer mixture, which we term OxBC, is active in supporting innate immune function. Immunological actions include priming of innate immune defenses to recognize and respond to early stage infections as well as a reduction in overzealous inflammatory activity.

In feeding trials with broiler poultry and pigs, dietary supplementation with low levels of OxBC (synthetically produced) led to reductions in *Clostridium perfringens* in broiler poultry and *E. coli* in piglets. Furthermore, supplementation with OxBC leads to lower incidence of disease and improved growth performance in piglets and chickens. Additional findings of increased immunoglobulin levels in colostrum and milk of sows receiving OxBC highlights the potential for further improvements in piglet health by enhancing passive immune transfer. The positive outcome of these trials has led to the successful commercial application of OxBC as an alternative to antibiotics for growth promotion and disease prevention in the Philippines and elsewhere in South East Asia.

The phytogenic origins of OxBC are confirmed by work showing the presence of β-carotene derived copolymers in a variety of plant-based feed stuffs. Other carotenoids, such as lycopene, lutein and canthaxanthin, also undergo spontaneous oxidation to form similarly active copolymer mixtures. The demonstrated activities of OxBC combined with the apparent ubiquitous presence of carotenoid derived copolymers in nature leads us to propose that these compounds are the actual source of carotenoid activities (apart from the well know provitamin A role of certain carotenoids). The copolymers represent a newly discovered class of phytogenic compounds with potential application as antibiotic alternatives in the feed industry. OxBC can be readily produced in highly pure form by synthetic manufacturing procedures and is easily handled, characteristics that further facilitate its commercial application.

Keywords: Oxidation, Carotenoids, Immune-Function, Phytogenic

PH12
Effect of a characterized citrus extract on poultry performances

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Nowadays, plants extract are more and more used in animal feed, as alternatives to antibiotic growth promoters. Some of them such as citrus extract had already shown beneficial effect on zootechnical performances and health. However, all citrus extracts can vary a lot in terms of concentration of active compounds. These variations may provoke efficacy differences. The study realised aimed to characterise a standardized natural citrus extract in order to identify the main components responsible for the effect. Then, the effect of this Standardized Natural Citrus Extract (SNCE) was evaluated through a meta-analysis in poultry.

After freeze drying the citrus extract at 50 mg/mL (Nor-Spice® AB, Nor-Feed SAS, France), three HPLC analysis methods were performed using HPLC-UV-DAD-DEDL (Shimadzu) and (HPLC UV-MSMS, Esquire 3000 Plus, Ion trap, Bruker) chromatographic systems. Analysis were performed using Hypersil RP-C18 column (4.6 mm, 250 mm, 5 µm) and data were analysed using DataAnalysis software. The main components of the product were identified using dereplication. In parallel, a meta-analysis was performed based on the results of seventeen trials realized between 1995 and 2018 with this Standardized Natural Citrus Extract. Each trial had 2 poultry groups, a control group (CTL group) fed with standard diet without supplementation and a SNCE group (SNCE group) supplemented with SNCE at dose between 250 and 400 ppm. Feed Conversion Ratio (FCR) and Average Daily Gain (ADG) were monitored in every trials. Trials were conducted worldwide. Statistical analysis were performed using Student test (T-test) and GraphPad Prism 7 (GraphPad software, USA).

Spectral data from HPLC analysis allowed to identify approximately 30 secondary metabolites among carboxylic acids, phenolic acids, coumarin, flavones, flavanones and flavanols.

Concerning the17 trials, 16 have shown a significant effect on FCR or ADG of broilers chickens ($p<0.05$, T-test). On average, SNCE supplementation on broilers significantly reduces the FCR by 2.3% and increases the ADG by 4.4% ($p<0.05$ Fisher Test)

According to these data, Standardized Natural Citrus Extract supplementation seems to be an effective solution in order to improve zootechnical performances of broiler chickens and to offer a good alternative to antibacterial growth promoters.

Keywords: citrus extract, standardization, microbiota, poultry, plant extract

PH13
In-feed resin acids improve small-intestinal mucosal characteristics of broiler chickens during dysbiosis challenge

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In-feed coniferous resin acids had previously shown to reduce both duodenal inflammatory T cell infiltration and small intestinal matrix metalloproteinase (MMP) activity towards collagen type I and type IV in the ileum of non-challenged broiler chickens, indicating a protective effect of resin acids on intestinal barrier integrity by preservation of the basal membrane and the extracellular matrix. Here we fed coniferous resin acid composition (40% natural resin acids in wheat flour) to study whether resin acids affect mucosal histology and collagen-degrading activity in a diet-induced challenge model, in which increased MMP expression and collagen breakdown were previously shown. Male Ross 308 hatchlings were randomly divided into three dietary treatments: 1) non-challenged control (NCC), 2) challenged control (CC), and 3) CC+ RAC at 250 g/ton (RAC); 10 replicate pens/treatment and 28 chicks/pen. The CC diet was produced by the inclusion of 7.5% rye (replacing an equal amount of wheat) for all dietary phases (starter d1-13, grower d14-26, finisher d27-39). One bird/pen was sampled for duodenal, jejunal and ileal tissue on d26. Duodenal samples were processed by routine histology into haematoxylin-stained slides and measured by light microscopy for the length of villi and the depth of crypts. Homogenized tissue samples from all intestinal areas were measured for the relative activities of collagen type I and type IV degradation by EnzChek™ kit (Thermo-Fisher Diagnostics, USA).

Birds performance, as measured by daily weight gain and feed conversion, was significantly lower for CC than NCC ($p<0.05$). Feed conversion was better for RAC than CC for the starter period ($p<0.05$), but otherwise performance was similar for CC and RAC treatments. Mortality was unaffected by the treatments. The relative ileal activity of collagen type I and type IV degradation was higher for CC than NCC (by +38% and +86%, respectively; $p<0.05$ for both). In RAC, the activity of collagen degradation was similar to NCC for both collagen types. In duodenum and jejunum, the collagen-degrading activity was unaffected by the treatments. The challenge significantly shortened duodenal villi and reduced the depth of crypts, thus thinning the entire mucosal surface (NCC vs. CC $p<0.05$ for both variables). In RAC, the length of villi and depth of crypts were similar to NCC and differed significantly from CC (RAC vs. DCC $p<0.05$ for both variables). In conclusion, the diet-induced challenge increased relative collagen-degrading activity in the ileum and caused alterations in histological parameters. In-feed resin acids prevented these diet-induced changes and maintained mucosal characteristics similar to those in birds without a dietary challenge. Thus, the dietary amendment by coniferous resin acids may partly protect intestinal mucosa of broiler chickens during intestinal disturbances.

Keywords: Resin acids, Feed additives, Intestinal Health, Gut Microbiota, Matrix metalloproteinases

PH14
The effects of mesobiliverdin containing algae on gut microbiota in broilers

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Gut inflammatory bowel diseases (IBDs) are costly and serious diseases in the livestock industry. New animal feeds that promote livestock gut health and control IBDs without antibiotics are needed. It exploits recent discoveries on mesobiliverdin (MBV), an analog of the animal metabolite biliverdin known to protect against IBDs and new synthetic strategies for producing it from kilogram (kg) amounts of microalgae. Two hundred and eighty-eight Arbor Acres broilers were reared from day 1 to day 30 and randomly allotted to 6 dietary treatments (four pens/treatment and 20 birds/pen). The settings of each group are as follows: a. control, b. antibiotic growth promoter (AGP, 0.1 % amoxicillin), c. 0.5% algae (AL), d. 1.0% algae (AH), e. 0.5% MBV enriched algae (MBVL), f. 1.0% MBV enriched algae (MBVH). The chicken feed of control group was no antibiotic addition and group of AGP containing 0.1 % amoxicillin. Group c and d were adding 0.5% and 1% spirulina algae powder respectively. Group e and f were adding 0.5% and 1% MBV enriched algae powder respectively.

During the whole experimental period, the chickens showed the good survival rate (99.7%). The live weight, average daily gain and feed efficiency did not differ for the different dietary treatments. For investigate the microbiota population, we showed that the effect of algae and MBV enriched algae on firmicutes/bacteroidetes (F/B) ratio in broilers. Firmicutes and Bacteroidetes are most common phylum in chicken ceca, with Proteobacteria and Cyanobacteria accounting for the remainder. Interestingly, we further analyzed the data of families and genera and found that algae powder can effectively increase the ratio of *Lactobacillaceae* and *Lactobacillus* respectively, and the most obvious is MBV enriched algal treatment group. For histological examination, current data indicated that antibiotic treatment decrease the villi length of duodenum and ileum in broilers and there have similar effect in jejunum. Moreover, we found that MBV containing algae can improve the intestinal health by elevated the villi length in duodenum, jejunum and ileum, especially the 1.0% MBV enriched algae treatment. In conclusion, we suggest that MBV containing algae can improve gut health in broiler chickens.

Keywords: algae, mesobiliverdin, biliverdin, gut microbiota, chicken

PH15
Potency of *Andrographis paniculata* and *Origanum vulgare* extracts in poultry

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The aim of this study was to determine the effects of phytobiotics combination of *Origanum vulgare* and *Andrographis paniculata* water extracts (FOA) given in feed on the performance, intestinal bacteria, and Eimeria spp. oocysts in feces, of broiler and layer chickens as alternative to Antibiotics Growth Promoter (AGP).

One-day old broiler chicks (Cobb) were divided into three groups of FOA, Zinc Bacitracin (ZB, as an AGP group), and Control. At day 28, body weight of FOA and ZB groups were significantly higher than the control group ($p < 0.05$). FCR was shown best in ZB group followed by FOA and Control. In the intestines, total number of *Lactobacillus* spp. and *Bacillus* spp. was higher in FOA group compared to ZB and control groups, meanwhile *Escherichia coli* and *Salmonella* spp. were lower.

In 14-days-old layer chickens that were infected with live coccidia vaccine (peroral, 5 doses), higher oocyst per gram (OPG) reduction after 7 days of treatment was observed in FOA and Amprolium (as an anticoccidial) group (82.53% and 92.02%, respectively) compared to the control group.

In conclusion, combination of *Origanum vulgare* and *Andrographis paniculata* extracts can be used as AGP replacements in feed.

Keywords: *Origanum vulgare, Andrographis paniculata*

PH16
The effects of *Thunbergia* on sulfatrimethoprim excretion in Nile tilapia (*Orechromis niloticus*)

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In aquaculture, antibiotics have been used mainly for therapeutic purposes and as prophylactic agents. The antibiotics are commonly administered by water immersion and medicated feed, which could obtain a uniform dose and avoid any potentials for high localized concentration. During a long cultivated period, animals are continuingly fed with antibiotics for disease prevention, so it might increase risk of antimicrobial residue in animal products. Unfortunately, very little information on the pharmacokinetics of various antibiotics was evident in each fish species. Among approved antibiotics in food animals, sulfonamide was presented a low rate of bacterial resistance by disc diffusion method. Since fish are poikilotherms, the drug excretion could probably be different from other species. Therefore, it might be interesting and worthwhile to understand the pharmacokinetic of sulfonamide in the fish. The study outcome should contribute to minimizing fish consumers' risk from antibiotic residues.

Thunbergia laurifolia Lindl. belongs to the family Acanthaceae, which is commonly known as Rang jeud in Thailand. This plant is traditionally used in Thailand for centuries as an antidote for several poisons and drug overdose. This experiment was performed in 4-month-old Nile tilapias (*Orechromis niloticus*), 10 fish for control and 10 fish for treated group. They were fed with commercial diet for 14 days before experiment started. At day 0, treated fish was supplemented with thunbergia at 500 mg/kg BW for 12 hours before single intramuscular injection with Sulfatrimethoprim at 50 mg/kg BW. Blood samples from all fish of both groups were collected at 0 (before sulfatrimethoprim injection), and at 0.5, 1, 2, 3, 6, 12, 24 hours and 7, 14 days and further examined for liver, kidney, muscle and also pancreatic functions. Biochemical tests; albumin, AST, BUN, cholesterol, creatinine, glucose, total protein and triglyceride were measured by Liquid Stable Reagent Trinder's, colorimetric, and kinetic methods. Results demonstrated that plasma concentration of sulfonamide was significantly low at the first thirty minute and unable to detect at the120th hour in thunbergia treated fish. All blood biochemical results of kidney, liver and pancreas were not different among control and thunbergia treated fish, which indicated that all fish did not have any adverse effects on their vital organ functions.

Therefore, it might be concluded that *T. laurifolia* extract had a detoxification effect by the significant reduction of sulfadiazine in fish plasma. Furthermore, *T. laurifolia* could be an alternative medicine as anti-inflammatory, antioxidant, anti-microbial, hepatoprotective effects.

Keywords: Sulfatrimethoprim, Thunbergia laurifolia Lindl, pharmacokinetic, Tilapia, alternative medicine

PH17
Raising pigs without antibiotics thanks to algae-based solutions

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Antibiotic resistance is one of the top five public health concern according to WHO. Consequently, Olmix has developed a complete and adapted program aiming at accompanying producers to decrease the use of antibiotics and thus limit the development of antibiotic resistance thanks to the use of natural algae-based solutions.

The program was implemented in a farrow-to-finish pig operation of 840 sows in France. The farm was submitted to a detailed audit in December 2015, from which an action plan was prepared and agreed with all collaborators. The action plan aimed at reducing antibiotic use from 100% of the pigs being systematically treated with one or several antibiotics, to a maximum of 10% of the pigs being treated with only one antibiotic.

Several recommendations constituted the action plan. An improved cleaning and disinfection protocol was implemented. Adjustments were made in the farrowing room in order to improve the comfort of newborn piglets. Biosecurity management was reinforced by a stricter quarantine program, more control of inputs to the farm and the action against rodents. The use of 5 innovative marine algae-based products, used in environment, feed or drinking water, completed these different measures. The action plan was implemented step-by-step from February 2016 to April 2017.

The results of this case study show that the use of antibiotic was strongly reduced to reach 94.5% of piglets raised without antibiotics. Moreover, global performance in maternity was improved. These results demonstrate that it is highly important to adapt the strategy to each situation and confirm the need of a global approach to reduce the use of antibiotics in farms.

Keywords: algae-based

PH18
Raising broilers without antibiotics thanks to algae-based solutions

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A major challenge in the animal farming industry will be to reduce the use of pharmaceuticals while maintaining growth performances in order to support the growing demand for meat. Interest for exploring natural bioactive products, able to improve animal health or act against infections, has increased. This study will present a case of using seaweed as an additive in an industrial poultry farm with the aim to reduce the use of antibiotics.

The experiment was undertaken on a total of 411,459 broilers receiving 4 seaweed- based products (11 batches) against a control population of 634,453 broilers (14 batches). Two different genetics of broilers were tested (JA957 and JA987) under high population density, 32 birds per square meter. Slaughtering was undertaken after 32 days. Results show that broilers which received seaweed-based products experienced a drastic decrease of antibiotic use (-92%) while maintaining and/or improving growth performances such as the weight at slaughter and the Food Conversion Ratio (FCR). Moreover, the condemnation rate at slaughter and economic mortality was reduced by 25 and 68% respectively for JA 987.

In conclusion, seaweed-based products introduced in the animal feed through an adapted program reduced the use of antibiotics without losing on animal growth performances. Such results indicate a global improvement of animal health despite intense farming practices which are inclined to progress worldwide within the coming years.

Keywords: Seaweed-based

PH19
Evaluation of efficacy of essential oil blend as alternative to anti-biotic growth promoters in broilers

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Essential oils are complex mixture of structurally diverse volatile compounds produced by aromatic and medicinal plants. Essential oils comprise structurally diverse volatile compounds, which are demonstrated to have unique bioactivity, especially when used in combination. Several studies confirmed the beneficial effects of essential oils in animal health and nutrition, especially as a performance enhancer for commercial poultry production. With the prohibition on the use of antibiotics as growth promoters (AGPs) in different countries and with increasing concerns about the microbial resistance, focus on the use of essential oils as an alternative for anti-biotic growth promoter in broiler diet is increasing exponentially. In this study, we evaluated the growth promoting properties of selected blend of essential oils (EOB), in comparison with different anti-biotics used as growth promoter in commercial boiler birds.
A total of 980 ROSS 308 birds were randomly divided in to 7 groups comprising, control, T1 (BMD) T2 (Tylosin), T3 (Virginiamycin), T4 (Oxy-tetracycline), T5 (EOB 250g/Ton of feed) and T6 (EOB 750g/Ton of feed). Anti-biotics – bacitracin, tylosin, virginiamycin and oxy-tetracycline were used as positive control. FCR, feed intake, body weight gain, mortality and histomorphological data were collected during the study period. FCR and body weight gain of EOB supplemented birds, from both dosage groups, resulted in highly significant ($p<0.001$) improvement when compared to control. Additionally, T6 (750g EOB supplementation) resulted in highly significant ($P<0.001$) difference in FCR and body weight again, when compared to BMD, tylosin, virginiamycin and oxy-tetracycline anti-biotic groups. Similarly, T5 (250g EOB supplementation) showed statistically significant ($P<0.05$) difference in FCR and body weight gain, when compared to oxy-tetracycline and virginiamycin supplemented groups. Among BMD, tylosin and T5 groups, there wasn't any significant difference in weight gain and FCR. Cumulative mortality was less in all groups including control, probably due to best in class farm practices, in spite of that, reduced mortality was observed in EOB supplemented groups, when compared to control. These results infer the dose dependent growth promoting activity upon EOB supplementation in broilers and its efficacy was better than anti-biotics virginiamycin and oxy-tetracycline and comparable to BMD and tylosin as growth promoter, even in the low dose (250g/ton of feed) EOB supplementation. Thus, even low dose of EOB can be effectively used to replace anti-biotics such as BMD, tylosin, virginiamycin and oxy-tetracycline in broiler diets, as an efficient alternate anti-biotic growth promoter.

Keywords: phytogenics, broilers, AGP, Zootechnical performance, alternatives to AGP

PH20
Combination program trial using two different plant extract additives improved immune and zootechnical parameters in broilers

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Phytogenics are secondary metabolites of plants with diverse bioactivity. During the last two decades, phytogenics have been widely used in animal health and nutrition because of their antimicrobial, antioxidant, immunomodulatory and growth promoting properties. Independent studies confirmed growth promoting and immune-modulatory effects of two different essential oil blends (EOB) in broilers. EOB1 comprises a blend of Oregano, Clove and Cinnamon essential oils and EOB2 contains blend of turmeric oleoresin, capsicum oleoresin and piperine. The aim of this study was to evaluate the efficacy of these two formulations in improving zoo-technical and immune-modulatory performance of broiler, in a combination program feeding trial.

A total of 1000 day-old ROSS 308 chicks were randomly divided in to 5 groups comprising negative control, positive control (BMD), T1 (EOB1 250g/t of feed), T2 (EOB2 250g/t of feed) and T3 (EOB2 and EOB1). In T3, birds were supplemented with EOB2 (250g/t of feed) for 14 days followed by EOB1 (250g/t of feed) from day 15 to 42 days of age. FCR, Feed intake, body weight gain, NDV and IBD anti-body titer levels were monitored. One-way ANOVA was used to compare different treatment groups using GraphPad Prism 6 software. Tukey's multiple comparison test was used, in order to draw treatment difference. Improvement (P<0.0001) in FCR and BW gain were observed in the positive control (1.69, 2367.7g), T1 (1.69, 2375.4g) and T3 (1.68, 2391.4g) groups, compared to that of control (1.72, 2329.6g) birds. FCR (P < 0.01) and BW gain (P<0.001) were found to be greater in T3 group birds, compared to BMD supplemented birds. Supplementation of BMD, EOBs alone and in combination did not affect the feed intake (P > 0.05) of birds compared to control. An increase (P<0.0001) in NDV antibody-titer levels was observed in the positive control (5073.4) and all three treatment (4767.1 to 8068.3) groups, compared to control (547.7) on 42d. T1 group NDV antibody titer value (8068.3) was greater (P<0.0001) than the positive control. Similarly, an increase (P<0.0001) in IBD antibody titer value was observed in positive control (872.9) and all treatment groups (803.5 to 892.7), compared to control (319.5). There was not any difference in IBD antibody titer values (P>0.1) among the treatment and positive control groups. These results show that supplementation of EOB2 in the starter phase, followed by EOB1 during the grower phase can improve both immune and zoo-technical parameters.

Keywords: plant extracts, broilers, zootechnical performance, AGP, immunity

PH21
Dietary resin acid supplementation improves the performance of sows and piglets

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Coniferous resin acids have anti-inflammatory and antimicrobial effects. We have previously shown that dietary resin acids improve the reproductive performance of sows and increased the production and immunoglobulin G -content of colostrum. Here we studied the effect of a tall oil -based resin acid composition (RAC) with 8.5% resin acids to the performance sows and their offspring.

Fifty-six Polish synthetic line 990 sows were allocated to two dietary treatments. The standard lactation feed was amended with 0 kg/tn (Control; C) and 1.0 kg/tn (Treated; T) of RAC from 2 wk before to 4 wk after farrowing. The parity number, backfat thickness at start and end of lactation, course and length of parturition, number of total born, born alive and stillbirth piglets, number and weight of piglets and weight of litter at weaning were recorded for the sow. The piglets were weaned at 4 wk of age. From both diet groups, 80 castrated male and 80 female weanlings were housed in groups of eight and allocated to C and T dietary treatments, to reach a total 320 piglets in the following four treatments: CC, CT, TC, TT. The piglets were weighed at 0, 2 and 6 wk after weaning, and recorded for daily feed intake, feed conversion ratio (FCR) and mortality. For statistical evaluation of the performance data, ANOVA was used.

Dietary RAC decreased the course of parturition, the number of stillborn piglets, and piglet mortality before weaning, increased piglet weight at birth, and resulted in an average of 0.96 more piglets per litter ($p<0.05$). For 2 wk post-weaning, piglet average daily gain (ADG) was lower for the CC than other dietary groups ($p<0.05$). ADG remained significantly lower in CC than CT group for 6 wk post-weaning. Average daily feed intake was higher in piglets of the CT group than in TC group ($p<0.05$). FCR was better in piglets of TT group than in piglets of the TC group. In conclusion, the performance of sows and piglets was improved by dietary RAC supplementation at lactation and nursery respectively.

Keywords: resin acid, sow, piglet, performance

PH22
Evaluation of the impact of garlic and cinnamaldehyde application on *Salmonella* recovery at end of broiler growout

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The final days prior to broiler transport is a critical time to reduce or eliminate foodborne pathogenic bacteria. Compounds such as allicin, deriving from garlic as well as cinnamaldehyde have shown to mitigate growth of gram-negative bacteria, including *Salmonella*. However, *in vivo* research implementing a combination of these compounds is limited. The objective of this study was to evaluate the effects of the Alliin Plus (AP) product, a combination of freeze-dried garlic and cinnamaldehyde, on reducing cecal, environmental and crop prevalence of *Salmonella* at the end of growout.

A total of 216 male broilers at 35 days of age were placed in one of the following treatments: 1) Control (CC), 2) AP in mash feed (AP-F), and 3) AP in drinking water (AP-W). Dosage of AP was 900 g per ton of feed or 1 g/L so that total expected daily intake per bird was equivalent for both treatments. Each treatment had 6 replicate pens with new litter (3 pens/room) and 12 birds/pen. A *Salmonella* Typhimurium (STM) challenge was performed 7 days post placement and all birds were gavaged with ~10^8 STM CFU of nalidixic-acid resistant strain. Impact of AP on STM challenge was measured in cecum. Four birds/pen were sampled on day 7 and 12 post-challenge (i.e. 14 and 19 days of treatment). The day before each sample collection (6 days and 11 days post-challenge), the litter surface of each pen was sampled using intermittently stepped-on drag swabs. Furthermore, crop samples were collected on day 12 post challenge (19 days of treatment). To test if *Salmonella* prevalence would change with feed-withdrawal practice, on the 12-day collection, two pens/treatment were either full-fed, 6-h off feed or 12h off-feed 6h off-water prior to sampling.

Although all litter was *Salmonella*-positive, AP-W at 14 days had the lowest prevalence (p=0.029) as there was no recovery of *Salmonella* with direct plating (>10^2 cells/mL sample). There was no difference on feed-withdrawal, therefore data was combined at the 12-day collection. This was also observed with cecal recovery of STM that was, although not significant, a trend of over a log reduction with the AP-W treatment (1.85 ± 0.51, p=0.470) relative to CC (3.07 log ± 0.48). Additionally, cecal recovery showed no statistical difference at 12 days post challenge. On day 12 post challenge crop also showed a trend of lower recovery of STM in AP-W (0.23 ± 0.15) relative to than in CC (1.39 ± 0.64; p=0.174). Results were similar in AP-F group where cecal recovery on day 7 trended of a log reduction relative to CC (1.49 log CFU ± 0.82; p=0.261) and crop recovery on day 12 was lower than CC (0.15 ± 0.15; p=0.133). These preliminary results suggest that garlic and cinnamaldehyde may help to reduce the prevalence of environmental and cecal colonized *Salmonella* prior to processing, therefore a possible water or future in-feed application at the end of grow out. Future studies at larger magnitude and dose titrations are warranted.

Keywords: *Salmonella*, poultry, allicin, cinnamaldehyde, water-application

PH23
Inclusion of lignocellulose in semi-purified diet on performance and duodenal morphology of broilers

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Antibiotics have been added in feed as growth promoters to reduce overall health problems and improve growth performance in past several decade. Nowadays, the use of antibiotic in modern broiler production has been prohibited from many countries throughout the world. However, one of the potential candidates as effective alternative to in-feed antibiotics is lignocellulose (LC). LC are the major structural component of plant cell wall and mainly composed of lignin and three polysaccharides: cellulose, hemicellulose, and pectin. Some reports exhibited that LC are profitable to gut health and structure as well as stimulating the hydrochloric acid secretion. Therefore, the current research was aimed to evaluate the effect of dietary LC in semi-purified diet on performance and duodenal morphological alterations of broilers. One hundred sixty 10-day-old Ross 308 chicks were divided into 4 groups, each with eight replicates of five birds. They were fed the semi-purified diet included with LC at 0 (control), 60, 80 and 100 g/kg until 45 days of age. Results showed that diets containing various levels of dietary LC clearly affected broiler performance and duodenal tissue. Compared to the control group, body weight and weight gain tended to be higher with increasing dietary LC levels and significantly increased ($p<0.05$) in the 80 and 100 g/kg LC groups. Whereas, feed intake significantly decreased ($p<0.05$) in both 80 and 100 g/kg LC groups, results in an improved feed conversion ratio in all the LC groups ($p<0.05$). With increasing dietary LC levels, crypt depth (CD) in duodenum (CD) was significantly shallow ($p<0.05$) in the 80 and 100 g/kg LC groups. Duodenal villus height (VH) and VH to CD ratio increased linearly ($p<0.01$) in all the LC groups than the control group. There were no significant ($p>0.05$) differences in duodenal villus area among the dietary treatments. In conclusion, dietary LC can be added in semi-purified diet up to 100 g/kg to enhance broiler performance as a result of stimulation of duodenal morphological maturation. These results suggest that LC have a high potential to use as alternative strategy to antibiotic growth promoters due to their ability to support gut development.

Keywords: Broilers, Duodenal morphology, Growth performance, Lignocellulose, Semi-purified diet

PH24
Sunflower meal inclusion rate and the effect of exogenous enzymes on broiler performance

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Worldwide, corn and soybean meal are the most used conventional feed ingredients for broiler diets. Nutritionists are constantly in search of alternative affordable and nutritious feed ingredients due to high feed costs. One promising ingredient is sunflower meal (SFM). It is rich in protein content, methionine, and does not have antinutritional factors. However, its use in broilers has been limited by low levels of lysine, high crude fibre and non-starch polysaccharides. This study was conducted to determine if inclusion levels of SFM can be increased, and if the efficacy of exogenous enzymes (EE) on broiler performance is altered by SFM inclusion rates.

All experimental procedures were approved by the Animal Ethics Committee at the University of Pretoria (Project No. EC042-18). A commercial 4-feeding phase of pre-starter (1-9d), grower (10-20d), finisher (21-28d) and post-finisher (28-35d) was followed. Two SFM inclusion rates were used; low sunflower meal (BSL) and high sunflower (BSH). SFM contained 36% crude protein. SFM inclusion in BLS was 3% throughout the phases, whereas in BSH, inclusion was 7.5, 10, 13 and 13.5%. Each SFM inclusion, had a Negative Control (NC); a Positive Control diet (PC) with additional 80kcal Apparent Metabolizable Energy (AMEn). Additionally, xylanase (X), xylanase plus beta-glucanase (XB), xylanase plus beta-glucanase plus protease (XBP), xylanase plus amylase plus protease (XAP) enzymes were added to the BSL and BSH NC diet formulation to make 12 treatments. One-day old male Ross 308 chicks (n=1,920) were placed on pens (2m*1m) and allocated to the treatments (n=8 replicate/treatment) from day 1 – 35. Pen bird weight (BW) and feed intake (FI) was determined on day 9, 20, 28 and 35d and feed conversion ratio (FCR) calculated. Data were statistically analysed (P<0.05) using a randomized two-way ANOVA model.There was no interaction (P>0.05) between SFM inclusion and Enzyme treatment for all parameters throughout the study period. Increasing SFM inclusion reduced FCR at 9d (1.15 vs 1.17), but affected performance parameters at any period thereafter. There was a significant main effect of enzyme treatment on BW gain to 35d. Birds fed the XAP enzyme gained more weight (2.69kg) than either the PC (2.61kg) or NC (2.62kg) while other enzyme combinations had no effect on 35d BW gain. A limitation of this study was that there were no significant differences between the NC and PC diet with 80kcal/kg more AMEn. This could be due to the energy density of the diets being above the requirement and limited the ability to separate enzyme effects on FCR. In conclusion, our data suggest that SFM can be increased to at least 7.5% in starter feed and 10% in grower and finisher diets without negatively affecting performance, and that the addition of XAP enzymes can improve BW gain of broilers grown to 35d of age.

Keywords: sunflower meal, non-starch polysaccharides, exogenous enzymes, efficacy, inclusion rate

PH25
Herb based complexes for improving the quality of the microbiome

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Herbal compounds possess antimicrobial properties and therefore can represent true alternatives to antibiotics. The greatest disadvantage of herb originated compounds is that the biological activities vary largely on the origin, the harvesting time or on the applied extraction procedure.

During our research an optimized extraction procedure was established for plant oils and extracts which enabled the minimizing of the biological variation and resulted in stable and reproducible antimicrobial properties for various herbal compounds towards multiple obligate and facultative pathogens of livestock. As a second step, during the screening procedure, a unique quality control system was introduced where instead of defining exact chemical compositions; plants, plant oils and extracts were categorized based on their biological activities observed in multiple *in vitro* studies towards isolates of the pathobiome, such as *E. coli*, *Clostridium perfringens, Brachyspira* species or *Lawsonia intracellularis.*

With using the optimized extraction procedure and the biological activity data of over hundred screened herb originated drugs, different feed additives were developed which prevents the pathobiome development and helps in maintaining the normal microbiome in livestock. The optimized compositions of phytochemicals were tested in target species such as poultry and pigs. *In vivo* trials were conducted on small scale laboratory farms and on large production farms, with either excellent or low hygienic circumstances.

Based on the results of several *in vivo* trials, it was concluded that the application of herbal extract containing feed additives can serve as alternatives to antibiotics, result in significantly less diseases and increased production parameters, meat quality and overall wellbeing, especially on farms with several hygienic issues and low farm management involvement. Stabilizing herbal compounds with optimized extraction procedures help in maintaining a stable and reproducible biological activity, which is a crucial parameter in terms of industrial scale production.

Keywords: pathobiome, selective inhibition, screening, minimal inhibition concentration

PH26
Study of cost effective feed additives to replace AGP in poultry chickens, improving productive parameters and reducing antimicrobial resistance spread

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Antibiotic growth promoters have been used for decades in animal production to maintain health and improve productive parameters. Global concern about the recurrent emergence and spreading of antimicrobial resistance is challenging the livestock producers to find cost effective alternatives. The use of phytogenic compounds, probiotics and organic acids appears as a feasible option due to their ability to emulate the bioactive properties of antibiotics. The present study was planned to compare (1) a standard AGP program against two antibiotic free programs: (2) a combination of probiotic- yeast moss - chestnut/quebracho extracts blend and (3) a chestnut/quebracho extracts blend. A total of 1,575 animals distributed in 3 treatments with 15 replicates each one was followed from 0 up to 42 days of life. Weight gain, feed conversion, live weight/feed conversion relation, mortality, and overall health were routinely measured and recorded. Intestinal health was evaluated at day 21 and 42. Cecal and litter samples were taken for microbiota analysis, moisture, bacteriological determinations and antimicrobial resistance environmental spread.

The weight gain and feed conversion were improved by both non-AGP programs when compared to AGP, 1.051 vs 1.077 and 1.072 for treatment 2 ($p>0.05$) and 3 ($p>0.05$) respectively at day 21. At day 41 no significant differences were observed among treatments, however birds in treatment 3 showed higher weight and improved feed conversion (2.978 vs 3.003 kg; 1.728 vs 1.715), while birds from treatment 2 only showed improved feed conversion (1.728 vs 1.714). Microbiota analysis show a differential effect of all treatments, particularly alpha diversity, number of species and specific groups of beneficial bacteria improved by non-AGP treatments. Food pad lesions and intestinal health was similar in AGP or non-AGP programs, with most in 0 lesion score. Both non-AGP treatments showed an important tendency to reduce antibiotic resistance in representative bacteria from intestine and litter. Bacteriological parameters, weight gain, feed conversion rate, reduction of mortality was improved by non-AGP, producing an improvement of productive parameters with costs a reduction. The available information supports the competitive use of these alternative programs and an important cost reduction by the use of specific mixture of polyphenols.

Keywords: phytochemicals, productivity, poultry, antimicrobial resistance, polyphenols

PH27
The dose of chestnut and quebracho polyphenols alters rumen microbiota profile and production of volatile fatty acids in bovines

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Previous studies showed that inclusion of polyphenol-rich plant extracts in feed improves performance in ruminants, but the effect may vary depending on the dosage used. It has been shown that polyphenols modify the digestive process through modulation of gastrointestinal microbiota and bypass of protein digestion. The rumen houses a highly complex microbiota which is able to convert indigestible plant mass into energy mainly through production of short-chain fatty acids (SCFA).

High-throughput sequencing of 16S rRNA gene was used to study the temporal changes in microbiota composition of eight rumen-fistulated steers supplemented with four doses of chestnut and quebracho plant extracts (0, 0.075, 0.15 and 0.30 kg per ton) in a 4x4 Latin-square design. In each sample, the pre- and post- prandial pH of the ruminal liquor was measured and the SCFA profile was determined by HPLC. Bioinformatics and statistical analysis of microbiota variability was performed using QIIME2 software.
6.724.119 reads of 16S rRNA gene V3-V4 region were obtained in total. A significant variation in rumen microbiota beta diversity was detected between animals and between samplings ($p<0.001$), but pre- and post-prandial samples showed very limited variation, with virtually identical patterns in both series of data. A smaller but significant effect was observed between polyphenol doses on alpha and beta diversity parameters. Steers with higher doses tend to have lower richness ($p=0.09$) and lower Shannon's diversity index ($p=0.03$), which indicates a more even and balanced microbial community in treated animals. When comparing with the not-supplemented control group, all three polyphenol doses reduced the relative abundance of phylum Euryarchaeota, which includes the methanogenic archaea responsible for greenhouse gas production. The two higher doses also increased the relationship between Firmicutes and Bacteroidetes (F/B). The dose of 0.15% was the one with the highest F/B ratio and the lowest relative abundance of methanogenic bacteria. Regarding SCFAs production and pH, a strong correlation was observed between these parameters and rumen microbiota diversity. Steers with lower number of bacterial species had a more acidic ruminal pH ($p=0.0004$), and higher ratio of C2/C3 in their SCFA profile ($p=0.0013$).

The results showed that the groups supplemented with chestnut and quebracho polyphenols have similar changes compared to the control group, but the modulatory effect on the diversity and composition of rumen microbiota and SCFA production profile varies depending on the dosage of extract used, reaching an optimal effect at intermediate doses. Therefore, the dose of polyphenols is a parameter that can be optimized since differentially alters ruminal physiology.

Keywords: microbiota, phytochemicals, rumen, bovines, polyphenols

PH28
Eugenol attenuates inflammatory responses and enhance barrier functions during lipopolysaccharide (LPS)-induced inflammation in porcine intestinal epithelial (IPEC-J2) cells

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Eugenol is an essential oil component which is known to possess anti-microbial, anti-inflammatory and anti-oxidative properties, however the effect of eugenol on porcine gut inflammation has not yet been investigated. In this study, an *in vitro* lipopolysaccharide (LPS)-induced inflammation model in porcine intestinal epithelial cells (IPEC-J2) was used.

The results showed that eugenol pre-treatment significantly suppressed the LPS-stimulated interleukin 8 (IL-8) level and the mRNA abundance of tumor necrosis factor α (TNF-α); enhanced the LPS-stimulated mRNA abundance of tight junction proteins *zonula occludens-1 (ZO-1), occludin (OCLN), claudin-1(CLDN-1), claudin-3 (CLDN-3)* and the mRNA abundance of the following nutrient transporters; *B^0-system neutral amino acid co-transporter (B^0AT1), system ASC sodium-dependent neutral amino acid exchanger 2 (ASCT2), apical sodium-dependent glucose transporter 1 (SGLT1), excitatory amino acid transporter 1 (EAAC1)* and *peptide transporter 1 (PepT1)*. In addition, eugenol improved the expression and even redistribution of ZO-1, tended to increase TEER value and maintained barrier integrity and tightness.

A low dose of eugenol showed to have a positive effect on attenuating inflammatory responses and enhancing selectively permeable barrier function during LPS-induced inflammation in the IPE-J2 cell line.

Keywords: eugenol, LPS-induced inflammation, inflammatory responses, barrier function, IPEC-J2 cells

PH29
Beneficial properties and mechanistic study of a phytogenic formulation, Rotam-CS, for avian coccidiosis

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In the interests of food safety and public health, edible plants and their compounds are now re-emerging as an alternative veterinary medicine for meat-producing animals. Here, we studied the impact of Rotam-CS, on coccidiosis, a protozoan disease, growth performance and drug resistance in chickens. First, we found that Rotam-CS was therapeutically effective against coccidiosis in chickens as evidenced by a survival rate, gut pathology, fecal oocyst excretion and anti-coccidial index. Next, we showed that Rotam-CS significantly increased body weight gain and decreased feed conversion ratio in chickens. Mechanistic study showed that Rotam-CS inhibited the life cycle of *Eimeria* species. Overall, this work suggests Rotam-CS as a potential remedy for avian coccidiosis via interference with protozoan life cycle.

SESSION 5
Immune-related products

ORAL PRESENTATIONS

Passive Immunity and IgG-like antibodies as an alternative to antibiotics

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With the surge of the world-wide antimicrobial resistance problem (see e.g. Review on Antimicrobial Resistance (2016), https://amr-review.org/) severe actions are needed to limit the use of antimicrobial drugs in the human as well as the animal sector. The animal production sector demands a huge consumption of antibiotics frequently used with little indication e.g. for animal growth performance enhancement and routine prevention rather than treatment of disease.

A prerequisite for alternatives to antibiotics to gain widespread acceptance in the animal production sector is that they are truly cost-efficient at the same level as antibiotics which as a class of drugs are very efficient, inexpensive and very broadly applicable. Non-vaccine immunization, also known as passive immunization offers an interesting alternative to antibiotics on one side and active immunization (vaccination) on the other side. Passive immunization offers a unique combination of sustainability, broad coverage, cost-effectiveness and absence of risk of anti-microbial resistance induction in production animals. In addition, passive immunization offers an often overlooked possibility of efficiently and safely treating or preventing infectious diseases with unknown and/or multi-factorial origin, making it particularly relevant for a range of major animal production diseases including perinatal as well as weaning associated intestinal disease.

The presentation will provide background on passive immunity approaches and give examples of applications within pig, cattle, poultry and fish production, showing recently published as well as unpublished research in the field. The performance of passive immunization approaches such as oral IgG and IgY, specific, polyspecific and non-specific immunoglobulin preparations, and modified immunoglobulins alone and in combination with other bioactive compounds will be compared with vaccination, maternal vaccination, breeding based strategies, and the use of probiotics and bacteriophages. Sources and production methods will be taken into account with a focus on sustainability, ease of use, low cost and efficiency, all of which are prerequisites for the widespread use of any alternative to antibiotics in the animal production sector.

Keywords: Passive immunization, animal production diseases, IgG, IgY, alternative

Host defence peptides with anti-microbial and immunomodulatory activities as antibiotic alternatives

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With the increasing emergence of antibiotic-resistant pathogens, antimicrobial peptides (AMPs) have been studied as alternatives to antibiotics based on their broad spectrum of bactericidal activity and selectivity. Cationic antimicrobial peptides are highly conserved in all organisms and are effective against many bacteria, including multidrug-resistant bacterial strains, by disrupting the bacterial membrane based on their cationic nature. However, the direct activity of cationic AMPs towards the microbial membrane is dependent on physiological conditions such as salt and serum. Increasing evidence indicates that direct microbial killing may not be the primary role of cationic AMPs in the body, and efforts to determine the role of cationic AMPs have focused on the immunomodulatory properties of cationic AMPs. The immunomodulatory activity of cationic AMPs is complex and includes anti-infective immune modulation such as the induction of chemokines and cytokines, pro/anti-inflammatory activity, direct chemotaxis, wound healing, angiogenesis, apoptotic activity and adjuvant activity. The immunomodulatory activity of cationic AMPs also varies depending on the cell type. Because of their ability to modulate the immune response, cationic AMPs are called host defense peptides (HDPs). Chicken NK-lysin (cNK-lysin) is a homologue of human granulysin which is found in the cytolytic granules located in human natural killer and cytotoxic T lymphocytes. It was previously demonstrated that cNK-lysin is hugely expressed in *Eimeria*-infected intestinal lymphocytes, suggesting a role in parasite infection. Subsequent studies have shown that cNK-lysin and cNK-2, the core α-helical region of cNK-lysin, can kill *Eimeria* sporozoites by disrupting the parasitic membrane. Interestingly, cNK-2 exhibits higher antimicrobial activity than the original peptide and even melittin indicating that the modification of the natural sequence can improve efficiency. In mammals, granulysin acts as an immunomodulatory peptide by serving as a chemoattractant for lymphocytes and modulating the expression of chemokines and cytokines. The present study demonstrates that cNK-2 has immunomodulatory properties as an HDP, including inducing chemokines/cytokines, an anti-inflammatory response, signaling pathway activation and internalization into chicken cells. By contrast, the antimicrobial effects of cNK-2 were reduced under physiological salt conditions. The responses of HD11 cells and primary monocytes to cNK-2 were also studied to understand the role of cNK-2 in innate immunity. The findings presented here provide advanced insight on how the chicken immune response is modulated by HDPs.

Keywords: antimicrobial peptide, host defense peptide, chicken, coccidiosis, alternatives to antibiotics.

Innovative enterobactin-specific egg yolk antibodies for controlling Gram-negative pathogens

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Passive immunization with specific egg yolk antibodies is emerging as a promising alternative to antibiotics for the treatment and prevention of various mucosal infectious diseases in food animals. Enterobactin (Ent)-mediated high affinity iron acquisition is a universal and critical contributor for Gram-negative mucosal pathogens to survive in the hosts. Given the bacteriostatic effect of lipocalin resulting from its potent Ent-binding ability, immune intervention directly targeting Ent is promising for iron-dependent pathogen control. In this study, we developed a novel Ent conjugate vaccine that has several significant advantages, such as ease of preparation, induction of high titer of Ent-specific antibodies in rabbits as well as chickens, and the ability of Ent-specific antibodies to bind various Ent derivatives including the salmochelins that help enteric pathogens evade sequestration of siderophores by host lipocalins. Notably, the *in vitro* growth assays provided compelling evidence that the Ent-specific antibodies function similarly as lipocalin to interfere with Ent-dependent growth of Gram-negative mucosal pathogens (e.g. *E. coli*, *Salmonella*, and *Campylobacter*) under iron-restricted conditions. Subsequently, we evaluated different vaccination regimens for production of hyperimmune Ent-specific egg yolk antibodies. Different layers (Barred Rock, Rhode Island Red), Ent conjugate vaccines (BSA-Ent, KLH-Ent, or CmeC-Ent), and immunization routes (intramuscular *vs* subcutaneous) were used in multiple immunization trials. The levels of specific antibodies (IgY) in serum and egg yolk were measured using immunoblotting and ELISA assays. The KLH-Ent triggered immune response in Barred Rock layers via intramuscular route, leading to significantly increased titer of Ent-specific IgY in serum (up to 4 fold) and yolk (up to 8 fold). Subcutaneous immunization of Rhode Island Red pullets with KLH-Ent or CmeC-Ent dramatically increased Ent-specific IgY in serum (up to 2,048 fold) and yolk (up to 1,024 fold). However, the BSA-Ent immunization did not induce anti-Ent IgY significantly. The lyophilized Ent-specific hyperimmune egg yolk IgY is being evaluated for its passive immunization efficacy in protecting chickens from the infections caused by *Campylobacter* and avian pathogenic *E. coli*. Together, this study reports an efficient method to prepare innovative Ent conjugate vaccines for inducing high level of Ent-specific antibodies. We also optimized vaccination regimen in layers and produced hyperimmune Ent-specific egg yolk IgY that has significant potential for prevention and control of Gram-negative infections in food animals.

Keywords: Egg yolk antibody, passive immunization, Enterobactin

High throughput screening for natural host defense peptide-inducing compounds as alternatives to antibiotics

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A rise in antimicrobial resistance demands novel alternatives to antimicrobials for disease control and prevention. As an important component of innate immunity, host defense peptides (HDPs) are capable of killing a broad spectrum of pathogens and modulating a range of host immune responses. Enhancing the synthesis of endogenous HDPs has emerged as a novel host-directed antimicrobial therapeutic strategy. To facilitate the identification of natural products with a strong capacity to induce HDP synthesis, a stable chicken HTC macrophage cell line expressing a luciferase reporter gene driven by an avian β-defensin 9 (*AvBD9*) gene promoter was constructed through lentiviral transduction and puromycin selection. A high throughput screening assay was subsequently developed using the stable reporter cell line to screen a library of 584 natural products. A total of 21 compounds with a minimum Z-score of 2.0 were identified. Secondary screening in chicken HTC macrophages and jejunal explants further validated most compounds with a potent HDP-inducing activity in a dose-dependent manner. A follow-up oral administration of a lead natural compound, wortmannin, confirmed its capacity to enhance the *AvBD9* gene expression in the duodenum of chickens. Besides *AvBD9*, most other chicken HDP genes were also induced by wortmannin. Additionally, butyrate was also found to synergize with wortmannin and several other newly-identified compounds in *AvBD9* induction in HTC cells. Furthermore, wortmannin acted synergistically with butyrate in augmenting the antibacterial activity of chicken monocytes. Therefore, these natural HDP-inducing compounds may have the potential to be developed individually or in combinations as novel antibiotic alternatives for disease control and prevention in poultry and possibly other livestock species.

Keywords: Host defense peptides, antimicrobial peptides, HDP-inducing compounds, high throughput screening, poultry

Making the transition from research trials to field application

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Since the mid 1990's, there has been a global shift away from the prophylactic or growth-promoting antibiotics (AGP) in poultry feed, due to legislation or consumer pressure. Therefore, there is a reasonably long history of the development of AGP alternatives. In spite of this history, the transition to AGP-free production is often not a smooth one for individual companies, or even individual farms within a company.

Ideally, a replacement product for AGP should be economically feasible, simple to apply consistently under field conditions, be accepted by consumers, not promote microbial resistance, and most importantly, be efficacious. The challenge faced by the industry is to replace a strategy that was broadly effective (i.e. AGP generally work under a wide variety of conditions, and the impact of AGP was typically most pronounced under poor production conditions). A transition to alternatives, however, will also require a higher standard of housing, husbandry, biosecurity, management, and nutrition. Controlled research trials are a necessary step towards commercial implementation, but caution must be used when evaluating research trial results.

Many proposed alternatives to antibiotics have been investigated in research conditions, with varying degrees of success. However, the translation to success under commercial conditions has often been problematic. There are several reasons for this. First, many controlled studies fail to include a proper negative control in which a challenge an be demonstrated. It is assumed that equivalent performance of a test product to antibiotics proves efficacy. However, in the absence of a demonstrated microbial challenge, it is impossible to determine whether the equivalent performance to AGP is due to an ability to reduce the effects of infection, or the lack of an infection. Second, controlled experiments usually utilize a specific disease challenge model. Efficacy against a single challenge organism may indicate usefulness, but the field challenges will vary from one region to the next, from one farm to the next, and even over time on the same farm. Third, even studies utilizing natural challenge models are limited by the specific environment in which the study is conducted. Finally, because of the variety of potential challenges that may be encountered in the field, a single alternative product with a specific mode of action is not likely to be effective against the variety of different pathogenic organisms that may be encountered.

A successful, commercially-viable AGP-free strategy will likely involve the utilization of multiple products, with complementary mechanisms of action, and will need to be tailored to each production unit. Although this may take time and effort, it is likely to be the only path to success.

Reprogramming the innate immune system as an alternative

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The aim of vaccination is to induce specific immunological memory that helps the adaptive immune response to mount an adequate response against a specific pathogen. Accruing evidence, however, suggests that some widely used vaccines protect in a-specific fashion against unrelated pathogens. This generic protection may be explained by innate immune memory or so-called "trained immunity". In recent years it was discovered that innate immune cells like macrophages and Natural Killer cells exhibit sustained memory that helps these cells to respond to re-infection. Various microbial products have been shown to "train" the innate immune system and their effects seem to be exerted by epigenetic changes.
Host defense peptides (HDPs) are small molecules present in all vertebrates and show both antimicrobial and immunomodulatory activities. Cathelicidins are cationic HDPs with an important function in the early vertebrate host response against invading pathogens. These peptides are secreted at mucosal surfaces by leukocytes and epithelial cells upon interaction with pathogenic microorganisms. If cathelicidins or cathelicidin-derived peptides are administered to experimental animals, it was observed that these animals were protected against subsequent infections. Here we report that the protective effects may be partly explained by "training" of the innate immune system.

It is concluded that reprogramming the innate immune system of farm animals by microbial products, HDP-derived products or combinations thereof may be a strategy to reduce antibiotic use.

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Efficacy of dried egg product administered to male broiler chickens during experimental necrotic enteritis

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Despite vaccination, medication, and management efforts, *Clostridium perfringens* (*Cp*) continues to cause necrotic enteritis (NE) worldwide resulting in devastating economic losses to the poultry industry. Coccidiosis is considered a trigger for NE outbreaks in flocks and thus efforts to control *Eimeria* spp. infection may ameliorate NE incidence and severity. Consumer apprehension about antibiotic usage in poultry is increasing and alternative solutions are needed. This study assessed the impact of a dried egg product (DEP) containing anti-interleukin 10 (IL-10) IgY neutralizing antibody on performance of broilers raised under antibiotic free (ABF) management practices during experimental NE challenge. A randomized complete block design was used to evaluate 6 doses (0, 143, 287, 358, 430, and 573 U/MT) of a DEP under experimental NE consisting of, Cocivac® B52 (Merck Animal Health, Kenilworth, NJ) spray vaccination on day of hatch according to label, and 10^8 cfu/bird/day of *Cp* in feed on d 18, 19 and 20. An additional *Cp* unchallenged control was included (NNC). Cobb 500 males were housed at 50 birds/pen in 70 floor pens (10 pens/treatment) for 42 d. Diets were administered in 3 feeding phases 0-14 (Starter), 14-28 (Grower), and 28-42 d (Finisher). A basal diet was mixed for each phase and DEP was added to each basal diet to make treatment diets, which were identical in ingredient and nutrient composition. Body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR), unadjusted and adjusted for mortality (MA), and production efficiency index (PEI) were determined for each feeding phase. On d 20, 4 birds were randomly selected from each pen for NE lesion scoring. Statistical analyses were conducted using JMP (v. 14.1, SAS Institute, Cary, NC) using pen as the experimental unit with treatment as a fixed effect and block as a random effect. During Starter, growth performance was not different (P=0.25 to P=0.78) between 0 DEP treatment compared to NNC. Inclusion of DEP during Starter linearly improved (P<0.01) BW, ADG, MA_ADG, FCR, MA_FCR, and PEI. *Cp* challenge during Grower markedly reduced (P<0.0001) BW, ADG, MA_ADG, FCR, MA_FCR, survival, and PEI and MA_ADFI (P=0.06), and increased (P<0.0001) NE lesions scores in the 0 DEP treatment compared to NNC indicating a successful *Cp* challenge. NE lesion scores reduced (P<0.0003) linearly with increasing DEP supplementation. Addition of DEP linearly improved BW, ADG, MA_ADG, FCR, MA_FCR, survival, and PEI during (P<0.0005) Grower; BW and PEI (P<0.0001) and FCR and MA_FCR (P<0.088) during Finisher; and BW, ADG, MA_ADG, FCR, MA_FCR, survival, and PEI during the 42-d trial (P<0.0006). Including 573 U/MT of DEP improved FCR (P<0.005) by 10, 15, 12, and 13 and MA_FCR (P<0.004) by 10, 13, 13, and 12 points during Starter, Grower, Finisher, and overall, respectively, compared to 0 U/MT of DEP. In conclusion, DEP significantly improved bird performance during experimental NE compared with unsupplemented birds under same environmental challenge conditions and coccidiosis vaccination practice.

Keywords: coccidiosis, necrotic enteritis, broiler performance, dried egg product, IgY

Yeast cell wall immunomodulatory and intestinal integrity effects on broilers challenged with *Salmonella* Enteritidis

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The objective of this study was to evaluate the immune effects and the dynamics of intestinal integrity in broilers challenged with *Salmonella* Enteritidis (SE) and treated with yeast cell wall (YCW). One hundred birds were housed in isolators at 1 day of age and divided into 4 treatments: G1- Birds challenged with SE; G2- Birds not challenged and supplemented with YCW (*Saccharomyces cerevisiae*, ImmunoWall® from ICC Brazil, at 0.5 kg/MT); G3- Birds not challenged and not treated/medicated and; G4- Birds challenged with SE and supplemented with YCW (same inclusion). The challenge was administered orally at 2 d with 10^8 CFU per bird. SE was quantified in crop and ceca contents at 8, 14 and 21 d. Circulating lymphocyte and monocyte subsets, as well as phagocytic cells were evaluated at the same time ages. Samples of ileum, ceca, and liver were collected at 14 d (8 birds/treatment) for histopathology. Specific IgA for SE in feces was evaluated also at 14 d. Intestinal mucosa permeability was assessed in 8 birds/group at 4, 8, 14 and 21 d by passage of a marker (Dextran-FITC, 3-5 kD) from intestinal lumen to blood. The data were analyzed by ANOVA and the means compared by Tukey's test at 5% of significance. At 4 d, G1 presented the highest intestinal permeability (significantly different from the treated group [G4]). Circulating leukocytes counts were higher in the non-SE challenged groups (G2 and G3). Despite this, challenged groups consistently presented higher numbers of various cell subtypes, especially at 14 d (APCs, monocytes, suppressor monocytes, and the series of helper T lymphocytes and cytotoxic T lymphocytes). Treatment was effective in controlling leukopenia and in preventing some of the immune subset fluctuations provoked by the challenge, such as for APCs and cytotoxic cells. The number of phagocytic cells was increased by challenge at 8 d, while the YCW decreased this effect. G4 presented the highest number of reactive animals, as well as the highest level of anti-*Salmonella* IgA. The challenge induced marked inflammatory responses in the intestine and liver (assessed by lymphocyte counts, section area, goblet cell counts, tissue architecture). Treatment was effective in improving tissue inflammatory signs such as lymphocyte infiltration in cecum, but not liver. The challenge with SE induced changes in all evaluated systems; however, intestinal integrity and some immune parameters were improved by dietary YCW in challenged birds.

Keywords: *Saccharomyces cerevisiae*; Poultry; intestinal permeability; IgA

SESSION 5
Immune-related products

POSTER PRESENTATIONS

IM1
Characterization of *in-ovo* administered innate immune stimulants for prevention of early chick mortalities due to yolk sac infection

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Early chick mortality (ECM) due to yolk sac infection (YSI) and colibacillosis causes substantial economic loss to the broiler industry of Saskatchewan. Moreover, at the sub-clinical level these infections lead to retarded growth, poor carcass quality, weak response to vaccination, and increased susceptibility to other infections. Currently, culling of chicks with navel defects and *in ovo* or post hatch administration of antibiotics is used for prevention of ECM due to YSI. However, increased antibiotic resistance in the general public, carry over antibiotic residues in the food chain and contamination of poultry meat with antibiotic resistant foodborne pathogens are emerging as significant food safety and public health risks associated with the use of antibiotics in poultry. In addition, the use of antibiotics in the animal industry poses a serious environmental hazard. Thus, to eliminate or reduce these risks and to preserve effective treatment options for public health, rational development of immune prophylactic/immune therapeutic alternatives to antibiotics (on the basis of immune principles and pathogen sensitivity) appears to be an urgently needed realistic approach.

In this context, we have previously demonstrated that *in ovo* administration of 50µg, 20µg or 10µg CpG ODN/embryo or a combination of 10µg CpG ODN plus 15µg poly I:C/embryo resulted >81% survival following severe experimental YSI compared with non-treated or PBS treated groups which exhibited 43% and 60% survival respectively. In the present studies we investigated additional combinations of innate immune stimulants with respect to control of ECM due to experimental YSI. We noticed a significantly improved immune synergetic effect of formulations including: (i) CpG20 µg + avian beta defensin 10µg or (ii) CpG 20µg + poly I:C 15µg or (iii) CpG 20µg + Cyclic polyphosphazene (CPZ) 10µg or (iv) triple combination of CpG 20µg + poly I:C 10µg+ CPZ 10µg. Though, *in ovo* administration of these immune stimulants showed no impact on cecal colonization of *Salmonella*.

Studies to investigate the effect of *in ovo* co-administration of innate immune stimulants and Marek's disease (MD) vaccine showed non-significant differences on post (pathogenic *E. coli*) challenge (PC) survival rates or levels of MD vaccine virus replication in groups treated with (i) innate immune stimulants (ii) Marek's vaccine (iii) co-administration of immune stimulants and MD vaccine. However, relatively lower PC survival rates (compared with other experiments) were observed in these studies. We presume that this may be the result of immune suppression due to live MD vaccine. While, this immune suppressive effect was reduced with an increased amount of CpG ODN.

In conclusion, our data suggest that the above described formulations may serve as potential candidates for replacement of antibiotics for the prevention and control of ECM due to YSI. Furthermore, on basis of these data we may assume that involvement of diverse immune pathways with the use of multiple innate immune stimulants may offer more robust protection from multiple pathogens. However, in this regard further characterization of a repertoire of innate immune stimulants will be helpful in achieving our final goal of increased productivity without use of antibiotics in poultry production.

Keywords: Innate Immune stimulants, Alternatives to antibiotics, Early chick mortality, Yolk sac infection, Avian Patogenic *E. coli* (APEC)

IM2
Dietary β-glucan alters gut health parameters and reduces *Salmonella* shedding in pigs

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Salmonella, while not typically a pathogenic agent in pigs, can cause disease in humans through contaminated food products. Therefore, methods to limit *Salmonella* colonization and/or shedding in pigs are warranted to reduce the incidence of food borne infections. The generation and spread of antimicrobial resistance genes are a major public health concern and non-antibiotic methods are needed to improve animal health and prevent the spread of food borne illnesses. The impact of dietary β-glucan, an innate immunomodulating agent, on intestinal gene expression, circulating monocyte response to innate agonists, and *Salmonella* shedding in pigs was investigated. At weaning (3 wks of age), pigs were fed a control diet or diet modified with *Saccharomyces cerevisiae* β-glucan for the duration of the study. Monocytes were isolated from whole blood at 2 and 4 wks post diets, stimulated *ex vivo* with various innate agonists, and TNF and IL-1β production measured by ELISA. Ileal and cecal tissues were collect at 4 wks post diet from a subset of pigs for gene expression and RNA scope analyses. The remaining pigs were challenged with *Salmonella enterica* serovar I,4,[5],12:i:- and monitored for *Salmonella* shedding over the next 3 weeks. In the cecum, expression of tight junction and mucin stabilizing genes were upregulated in β-glucan fed pigs compared to controls. Increased expression of MUC2 (porcine mucin gene) in the cecum villi and crypts of β-glucan fed pigs was also detected. However, when stimulated with Pam3CSK4 (TLR2 agonist) monocytes from β-glucan pigs produced less IL-1β compared to cells from control pigs. Pigs fed dietary b-glucan had significantly reduced *Salmonella* shedding over the 3 wk period. The reduction of *Salmonella* shedding may be possible through induction of a tolerant phenotype, as circulating monocytes had reduced responses to TLR agonists. The altered gut health parameters may also indicate that epithelial barrier functions were enhanced in β-glucan fed pigs. Overall, β-glucan may serve as a non-antibiotic dietary additive to limit the shedding of the foodborne pathogen, *Salmonella,* through alterations of the local and peripheral immune responses.

Keywords: Beta-glucan, pig, immunomodulation, *Salmonella*, intestine

IM3
Novel hyperimmune egg yolk IgY antibodies developed against protective antigens of *Eimeria* and *Clostridium perfringens* protect against coccidiosis and necrotic enteritis

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Passive immunization with pathogen-specific egg yolk antibodies (IgY) is emerging as a potential alternative to antibiotics for the treatment and prevention of various human and animal diseases. The use of IgY offers several advantages in that laying hens are an excellent source of high-quality polyclonal antibodies, which can be collected noninvasively from egg yolks. With the increase in regulations on the use of antibiotic growth promoters and rise in consumer demand for poultry products from 'Antibiotic Free' or 'No Antibiotics Ever' flocks, the quest for alternative approaches intensified in the recent years. Successful strategies developed as antibiotic alternatives should be both safe for humans and animals, be easily administered, economically feasible and have significant beneficial impact on health and performance. In this report, we describe successful application of egg yolk IgY antibodies to prevent and treat coccidiosis and necrotic enteritis (NE), two most important enteric diseases of poultry which cost industry more than $ 10 million. A series of experiments were conducted to investigate whether passive immunization with oral supplementation of these egg yolk powders as source of *Eimeria*- and *Clostridium perfringens* (CP)-specific antibodies would have any protective effect upon coccidiosis and NE infection in broiler chickens. Four antigens selected from *Eimeria* and CP were proven to be the best therapeutic antibodies in newly hatched broiler chickens when using avian coccidiosis and NE disease challenge models developed at ARS. This is the first report that shows the effectiveness of *Eimeria*- and *Clostridium*-specific egg yolk IgY antibodies against the prevention and treatment of coccidiosis and NE.

Keywords: egg yolk antibodies, passive immunity, coccidia, *clostridium*, necrotic enteritis

IM4
Characterization of NK-lysin antimicrobial protein genes, and their activities, in rainbow trout (*Oncorhynchus mykiss*)

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The regulation, and number, of antimicrobial peptides (AMPs) is not well understood in commercially-important fish species. Based on the new rainbow trout genome assembly, we identified 6 new saposin-like AMP genes and their chromosomal locations. Protein sequence alignments and in silico modeling show that the proteins encoded by those genes belong to the Nk-lysin AMP sub-family (termed: Nkl 1, 2, 3, 4 and Nkl-like a & b).Transcriptomic data show that expression of nkl1-nkl4 mRNAs occurred in many tissues. By contrast, the nkl-like a & b mRNAs are mostly expressed in immune-related tissues. The effects of various aquaculture stressors, and a disease challenge (*F. psychrophilum*; Fp) in rainbow trout, were examined using RNA sequencing. Abundances of nkl1, nkl2, nkl4, and nkl-like a were downregulated by high-temperature and salinity stress, and nkl3 and nkl-like b were downregulated by high-temperature. In the Fp challenge study, abundances of nkl3, nkl4, nkl-like a and nkl-like b, were significantly affected by genetic line (resistant vs non-resistant) and treatment (PBS or Fp), which were further verified by qRT-PCR with spleen tissue sampled at 4 post-challenge time points (6 h, 24 h, 48 h and 144 h). This work represents an initial characterization of these AMPs in rainbow trout, with ongoing in vitro work to characterize how these AMPs affect flavobacterial pathogens, their biofilms, and survival and replication of novirhabdoviral pathogens. Understanding the distribution, regulation and bioactivity of these AMPs may enable rational design of approaches to reduce infectious disease in commercial aquaculture.

Keywords: Antimicrobial Peptides, Nk-lysin, Rainbow trout

SESSION 6
Regulatory pathways to enable the licensing of alternatives to antibiotics and incentives from stakeholders to support their development

ORAL PRESENTATIONS

US FDA's Regulatory Pathway for Alternatives to Veterinary Antimicrobials

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Increased awareness of antimicrobial resistance drives the need to identify and develop alternative products that can be introduced into the marketplace. In the United States, the Agency responsible for the evaluation of the products depends on the product, the primary mechanism of action of the product, and the specific marketing claim. FDA-CVM is responsible for the approval of food additives and new animal drugs. Alternatives to antimicrobials that do not act primarily through direct stimulation of the immune system and are intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease or to affect the structure or function in man or other animals are evaluated as new animal drugs.

New animal drugs are approved for specific indications and conditions of use (e.g. dosage regimens, species, animal class, withdrawal times, prescription status, etc.) which are developed based on a demonstration of effectiveness while balancing any risks associated with target animal safety, human food safety, human user safety, and environmental impact. Congress established the statutory standards for the evaluations of these components.

These regulatory standards can be met through "non-standard" approaches, including the use of published studies, foreign studies, or validated model studies. FDA-CVM has developed processes to assist sponsors with innovative and novel technologies, including those to be used for antimicrobial alternatives, to discuss approval pathways early in the project development plan. FDA-CVM also collaborates with international regulatory agencies to reduce divergent studies for global registration and therefore facilitate drug approvals. FDA-CVM also incentivizes drug development through fee waivers that may be applicable to a sponsor interested in developing an alternative to antimicrobials.

Promoting the authorization of alternatives to veterinary medicinal antimicrobials in the European Union

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Antimicrobial resistance (AMR) to medicinal products for human use and veterinary medicinal products (VMPs) is recognised as a major threat to human and animal health in the European Union (EU) and worldwide. Encouraging and fostering the development of new, alternative medicines that could prevent or treat resistant infections and reduce the need for use of conventional antimicrobials is one of the pillars of fighting against the AMR threat and a high priority for the European Medicines Agency (EMA) and the European medicines regulatory network. The 2016-2020 strategy on antimicrobials of the Committee of the Veterinary Medicinal Products (CVMP) of EMA includes an action to explore measures that could be taken to promote the development and access to market of alternatives to antimicrobials. The new Regulation (EU) 2019/6 strengthens actions to tackle AMR and foster innovation.

In this context, the CVMP has drafted a Reflection Paper 'Promoting the authorisation of alternatives to antimicrobials in the EU' aimed at performing a gap analysis of the measures currently in place and additional measures that could be implemented to promote the development, authorisation and use of VMPs that may represent an alternative approach to the use of conventional antimicrobials, with special focus on alternatives to antibiotics, in animals. Existing gaps were identified through reflection on previous experience with such products at EMA, discussion with regulators from other regions, feedback from stakeholders, and review of the outcome of previous conferences on the subject. Some additional measures and activities to fill the key gaps identified are proposed in the document.

Gaps were identified in a) the existing EU regulatory framework (e.g. lack of consistent terminology, uncertainty on product classification and the existence of new regulatory paradigms for which specific guidance is not current available) which indicate the need for establishing appropriate, harmonised requirements in the legislation and specific guidance on the technical requirements for the authorisation of this type of products. Gaps were also identified in b) the support given to developers and applicants who would benefit from additional measures such as early access to scientific, regulatory and procedural advice, increased incentives to small, medium companies and from the creation of pull incentives to help bringing the most promising or relevant alternatives to the market. Finally, some gaps in c) the area of collaboration and communication with stakeholders were also recognised. The creation of a platform of communication and dialogue with stakeholders on development of alternatives to antimicrobials and the drafting of a roadmap to establishing priorities and setting targets to monitor success of measures implemented are amongst the potential measures proposed.

In conclusion, the results of the analysis identified a number of gaps for which additional measures are proposed and could be implemented to promote the development, authorisation and use of alternatives to antimicrobials in veterinary medicine in the EU. This will require a long-term approach and a set of coordinated actions with stakeholder engagement across the regulatory network and industry. The CVMP Reflection Paper was recently published for public consultation on the EMA webpage (https://www.ema.europa.eu/en/cvmp-reflection-paper-promoting-authorisation-alternatives-antimicrobials-eu) and is open for comments until 30 April 2020.

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Legal framework for the approval/designation of alternatives to antibiotics

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Ministry of Agriculture, Forestry and Fisheries of Japan is promoting the developments of alternatives to antibiotics (ATAs) to combat antimicrobial resistance in veterinary fields. ATAs include, but are not limited to, vaccines, cytokines, enzymes, immunomodulators, immunostimulants, organic acids, probiotics, herbal medicines and bacteriophages. In Japan, these products are divided into two different categories based on their active ingredients and label claims (purposes for use), veterinary medicinal products (VMPs) and feed additives, regulated by different laws.

The Act on Securing Quality, Efficacy and Safety of Products Including Pharmaceuticals and Medical Devices (Act No. 145 of 1960) regulates products, including human medicinal products and VMPs, at each stage of development, manufacturing (importing), marketing, retailing and usage. The purpose of the Act is to improve health and hygiene by providing the control required for securing the quality, efficacy and safety of pharmaceuticals, quasi-pharmaceutical products, cosmetics, medical devices, regenerative medicine products (hereinafter referred to as "pharmaceuticals, etc.") and for preventing the occurrence or spread of health and hygiene-related hazards caused by the use of those pharmaceuticals, etc. by taking measures against designated substances, and by taking necessary measures for the promotion of research and development of pharmaceuticals, medical devices and regenerative medicine products which fulfill particularly high medical needs. In accordance with the Act, a person intending to market a VMP shall, for each product, obtain marketing approval of the Minister of Agriculture, Forestry and Fisheries with respect to its marketing. The approval shall not be granted when the VMP does not possess effects indicated in the dossier, has harmful action outweighing its effects or does not have appropriate quality.

The Act on Safety Assurance and Quality Improvement of Feeds (Act No. 35 of 1953) regulates feeds and feed additives. The purpose of the Act is to contribute to public safety and stable production of livestock products by regulating the production of feeds and feed additives, setting official specifications for feeds, conducting tests of feeds in conformity with the official specifications so as to provide the assured safety and improved quality of feeds. Feed additives in the Act refer to those used in feeds by methods such as addition, mixture and infiltration to prevent deterioration of quality of feeds, to supply nutrient ingredients and other effective ingredients of feed and to promote efficient use of feed nutrient ingredients, which are designated by the Minister of Agriculture, Forestry and Fisheries after consultation with the Agricultural Materials Council. The Agricultural Materials Council reviews efficacy, residue and safety of candidate feed additives by the dossier submitted by the company.

In this presentation, processes toward approval of VMPs and designation of feed additives in Japan are shown and discussed.

Industry perspective on the registration of alternatives to antibiotics

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There is a clear and undisputed need to fight antimicrobial resistance, in the interest of both animal and public health. Also given the decreased entry of new veterinary antibiotic treatment options in the last decades, it is imperative to find and register alternatives for the currently available antibiotics. Health for Animals is aware and committed to this fight. There are numerous types of "alternatives to antibiotics" (ATA). The definition of ATA is not evident, as most ATA would also respond to the usual functional definition of an antibiotic. In addition, the definition of what a veterinary medicinal product is can be different depending on the geography. There are both "classic veterinary medicinal products" (vaccines, phytochemicals, etc…) and additives (organic acids, herbal, botanical and mineral compounds, etc.) and there are solutions that are not easily classified in the previous categories (bacteriophages, in feed antibodies, a-specific immunostimulators, gene editing technology derived products…). The hurdles from a regulatory perspective are numerous, from an industry and from an authority perspective.

A first hurdle lies in the definition and classification as alluded to above. In particular, new technologies risk to be confronted with an unclear classification, which leads to the second hurdle. The second hurdle is within the type of regulatory pathway and the inherent lack of predictability associated it Current frameworks are based on precise and clear requirements for a specific product-claim combination and cannot easily deal with ambiguity and the specificities of new technologies. Regulators so far have proven hesitant to allow claims such as reducing the need/use of antibiotic treatment. The assessment of safety and efficacy of new technologies and approaches will require new assessment paradigms, while a benefit-risk assessment will learn to consider more the secondary benefits. A lack of regulatory convergence globally is a third hurdle. VICH should be the place where we proactively define technical requirements, also for new technologies. We continue to observe a *tendency* to start from a regional or local framework. The animal health industry and all stakeholders must support OIE initiatives to obtain modern and flexible regulatory systems, to control illegal and falsified medicines and to implement the OIE standards in general. A fourth hurdle that should be taken into account proactively is the public and consumer acceptance of new technologies, which has proved not easy and should not be taken for granted even when we know the science backs us up. The fifth and crucial hurdle is creating a regulatory environment that can foster and stimulate innovation even and especially in an environment characterized by risk and uncertainty. The main driver for establishing such regulatory environment is sufficient protection of the data created, allowing sufficient time for an appropriate return on investment. HealthforAnimals and the animal health industry in general have a long-standing commitment regarding antibiotics. Since 2017, we have defined five principles in our "Antibiotics Commitment". These guiding principles include the judicious and responsible use of antibiotics, the promotion of disease prevention and an increased access to products and expertise and the Investment in development of products for prevention and treatment, including of course, alternative to antibiotics.

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