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TOGETHER WE CAN ACHIEVE SUCCESS

A good product is just the beginning.
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HENDRIX GENETICS

Data analysis and feedback to enhance performance and genetic progress

Sylvain Briere
Product Manager, Hendrix Genetics Turkeys, France

Introduction

As a breeding company, part of our mission is to collect, analyze and explore the genetic performance of our products at different steps such as breeders, hatcheries, commercial flocks and slaughterhouses. For several years, we have collected a large amount of data from our own flocks (pure lines to breeders) as well as data from customers. The purpose of collecting data is to be able to understand product performances in different conditions, generate technical advice, update production standards and predict market evolutions. Accurate data analysis is essential to improve product performance and sometimes solve problems from the field.

Understanding Big Data

"Big Data" refers to the large amount of data that is openly available. In livestock production, most of this data is generated from barn sensors or devices (temperature, feed and water intake, body weights, etc.) and from outside activities (hatcheries and slaughterhouses).

Based on the variety of sources and type of data we collect, one of the first steps is to measure the quality of this information: volume (amount of data collected), veracity (quality and accuracy) and value (insights derived from the data). The next steps aim to connect different sources of data together: from breeder performance to slaughterhouse performance.

Data Analysis

Data analysis refers to different actions realized from or around the data to be able to use these in an efficient way. Among all these actions, we can mention inspection, cleaning transforming and finally modeling. The last one is crucial from a production and primary breeder perspective because it supports decision-making.

The process generally involves several steps:

How do we use data in Hendrix Genetics Turkeys?

*** Breeder data**

Internal breeder flocks' data are regularly collected and analyzed for production and breeding purposes. On the production side the aim is to confirm if the production level and capacity are aligned with technical budget and sales planning. On the breeding side the aim is to confirm (or not) genetic projections and direction for each of our products. The breeder data is also benchmarked with PS customers data to give us a wider view of production capacity of our turkeys.

*** Customer data**

Customer data includes hatcheries, production farms and slaughterhouses from different countries and companies. We have collected data from customers since 2013 to analyze products and market trends. This database is the biggest one we use nowadays as each single flock is considered individually and anonymously:

	2015	2017	2019	2021	2023
Number of countries	4	6	7	5	5
Number of flocks	2 458	2 991	4 124	2 238	2 376
Number of birds	26 300 000	41 700 000	42 850 000	20 640 000	21 680 000

Currently, our database is consistent with around 5 different countries in EMEA (Europe, Middle East and Africa) sharing flock performances with us. This database represents more than 2000 flocks every year which correspond to more than 20 million birds placed at day-old. Obviously, not all these flocks are kept for detailed analysis as part of them might have been impacted by diseases or technical challenges.

*** Database Structure**

Our database is structured to combine different types of information or data we collect from the market and/or the customers. Here is the list of the main types of information we are able to connect all together for further analysis:

- CS Flock: the main "table" with all technical data from farms
- Strains: list of all the genetic strains and genetic type (heavy, medium or alternative)
- Grower Organization: hierarchical structure of the companies
- Processing data
- Product Type: standard, welfare oriented, ABF, organic production, etc.

From this structure, for example, we can estimate the impact of organic conditions on the performance of the same strain(s) in the same country or organization or we can benchmark different OpCos from the same company.

*** Case Study of Customer Improvement**

	2022	2023	2024
Toms ADG (g/d)	112.7	114.2	115.0
Flock FCR (kg/kg)	2.61	2.59	2.54
Flock Livability (%)	91.6	93.7	94.7

This table shows, as an example, the performance of one of our customers. Based on their data and technical exchanges and advice, we worked closely to improve their results between 2022 and now. We improved together their toms' average daily gain by 2% from 112.7 g/d in 2022 to 115 g/d in 2024. During the same time, technical feed conversion improved from 2.61 to 2.54 which means 3% improvement and livability by 3% from 91.6% to 94.7%.

*** Genetic Improvement**

Following and analyzing the performance of breeder and commercial flocks helps us to highlight genetic needs from the market. Through regular exchanges with the geneticists from Hendrix Genetics Turkeys we improve and enhance our breeding direction to fit the context of the market or meet future industry needs: from fine tuning our products to completely changing a pure line within our breeding program.

Conclusion

Today, data analysis is a valuable focus area where a structured approach is required. Data is necessary to improve the results of livestock production by underlying the best practices within a company or identifying technical issues through the data. Of course, the quality, quantity and reliability of the information exchanged are crucial for efficient and accurate decision making within the breeding program. With this information, we aim to support the turkey industry with high performing products that deliver value for our customers and the industry as a whole.

Field evaluation of a Live Mycoplasma synoviae vaccine in turkey breeders, by MS Specific PCR and Serology

Xavier Dubord [1], ***Godener Claire*** [2]
[1] LABORATOIRE LCV - Z.I. Plessis Beucher - 35220 Châteaubourg, France
[2] HENE VERT - 4 Rue Théodore Botrel – 22600 Loudéac, France
x.dubord@laboratoirelcv.com

Abstract
Mycoplasma synoviae (Ms) is a major poultry pathogen. In turkeys, Ms can cause significant mortality associated with airsacculitis, infra-orbital sinusitis, arthritis and/or synovitis. The Ms infection is one of the main causes of early slaughter of turkey breeder flocks in France. Thus, it was decided to evaluate an attenuated live vaccine, authorised in chickens, in turkey breeders from a hatchery historically confronted with episodes of Ms infections. Between June 2017 and August 2021, 90 flocks free of Ms were vaccinated at 7 weeks of age with MS-H Vaccine by eye drop (+ booster at 65 weeks in case of extended laying periods). Monitoring was organised to assess vaccine uptake and identify field strain Ms challenge: PCR was performed from tracheal swabs every 4 weeks on all flocks and on 6 flocks of broiler turkeys at 1 and 21 days of age from vaccinated parents. The DNA obtained from these PCRs was then tested with a DIVA PCR. Ms ELISAs were performed from 20 flocks of turkey breeders and from 6 flocks of broiler turkeys sampled at 1 and 21 days of age from vaccinated parents. The first observation was that there was no clinical reaction after administration of the vaccine. The vaccine strain was not detected in progeny by PCR. MS-H PCR positivity rate is variable but on average very good 9 weeks after vaccination (96%). This positivity rate decreases with increasing age and increases again after the booster vaccination carried out in flocks with extended laying periods. The Ms ELISA titres were very low and progressively increased with age. A positive titre was detected in one single day-old turkey from parents at 64 weeks of age. These maternally derived antibodies disappeared after 21 days of age. Since 2017, no contamination with field strains of Ms was identified in the company. In conclusion, PCR monitoring is an appropriate method to assess the vaccination uptake of turkeys vaccinated with MS-H Vaccine.

Introduction
Mycoplasma synoviae (Ms) is a major and widely distributed pathogen in poultry. In broiler turkeys, Ms can cause significant mortality following vertical or horizontal contamination. Clinical signs associated with infection with this bacterium can be airsacculitis (increased condemnations at the slaughterhouse), infraorbital sinusitis, arthritis or even synovitis, requiring costly antibiotic treatments (Van Meirhaeghe *et al.* 2015).

The infectious pressure of Ms in France is high, as highlighted by previous prevalence studies: in particular, 60% seroprevalence in laying hens (Bouchardon *et al.* 2012) and 9% (Dheilly, 2003) to 10% (Kermogant, 1998) in broiler turkeys. Although the prevalence varies from one year to the next, the infectious pressure on breeding turkey farms remains high because the number of birds potentially Ms positive and living outdoors is increasing.

In turkey breeders, the prevalence is very low. Infected animals are often older than broiler turkey when infected by Ms and show no symptoms. However, Ms infections are one of the main causes of early slaughter of turkey breeders in France because the risk of vertical contamination has a significant impact for broiler turkey farms.

In France and other countries around the world, vaccines are used to protect poultry against the clinical and economic consequences of Ms infections.

MS-H vaccine (MS-H) is indicated for use in future laying hens and broiler/layer breeders. This vaccine has been authorised in the European Union since 2011 with an indication to reduce air sac lesions and the number of eggs with eggshell apex abnormalities due to Ms.

In 2007, this vaccine was tested in broiler turkeys to assess its effectiveness against the clinical consequences of Ms infections (Noormohammadi *et al.* 2007). According to this trial, MS-H Vaccine by eye drop or spray colonised the upper respiratory tract and induced a serological response, without causing damage to the air sacs, joints, or tracheas. Histopathological examination of turkeys vaccinated after exposure to a virulent Ms strain revealed that administration of MS-H by eye drop or spray, at the recommended dose for chickens, protected turkeys from macroscopic/microscopic lesions and colonisation of the trachea by field Ms strains. Spray administration of MS-H to broiler turkeys placed in an isolator had provided better results than the eye-drop application. However, it is likely that in the field this would have been different because the spray administration technique in commercial livestock farming results in a significant loss of vaccine dose (de Wit *et al.*, 2013).

Following this finding, it was decided to evaluate the safety, efficacy and vaccine response to MS-H in turkey breeders from a hatchery historically confronted with episodes of Ms infection.

1. Materials and Methods

1.1. Choice of farms

A French hatchery for turkey breeders was chosen because this company has historically been confronted with episodes of Ms on its farms, resulting in the slaughter of flocks (5 contaminated by Ms between 2014 and 2017). Among these cases, a farm had been contaminated twice in a row without the farmer being at fault. For the company, it is very complicated to no longer work with certain farms even if there are recurrences. The farms are all located in the North-West of France, an area with a very large density of poultry and therefore high pressure of Ms.

The biosecurity of farms is therefore important (showering, change of clothing, etc.) but not infallible in the face of a pathogen that can spread by air up to 8 km on dust or dander particles (Hy-Line International 2020).

In addition to the economic impact of early slaughter, there is a significant human impact on the company's staff, due to the significant disruptions to the production schedule, and for farmers who lose animals early without apparent symptoms.

1.2. Animals

The genetics (male and female) of the turkey breeders were *Premium* (Aviagen Turkeys) and *Grade Maker* (Hybrid). Between June 2017 and August 2021, 430,000 animals (90 flocks of males and females in production) were followed after their vaccination with MS-H. Their feed was classic. The animals were transferred to the production site at circa 29 weeks of age and the start of production was at circa 32 weeks of age. The end of normal hatching egg production for these turkey breeders was circa 55-57 weeks of age. Some flocks were in lay for an additional egg-laying period of 67-70 to 87-90 weeks of age.

1.3. Choice of vaccine

MS-H is a commercial vaccine against Ms authorised in chickens and consists of a live attenuated thermosensitive vaccine strain called MS-H. Each 30µL dose of vaccine contains a minimum of $10^{5.7}$ colour changing units. The name of this vaccine is MS-H Vaccine eye drops suspension (Pharmsure Veterinary Products Europe Ltd).

All animals were vaccinated at 7 weeks of age by eye-drop (one dose per animal as in chickens and as had been successful in the trial of Noormohammadi *et al.* 2007) and for some flocks that were expected to do an additional egg-laying period, a second eye-drop application was made at around 65 weeks of age.

A PCR Ms analysis was performed 2-3 days before vaccination to ensure that all animals to be vaccinated were Ms negative.

1.4. Choice of PCR kits

The PCR kit chosen to detect field or vaccine Ms strains was a classic real-time Mg/Ms PCR kit targeting 16s RNA: *Adiavet® Myco Av Fast Time* (Adiagene). The DNA obtained from this PCR was then tested with a DIVA

(Differentiating Infected from Vaccinated Animals) real-time PCR: *Adiavet™ MS-H DIVA Fast Time* (Adiagene). This kit specifically detects Ms strains (100% in the validation dossier, reference ADI561-100), while differentiating infections by field Ms strains from the MS-H vaccine strain.

1.5. Choice of ELISA kits

The Ms ELISA kit used in this study was from Biochek because it clearly specifies the possibility of antibody analysis from turkey serums. According to the supplier's specifications, a titre was considered positive from 594.

1.6. Monitoring of vaccinated flocks

The purpose of this monitoring was to assess vaccine take and to identify field strains of Ms.
For the PCR analysis, 40 tracheal swabs were taken in females and 20 in males (analysed in pools of 3) every 4 weeks for all flocks.

Concerning the broiler turkeys from vaccinated parents, 10 tracheal swabs were taken on 6 flocks at the age of 1 and 21 days.

Blood samples for Ms ELISA serology were taken from 10 turkey breeders per flock (19 flocks of females and 1 flock of males) at 28, 32, 44 and 52 weeks of age.

For broiler turkeys from vaccinated parents, 20 blood samples per flock (6 flocks) were taken at 1 and 21 days of age.

2. Results and Discussion

MS-H PCR results in female turkey breeders (Figure 1) at 16 weeks of age were very positive (96% on average). Only 2 negative flocks at 24 weeks of age had to be revaccinated to detect the strain by PCR. The lack of detection of the vaccine strain by PCR in these two flocks could be due to an error in the use of the vaccine, or the levels present being below the level of detection of the PCR technique, which must be applied within 2 to 3 hours after thawing in water below 35°C to avoid a decrease in the viability of the vaccine strain.

The rate of positive MS-H PCR decreased with age (approximately 61% MS-H positive PCR 49 weeks after vaccination (= 56 weeks of age): the end of a normal production period) and increased again after a booster vaccination performed at 65 weeks in flocks with extended laying periods (Figure 2).

Broiler turkeys 1 or 21 days old from vaccinated parents were all PCR negative (data not shown).
The MS-H positive PCR rate in males (Figure 3) is higher than in females (p-value=$8.33x10^{-21}$ Aspin-Welch).

Ms ELISA titres increased gradually with age (Figure 4) even though the correlation between the Ms ELISA positivity rate and the age of the animals was weak (23% of sera weakly positive 20 weeks after vaccination and 55% 45 weeks after vaccination). Seropositive females appeared to be able to transmit detectable antibodies to day-old turkeys (Figure 5), although this was rare and more likely at the end of egg-laying when parental titres were at their maximum. Maternally-derived antibodies were detected only in a 1-day-old turkey from 64-week-old parents; unless it was a possible non-specific reaction.

Maternally-derived antibodies were undetectable when the offspring were 21 days old (data not shown).

The first observation of this study was that MS-H Vaccine by eye-drop did not cause any adverse vaccine reaction in turkey breeders. The vaccine strain was also not detected in the offspring by PCR (no vertical transmission of the vaccine strain).

Secondly, regarding vaccine take, the percentage of positive MS-H swabs (PCR) was very high at the beginning of the production period and then decreased with age. In males, these results were somewhat higher than in females, perhaps

because these animals were vaccinated more slowly and are housed in smaller bird groups that females that facilitate easier handling of birds; unless there is a gender related effect.

The variability of the PCR results (R2 < 0.006) could be explained by the sampling technique (tracheal sampling potentially being more complicated/less sensitive than in the cleft palate).

For comparison, the percentage of positive MS-H PCR in turkey breeders was 97% 10 weeks post-vaccination and 61% 49 weeks post-vaccination. In the study by Moronato (2018), 100% of PCRs were positive 10 weeks after MS-H vaccination of broiler breeders and up to 49 weeks post-vaccination (vaccine done at 4 weeks of age).

Regarding the Ms ELISA titres of the turkey breeders in our study, they were low and increased slowly with age. Compared to results obtained in broiler breeders (Moronato *et al.*, 2018 and Todte, 2014), humoral antibody titres therefore appear to be lower in turkey breeders; an observation that should perhaps be put into perspective because the ELISA kit contains antibodies against chicken antibodies. In broiler breeders, 72% of ELISA titres were positive 5 weeks after MS-H vaccination (mean Biochek titres 1540 versus 400 in turkey breeders and 0% positive ELISA) and 100% positive at 37 weeks after vaccination (mean Biochek titres 2851 versus 670 in turkey breeders and 55% positive ELISA). Thus, the ELISA titres of the offspring appeared to be higher in chickens (37% positive ELISA for the offspring of broiler breeders vaccinated 37 weeks earlier and 100% positive ELISA for the offspring of broiler breeders vaccinated 47 weeks earlier; compared to 0% positive ELISA for 1-day-old broiler turkeys from turkey breeders less than 57 weeks of age). It should be noted that the field challenges of Ms may not be detected by PCR and could potentially influence Elisa titres.

At 57 weeks of age, the veterinarian working for the hatchery concerned decided to revaccinate flocks with extended egg-laying periods with MS-H by eye-drop. This increased PCR positivity rates and perhaps thanks to this, better protected the animals. As studied in chickens (Feberwee *et al.* 2017), we can hypothesize that the presence of the MS-H strain in the upper respiratory tract may be correlated with the reduced ability of field Ms strains to colonize the respiratory tract of birds and thus provide better protection against Ms.

Since 2017, no contamination with a field Ms strain has been identified in the company concerned, which seems to demonstrate the effectiveness of MS-H in protecting turkey breeders against infections by field Ms strains. The company's staff is therefore less stressed by possible contamination by a field Ms strain since the implementation of vaccination with MS-H.

Conclusion

PCR monitoring is an appropriate tool to assess the vaccination take of turkey breeders vaccinated with MS-H by eye-drop.

Despite the registered efficacy claim for MS-H in chickens, the vaccine strain appeared to colonize the upper respiratory tract of turkey breeders less intensely and durably than in broiler breeders, resulting in fewer humoral antibodies, unless the sampling technique or the nature of the ELISA kit were responsible for these differences.

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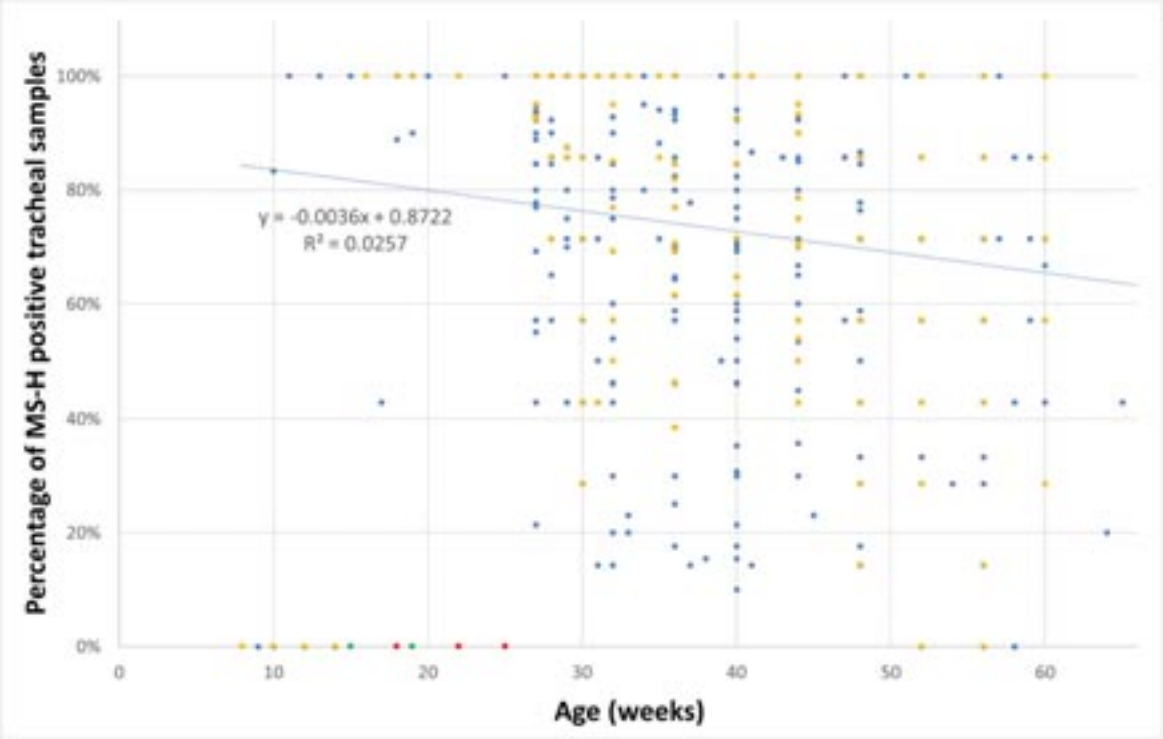
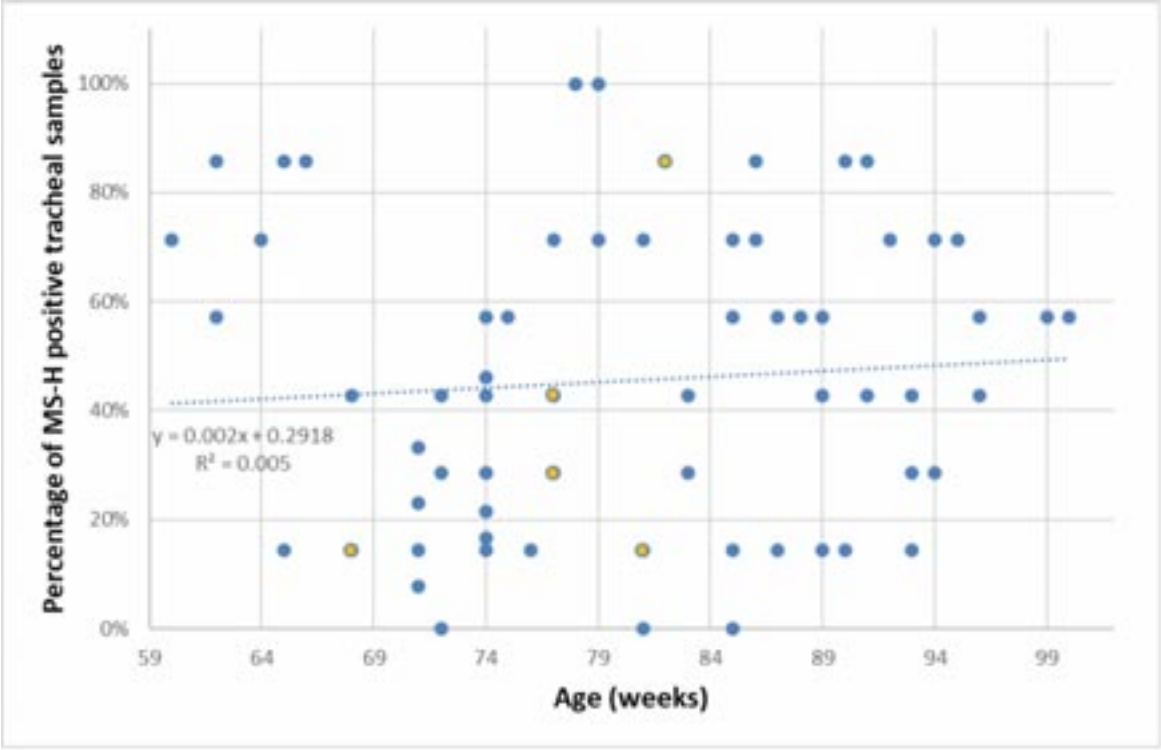
Figure 1. *Percentage of positive PCR MS-H tracheal swabs for females (normal production period)*



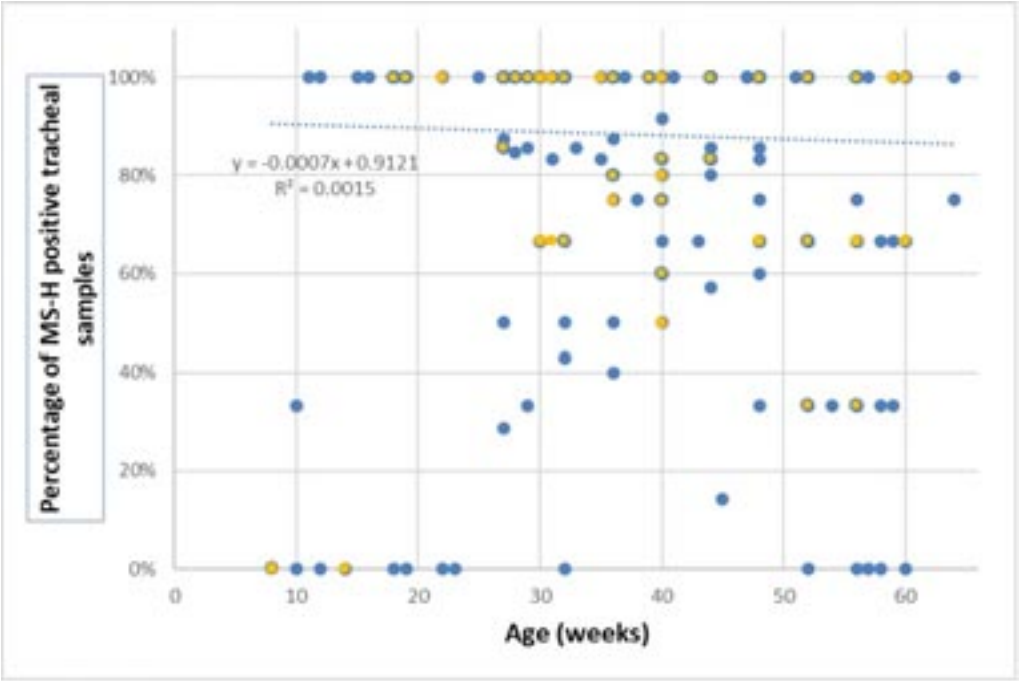
● First flock vaccinated twice ● Second flock vaccinated twice
● Means one flock ● Means several flocks with the same results

Figure 2. *Percentage of positive PCR MS-H tracheal swabs for females (2nd egg-laying period)*



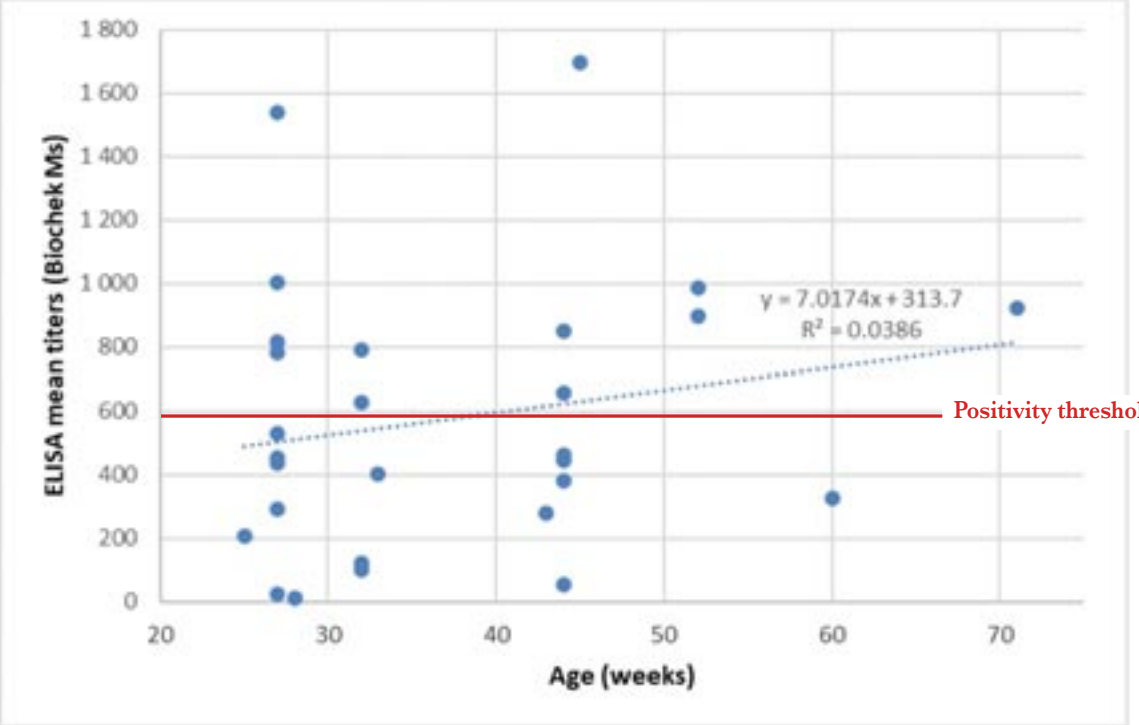
● Means one flock ● Means several flocks with the same results

Figure 3. *Percentage of positive PCR MS-H tracheal swabs for males*



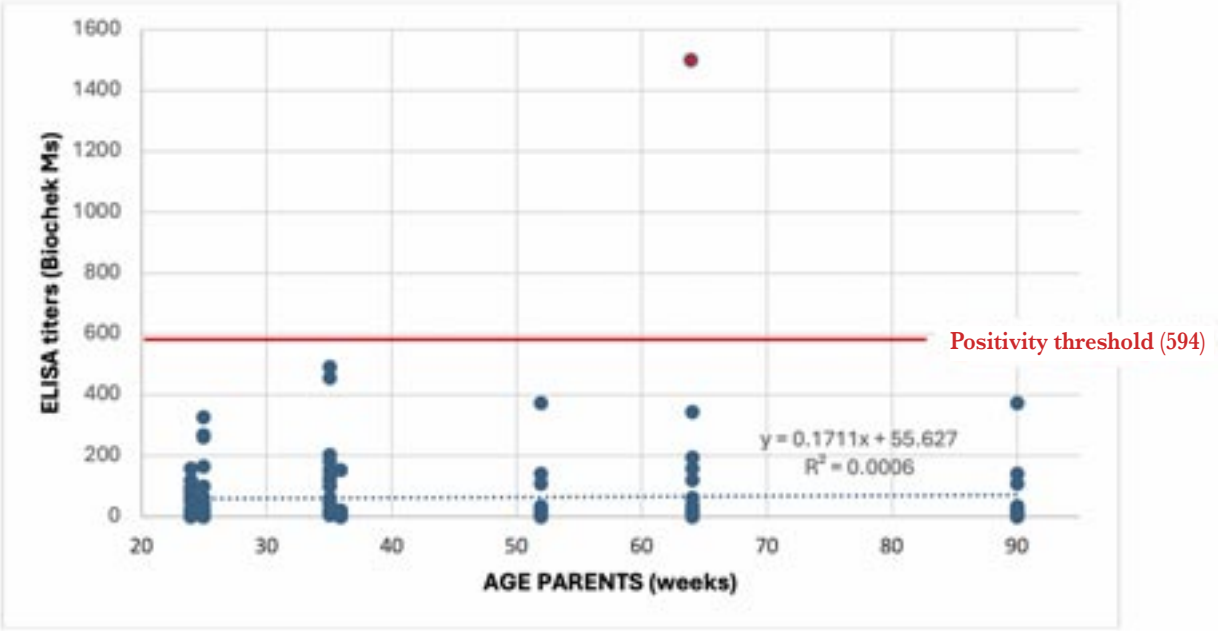
● Means one flock ● Means several flocks with the same results

Figure 4. *Mean ELISA Ms titres (normal egg-laying period, females and males)*



● One flock

Figure 5. *ELISA Ms titres on day-old turkeys (depending on the age of vaccinated parents)*



● Seropositive turkey poult ● Individual titre of turkey poults

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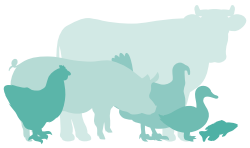

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INVAC International GmbH
Mielestraße 1
D-14542 Werder (Havel)

Fon +49(0)3327 46595 -0
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Turkey Grade and Condemnation

Greg M. Hansen
Poultry Intellimetrics, Inc. USA – on behalf of Aviagen Turkeys Ltd, UK

Producing poultry of Grade A quality is an immense team project and requires everyone involved to do their part in preventing defects and downgrading on every single bird. It starts with the primary breeders and ends with very the last employee who puts the product in packaging. Everyone in between from hatcheries, feed mills, growers, catchers, and plant employees are tasked with the responsibility to pass along a Grade A bird to the next member of the team. Downgrading not only results in loss of value and possible condemnation but also efficiency, and product flexibility. Historically there has been mystery, suspicion, and even finger-pointing as to the nature and source of downgrading, but as the components of downgrading are isolated and identified, they become easier to understand and correct. When managed in this light, their prevalence can be decreased, leading to improvements in quality and value. An old adage says: "*If it can be measured, it can be managed.*" This presentation provides an understanding of tools used to identify and measure the individual sources of downgrading from the farm, catching, and in the plant as well as current trends and practical ideas to help improve.

The trend for % Grade A for hen turkeys has been on a steady declining trend in recent years. The top plants historically were able to achieve 90% consistently, and the industry average was commonly near 84%, but recently few plants are able to achieve an average of 84%, and the US Industry average has slipped to the high 70's. There are many elements contributing to this decrease and tend to be more related to the changing labor force than the grade potential in the modern turkey. Grade is a team project, and with labor challenges the strength of the team has struggled. The leading factors for the decline in hen grades include:

- **Product changes:** What was once a valuable option for downgraded carcasses the whole bone-in-breast has become a major product in recent years resulting in the loss of focus on whole bird Grade A/downgrading, especially related to wing defects.
- **Automation:** After struggling with the labor shortages during and after COVID, there has been an unprecedented amount of automation introduced to turkey primary processing. While this has eased some of the production stresses related to a shortage of labor, machine error or mutilation has become an added component of downgrading that did not exist when most tasks related to primary processing were performed manually.
- **Changing Workforce:** As the labor market has changed with many plants experiencing higher turnover, workmanship has also declined not only for plant employees but farm and catching crews. This has not only contributed to the amount of downgrading but also the ability of the processing facility to manage slight defects and still obtain Grade A quality product at the end of the process.
- **Chiller Anti-Microbials:** A water-bath is the most commonly used chilling method in North American plants, and some of the anti-microbial products used in the chiller water have an adverse impact on carcass quality. While pure water can wash out many of the smaller bruises and increase the number of Grade A carcasses 1-2%, some of the peracetic acid based products commonly used today react to the bruises and blemishes, darkening them, which results in the loss of Grade A quality.

The ability of the hens to reach 12-13+ kg has almost eliminated most production programs geared toward raising toms for whole-bird sales. However, unlike the hens the potential for Grade A toms has significantly increased in recent years. While some of the change can be linked to more plants adding CAS systems to reduce carcass damage, more of the improvement for toms has to do with improved genetic potential. Twenty years ago the Industry struggled to achieve 50% Grade A carcasses and the top performers were under 70%. Now the industry average is near 65% and the top performers are close to 75%. The single largest difference has been related to breast blisters.

The production of Grade A quality turkeys, is a complex and collaborative effort that involves every stage of the process. By understanding and addressing the factors contributing to downgrades, the industry can achieve higher standards and deliver superior products to consumers.

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The effects of toxin mitigating strategies on poultry physiology and hatchability of turkey eggs

Dr. Cornelia Becker[1], Dr. Alexandra Schlagheck[1], Ákos Kálmán[1]
[1]Biochem Zusatzstoffe GmbH, Küstermeyerstr. 16, 49393 Lohne, Germany
Corresponding author: cbecker@biochem.net

Introduction

Mycotoxins are secondary metabolites of moulds with an uprising prevalence over the last years, promoted not only by improved analytical methods but also by increasing severe climatic conditions. Clinical diagnosis is often very complicated, as typical symptoms rarely occur, especially in the case of contamination below the EFSA limits. Instead, there is a non-specific reduction in performance and health due to the reduced efficiency of certain organs that have been damaged by the mycotoxins at the cellular level. There are already many scientific studies on the short and long-term effects of mycotoxins on the physiology of chickens, but very few well-founded studies on turkeys. In principle, the mode of action of mycotoxins should be transferable between the two poultry species, but as some metabolic pathways are different in turkeys compared to chickens (Arsenault et al., 2014), experience still needs to be gathered regarding the actual effects, particularly under field conditions.

Mycotoxins evolve their impacts on an invisible cellular level by their cytotoxic effects. For example, various mycotoxins inhibit the protein biosynthesis (Van De Walle et al., 2010), causing a lack of important proteins such as building proteins or enzymes. Among others, this leads to an altered gene expression. The clinical consequences are shown by a malfunction of organs built up by such sabotaged cells. Particularly fast-growing organs or tissues, such as the immune system, react sensitively. One potential visible outcome is an increased susceptibility to pathogens. In chicken, scientific studies have shown an enhanced growth and toxin production of Clostridium perfringens in the presence of deoxynivalenol (DON) or an increased colonization of Salmonella typhimurium in the presence of T-2 toxin (Park et al., 2015). Both bacterial categories evolve their pathogenic potential i. a. based on their exotoxin forming abilities. Consequently, the presence of DON indirectly increases exotoxin-associated diseases.

Another tissue suffering extremely from the cytotoxic effects of mycotoxins is the gut epithelium. Even at low levels, mycotoxins lead to functional disorders of enterocytes and a reduced feed efficiency. The function of the goblet cells is also impaired, resulting in a reduced mucus production. Additionally, the formation of certain building proteins of the tight junctions is reduced, altogether leading to a disrupted barrier function and an increased gut permeability (Van De Walle et al., 2010). This again may lead to an increased translocation of pathogens and uptake of other unwanted substances such as endotoxins. Being building parts of gram-negative bacterial cell walls, endotoxins are always present in the gut lumen. But if gut integrity is impaired, it comes to an uncontrolled uptake into organism leading to a dose-dependent inflammatory response (Mani et al., 2012). This reaction is at least an energy guzzler, but together with other factors such as heat stress it can lead to serious clinical complications such as death. Thus, once more mycotoxins are door-openers for another toxin category.

Finally, mycotoxins increase oxidative stress (Da Silva et al., 2017), by that increasing metabolic stress in cells. For example, this may lead to a reduced hatchability (Shah Alam et al., 2024), since the egg tissue is very rich in polyunsaturated fatty acids and therefore extremely vulnerable to oxidative damages.

The aim of this short paper is to show how mycotoxins can become drivers of such secondary pathological lesions and how an efficient mycotoxin neutralising strategy can prevent these effects, by using two different types of trials. While the first trial is conducted under strict scientific conditions, focussing on differentiated physiological and histopathological parameters, the second trial gives an impression about the impacts of a low mycotoxin contamination on the hatchability of turkey eggs under commercial field conditions.

Trial I

Objectives: This scientific trial investigates the efficiency of a toxin binder (TB) in preventing the adverse effects of a high multitoxin-contamination on different physiological parameters in broiler chicken taken as representative poultry category.

Materials: In a floor-pen trial conducted at an unbiased institute (Samitec, Brazil), 1,080 day-old Cobb 500 male broiler chicks were randomly allocated to four feeding groups (n=9; 30 chicks per box): the negative control (NC) receiving a basal diet, the second control group (NC2) with additionally 0.2 % TB (B.I.O.Tox®Activ8, Biochem), the positive control (PC) artificially contaminated with 1.5 ppm aflatoxins, 1.0 ppm T-2/HT-2 toxin and 50.0 ppm fumonisins and the trial group (PC1) with the same multi-contamination as PC plus 0.1 % TB. After a trial duration of 35 days, the following parameters were collected or calculated:

- histomorphology of jejunum shown by villus height/crypt depth ratio (VH/CD ratio)
- histopathology of liver shown by periportal heterophilic inflammatory infiltration with the following scoring: absent (score 0), mild (score 1), moderate (score 2), marked (score 3)
- histopathology of bursa of Fabricius shown by lymphoid depletion and abscesses applying the same scoring as for the liver
- liver function derived from total plasma protein (TP), g/dl calculated as average per group
- oxidative stress level measured by thiobarbituric acid reactive substances (TBARS), nmol MDA (malondialdehyde)/mg protein calculated as average per group
- zootechnical performance represented as average per group by feed intake, body weights, body weight gains, feed conversion ratio (FCR)

Statistical analyses were performed with software package SPSS (Version 29.0). Normally distributed data were analysed by one-way-ANOVA, followed by post hoc Duncan test. Not normally distributed data were analysed by Kruskal-Wallis Test or Median Test.

Results and discussion: The high multitoxin-contamination in PC significantly reduced VH/CD ratio compared to the negative controls and PC1 ($p<0.001$), indicating an impaired gut morphology, which was completely prevented with the use of TB (fig.1). In the liver, heterophilic infiltration was increased in PC to a median score of two and a median score of one in PC1 compared to zero in NC and NC2. Taking a closer look at the distribution of the single scores, the lesions were clearly milder in PC1 supplemented with TB compared to PC (fig. 2, left side). In the bursa of Fabricius, lymphoid depletion was clearly increased in PC to a median score of one compared to zero in NC, NC2 and PC1. Regarding abscesses, all four groups kept median score zero, although PC revealed the highest grades detected (fig. 2, right side).

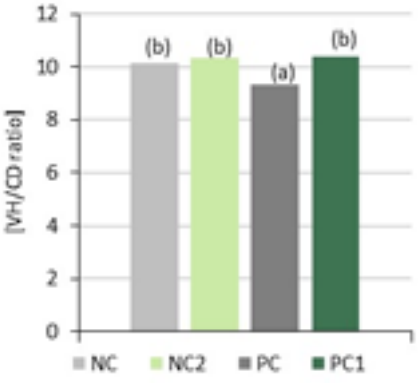

Fig. 1: *VH/CD ratio; a-b values labelled with a different superscript differ significantly ($p<0.001$)*

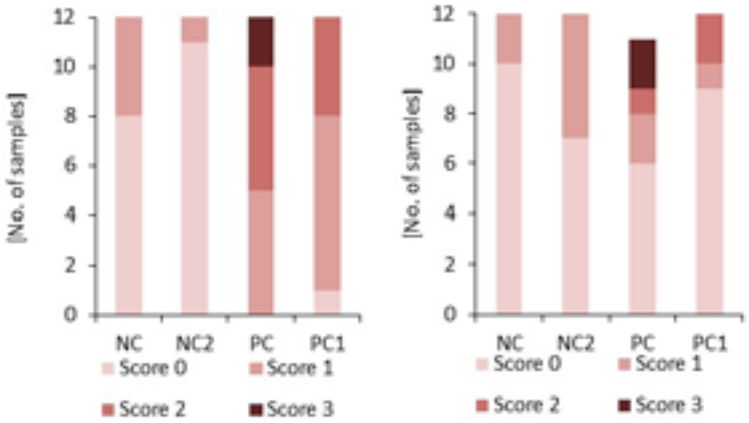

Fig. 2: *Heterophilic infiltration in liver tissue (left); abscesses in bursa of Fabricius (right), one sample was discarded in PC*

TP was significantly reduced ($p<0.001$) and TBARS were significantly increased ($p<0.014$) in PC compared to controls (fig. 3). Both parameters were completely preserved in PC1, pointing to a maintained liver protein forming capacity and oxidative stress level. Performance parameters were significantly impaired in PC and PC1 but revealing significantly better results in PC1 compared to PC ($p<0.001$, fig. 4). Regarding FCR, no significant differences were observed between groups. Taken together, the TB successfully reduced the adverse effects of applied mycotoxins on the physiology and performance of broiler chickens.

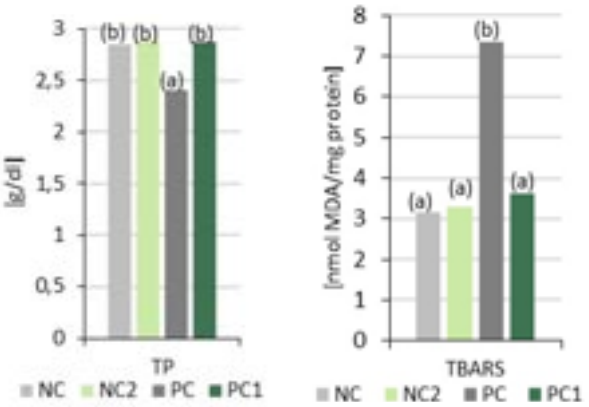

Fig. 3: *TBARS/TP; a-b Values labelled with a different superscript differ significantly ($p<0.001/p<0.014$)*

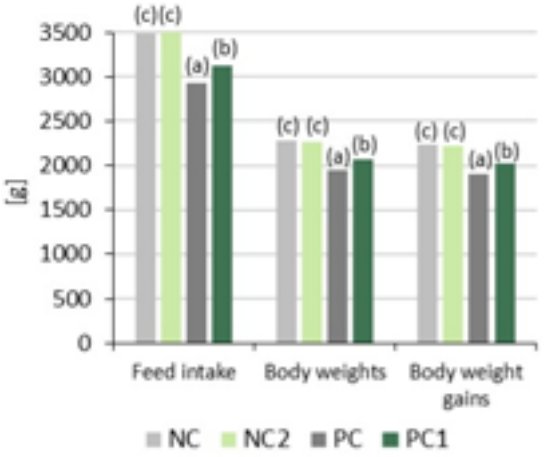

Fig. 4: *Performance; a-c Values labelled with a different superscript within a row differ significantly ($p<0.001$)*

Trial II

Background and objectives: The following field trial was performed in a turkey parent flock under commercial field conditions on a farm in Eastern Europe producing more than one million eggs per year of the Hybrid converter NOVO breed. Prior to the trial, the farm used a simple toxin binder (TBS) targeting aflatoxins. As the hatchery results still showed a fluctuating hatching rate, the use of an advanced, broad-spectrum binding product (B.I.O.Tox®Activ8, A8, Biochem) was to be tested in two houses of the farm.

Materials: In the control group TBS, 14,644 birds continued to be fed the original toxin binder at a dosage of 1 kg/t final feed, while the trial group A8 with 2,800 birds received the broad binding product A8 at a dosage of 0.7 kg/t final feed. Figure 5 shows the results of an in vitro binding comparison between the two toxin binders, which was conducted with 20 ppb aflatoxin B1, 450 ppb zearalenone, 150 ppb T-2 toxin, 30 ppb ochratoxin A, 1,000 ppb fumonisin B1 and 450 ppb deoxynivalenol, an inclusion rate for both binders of 0.1 % and at pH 3 followed by pH 6.5 (Trilogy Analytical Laboratory, USA). Table 1 summarises the mycotoxin contaminations of the three diets fed during the trial period of 30 weeks (pre-laying phase plus full laying period) analysed via liquid chromatography-mass spectrometry (LC-MS/MS).

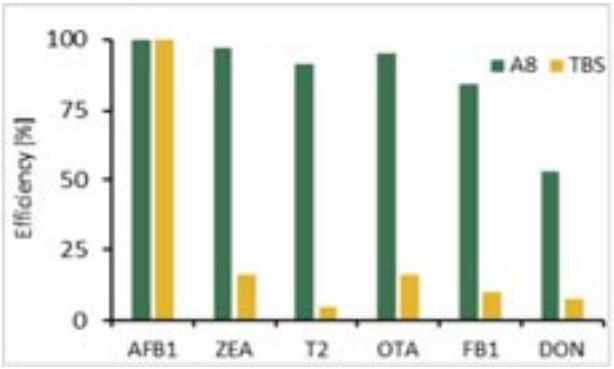

Fig. 5: *Comparison in vitro binding efficiency of TBS & A8*

Mycotoxin		Unit	Pre-lay (week 26 - 32)	Layer I. (week 32 – 44)	Layer II. (week 44 – 56)
Aflatoxin B1	AFB1	µg/kg (ppb)	2.1	-	-
Aflatoxin B2	AFB2		-	-	-
Aflatoxin G1	AFG1		-	-	-
Aflatoxin G2	AFG2		-	-	-
Ochratoxin A	OTA		-	-	-
Fumonisin B1	FB1		552	131	142
Fumonisin B2	FB2		-	30.8	59.2
T-2 Toxin	T-2		-	-	-
HT-2 Toxin	HT-2		-	-	-
Zearalenone	ZEA		-	-	13.3
Deoxynivalenol	DON		60.1	92.5	82.2

Tab. 1: *Results mycotoxin analysis of the three feeding phases*

At the end of the trial, the differentiated data of the hatchery receiving the eggs of both groups were collected and analysed.

Results and discussion: The hatchery received a total of 166,347 eggs from the TBS group (110.81 eggs/turkey) and 31,205 eggs from the A8 Group (111.97/turkey). Since the turkeys were artificially inseminated and thus the insemination management has a significant influence on the laying rate, only the hatchery results under the influence of the two different toxin binders will be considered here. The overall hatching results revealed clearly fewer infertile eggs and a higher hatching rate in eggs laid by turkeys receiving feed supplemented with A8 compared to the TBS despite a lower inclusion rate (tab. 2). This implicates that although the multiple contamination with mycotoxins analysed in the feeds was quite low, it could still have had an economically relevant impact on hatchability. This in turn suggests that the use of such a broadly binding, advanced toxin binder may be a relevant, worthwhile investment.

	Parameter	Group TBS	Group A8	A8 vs. TBS (relative)
Hatchery	**Infertile eggs**	**3.1 %**	**2.8 %**	**- 9.7 %**
	Necrotic + haemorrhagic eggs	5.2 %	5.6 %	+ 7.7 %
	Eggs remaining in incubator	91.7 %	91.6 %	- 0.1 %
	Dead-in-shell embryos	4.6 %	4.5 %	- 2.2 %
	Spoilt eggs (broken, impure)	9.4 %	6.9 %	- 26.6 %
	Rotten eggs	0.11 %	0.07 %	- 36.4 %
	Hatching rate	**77.7 %**	**80.2 %**	**+ 3.2 %**

Tab. 2: *Data hatchery*

Summary conclusion

The two trials presented here offer two completely different perspectives on the impact and relevance of toxin mitigation strategies in turkeys and point to various aspects that need to be further clarified in the future.

Trial I, which is strictly scientific in nature, provides a clear overview of the specific impacts of mycotoxins on birds and paints a comprehensive picture of how mycotoxins exert their harmful impacts and lead to secondary pathological lesions. The reduced VH/CD ratio in the positive control is a clear sign of a damaged intestinal epithelium, which in turn leads to a diminished feed efficiency. The increased number of heterophilic cells in the liver indicates inflammatory processes and the reduced TP is an indicator for reduced protein forming capacity of the liver. The lesions detected in the bursa of Fabricius can lead to an impaired immune response and consequently to an increased susceptibility to pathogens. The strongly increased TBARS, on the other hand, are a clear sign of an increased oxidative stress level in the birds. The trial also objectively evaluates the effectiveness of a TB in reducing the adverse effects of mycotoxins in live animals. The investigation of such processes certainly requires very scientific conditions. However, the artificial mycotoxin contamination in such a scientific trial must be set unrealistically high compared to field conditions in order to provide such clear results. The environmental conditions are also ideal, so that other influencing factors can be ruled out. Consequently, such a kind of trial does not take into account the interaction of other environmental factors such as high animal density, pathogen pressure or management and cannot provide a full assessment of the relevance of the issues investigated under commercial field conditions.

Trial II, on the other hand, provides information on the relevance of mycotoxins and strategies for their control under commercial field conditions and shows the effects on farm economy. In this particular case, the fertility and hatchability of eggs were affected. Even though the mycotoxin contamination detected in the feeds was relatively low, the result is comprehensible. Mycotoxins are not evenly distributed in feed and certain amounts can be present as so-called masked variants that are not detected by a general screening. Therefore, the real contamination level could have been higher. Furthermore, there are always additional stress factors under field conditions, which can also increase for example oxidative stress level. The scientific broiler trial markedly showed that mycotoxins directly contribute to this issue. In trial II, the neutralisation of the detected mycotoxins seemed to have made the decisive difference. Certainly, such field trials do not have extremely high scientific and statistical power. However, with an increasing number of such

studies and in conjunction with a highly scientific trial in which the efficacy of the product has been proven, this can still be a very interesting tool to obtain more information about the problem and the relevance of the product under field conditions. Accordingly, it is highly recommended to continue collecting such field experiences in each individual poultry species in order to preserve animal health and farm profitability. Thus, although turkeys may be anatomically very similar to chickens, the severity of the manifestation of clinical symptoms is likely to vary due to different metabolic pathways.

Taken together, both trials offer a comprehensive and interesting insight into the way how mycotoxins exert their adverse health effects, starting at an invisible cellular level, through the impairment of organ functions to complex clinical problems, investigating parallelly the efficacy of mitigating strategies.

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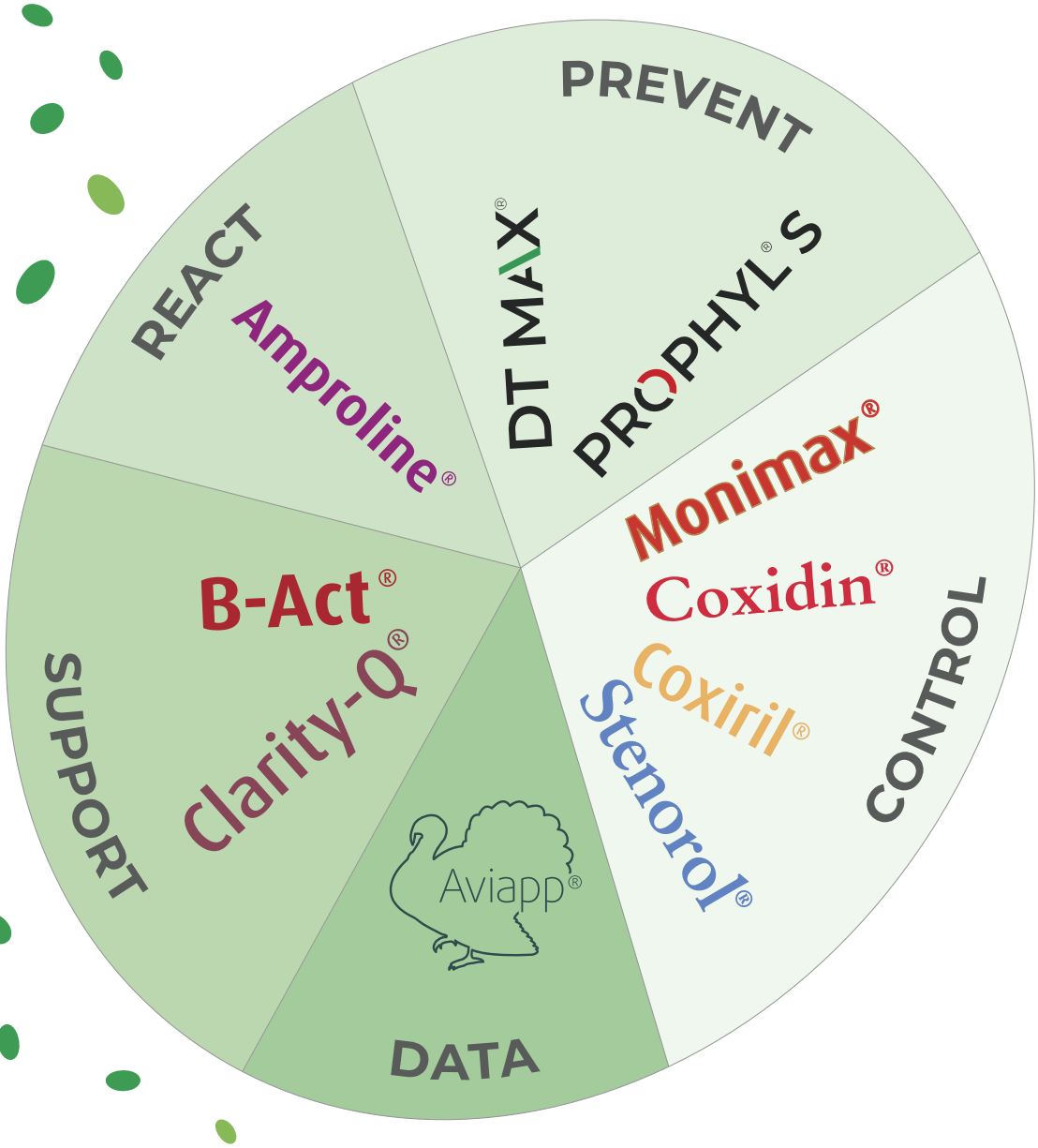

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New veterinary legislation and off-label use: the devil is in the details

Wouter Depondt
Huvepharma NV, Belgium

Today, veterinary medicines and medicated feed in EU are regulated by mainly 2 regulations. The first one is regulation 2019/6 (https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32019R0006) , setting the regulatory framework for the placing on the market, manufacturing, import, export, supply, distribution, pharmacovigilance, control and the use of veterinary medicinal products. The second one is regulation 2019/4 (https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32019R0004) , setting the regulatory framework for the preparation, placing on the market and use of medicated feed. Medicated feed is one of the routes for the oral administration of veterinary medicinal products, but as such not considered as a veterinary medicine anymore. This is why there is a different regulation for medicated feed. In this article, some specific parts of the regulations, that are important on a daily base for the veterinary practitioner.

Regarding regulation on medicated feed:

1. A veterinary prescription for medicated feed shall be issued only after a clinical examination or any other proper assessment of the health status of the animal or group of animals by a veterinarian and only for a diagnosed disease. However, if it is not possible to confirm the presence of a parasitic infection, a veterinary prescription for medicated feed containing anti-parasitics without antimicrobial effects may be issued based on the knowledge of the parasite infestation status in the animal or group of animals (article 4).

2. In the original regulation, no maximum allowed carry-over level was set. This has now been established by a delegated act at a maximum carry over level at 1%. (https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=OJ:L_202401229)

3. Medicated feed containing antimicrobial veterinary medicinal products shall be used in accordance with Article 107 of Regulation (EU) 2019/6, meaning for example that the same rules on off-label use for veterinary products also apply for medicated feed.

Regarding regulation on veterinary medicines:

- Off-label use (or extra-label as it named in US)
  In the case of food-producing terrestrial species if there is no authorised medicine OR the product(s) are not available on the market, to treat a condition, a veterinarian may administer/ prescribe, to avoid causing unacceptable suffering, medicinal products outside the terms of the marketing authorisation or SPC. There are different options, but for food-producing animals, only below options are being used in practice:
  "*a veterinary medicinal product authorised in the Member State or in another Member State for use in the same or in another food-producing terrestrial animal species for the same indication, or for another indication.*"
  Important to note, is that a practitioner can chose between options above, and it is no longer necessary to first select for example a product in the same member state for the same species. Also a combination is possible, like a product from another member state for another species with another indication.
  If above is not possible, products can also be used in following descending order of suitability (or also called cascade system):

- relevant Member State for use in a non-food-producing animal species for the same indication
- a medicinal product for human use
- a veterinary medicinal product prepared ex-temporaneously
- veterinary medicine authorised in a third country (e.g., UK) for the same species and same indication (not for immunological products)

Most important is to realise that, in all cases of off-label use, the prescribing veterinarian is responsible for all possible adverse events (safety, efficacy,...) that can happen.

Important to note, is that Regulation (EC) No 470/2009 always stays valid. It means basically, if a veterinary product is used for which no maximum residue level (MRL) is established, any residue found in foodstuff, can be considered as a non-allowed substance with severe consequences, for the veterinary practitioner, who is responsible for all risks related to off-label use. Regulation clearly states only pharmacological substances with a species specific MRL, a provisional species specific MRL absence of the need to establish MRL can be administered (article 16 in accordance with article 14(2)a, b, c and can the foodstuff can be placed on the market.(article 23)

So, the advice could be to only prescribe off-label if a MRL is available for the concerned species. Luckily for the poultry sector, most MRL's are set as poultry (or all food producing species), what also includes turkeys.

The list can be found in this link:
https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:015:0001:0072:en:PDF

Setting withdrawal time in case of off-label use is straight forward:

For meat and offal from food-producing mammals and poultry and farmed game birds the withdrawal period shall not be less than:

(i) the longest withdrawal period provided in its summary of the product characteristics for meat and offal multiplied by factor 1,5;
(ii) 28 days if the medicinal product is not authorised for food-producing animals;
(iii) one day, if the medicinal product has a zero withdrawal period and is used in a different taxonomic family than the target species authorised; turkeys fall under the same taxonomic family as chickens.

If a product is used off-label for a non-registered indication but for the same species, at the registered dose, the withdrawal time remains as it is on the label.

There are some exceptions concerning antibiotics.
(https://www.ema.europa.eu/system/files/documents/regulatory-proceduralguideline/vet_reg_cascade_list_report_en.pdf)

Some examples:

- Amoxicillin-clavulanic acid cannot be used in poultry.
- Florfenicol, 3rd and 4th generation cephalosporines,quinolones and fluoroquinoles can only be used after an antibiogram has been performed and no antibiotic can be used that has lower AMEG classification.
- Some products cannot be used to treat Salmonella for group treatment, like fluoroquinolones and 3rd and 4th generation cephalosporines

Finally, there is also a new delegated regulation regarding the effective and safe use of veterinary medicinal products authorised and prescribed for oral administration via routes other than medicated feed and administered by the animal keeper to food-producing animals. Several points are discussed, like the use of so-called oral granules for individual use. More relevant for the poultry industry, are Biocidal products, feed additives or other substances used in drinking water. They shall not be used simultaneously with a veterinary medicinal product where there is evidence of negative interactions or incompatibilities between those products and the veterinary medicinal product when added to drinking water.

(https://eur-lex.europa.eu/legalcontent/EN/TXT/PDF/?uri=OJ:L_202401159)

GB legislation is a bit different than EU legislation regarding off-label use:

Where there is no suitable veterinary medicine authorised in your territory for the specific condition in the animal being treated, to avoid unacceptable suffering, you are permitted to use your clinical judgement to treat animals under your care in accordance with the cascade.

The steps, in descending order of suitability, are:

- Veterinary medicine with a Marketing Authorisation valid in GB or UK wide for indicated species and condition
- Veterinary medicine with a Marketing Authorisation valid in NI for indicated species and condition, in accordance with a Special Import Certificate granted by the VMD
- Veterinary medicine with a Marketing Authorisation valid in GB, NI or UK wide for a different species or condition. For products not authorised in GB or UK wide a Special Import Certificate from the VMD is required

All substances contained in the medicine must be substances which have a Maximum Residue Limit (MRL), but not necessarily in the species for which it is intended to be used! **This is different from EU legislation!**

The vet responsible for prescribing the medicine must specify an appropriate withdrawal period

- the longest withdrawal period provided in the SPC for meat and offal, multiplied by a factor of 1.5
- 28 days, if the product is not authorised for food-producing animals
- 1 day, if the product has a zeroday withdrawal period

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The use of enzymatically-processed soybean meal in turkeys

Dr. Megan Bible
Hamlet Protein, Horsens, Denmark
mbi@hamletprotein.com

Introduction

Soybean meal (SBM) is the most commonly used protein source in animal livestock diets. This protein ingredient has highly digestible amino acids at a reasonable price. Known for being high in lysine and tryptophan, SBM complements the cereal grains (corn, wheat, etc.) added to diets. Turkeys require a high protein diet with 60% to 80% of their protein coming from SBM (Lee et al., 1991). During the first six weeks of life for a poult, the protein requirement is between 24% to 28%. On average, a turkey consumes approximately 9 kg (20 lb) of SBM in its lifetime (Iowa Food & Family, 2018).

Anti-nutritional Factors

Although SBM is a cost-effective, good source of protein, there are compounds in SBM which will have a negative effect on the animal's ability to digest and absorb nutrients. Research has demonstrated numerous times that high levels of SBM in young animal diets are detrimental to growth performance and health. Anti-nutritional factors (ANFs) are present in SBM to provide defense against bacteria, mold, and over-consumption by wildlife. There are three main ANFs present in SBM: trypsin inhibitors, oligosaccharides (raffinose and stachyose), and β-conglycinin.

Trypsin inhibitors are present in raw soybeans but are decreased during heating when producing SBM. Trypsin inhibitors will increase pancreatic weight and decrease the nutritive value of SBM by binding to the enzymes trypsin, chymotrypsin, and other gastrointestinal proteases. However, overheating or heat treatment for a long duration will cause Maillard reaction, which will damage the lysine.

Stachyose and raffinose are the main oligosaccharides present in SBM. These galacto-oligosaccharides are heat stable but can be broken down by β-galactosidases. Unfortunately, young animals have an undeveloped large intestine microbiota that is not able to break down these compounds. This leads to digestive disturbance causing flatulence and scours at extreme levels.

Lastly, the storage protein, β-conglycinin is a heat stable compound known for its antigen effects in young animals. Beta-conglycinin will initiate an immune response causing a reduction in nutrient absorption, villi atrophy, and intestinal cell death. Furthermore, β-conglycinin can cause "leaky gut" syndrome in young animals, leading to hyper-permeability of the intestinal epithelium resulting in an increased passage of e.g. bacteria, toxins, and undigested nutrients across the gut barrier, ultimately leading to diarrhea.

There is a huge variation in ANFs in SBM as demonstrated by the evaluation of the top producing countries, USA, Brazil, and Argentina (García-Rebollar et al., 2016; Table 1).

Table 1. *Levels of trypsin inhibitor activity and soy oligosaccharides in different SBM sources (adapted from García-Rebollar et al., 2016).*

	USA[a]		BRA[a]		ARG[a]	
Anti-nutritional factor	Range	CV, %	Range	CV, %	Range	CV, %
TIA[b], mg/g DM[c]	1.4-5.5	22.1	1.8-4.7	18.9	1.4-4.6	20.2
Stachyose, %	4.32-8.26	8.4	3.65-7.34	10.1	3.73-7.10	9.5
Raffinose, %	0.6-1.86	25.9	0.9-2.57	17.2	0.9-2.01	14.2

[a]USA=United States of America, n=180; BRA=Brazil, n=165; ARG=Argentina, n=170.
[b]TIA=trypsin inhibitor activity. [c]DM=dry matter at 88.5%.

Due to the variability of ANFs in SBM, it makes it practically impossible to predict the levels. Also, it is quite possible that turkey poults may be the most sensitive due to the high inclusion of SBM in starter diets. Overcooking SBM is not an option as it leads to a decrease in growth in turkeys (Lee et al., 1991).

Enzymatically-processed Soybean Meal

A patented bioconversion process transforms SBM into an enzymatically-processed SBM (ESBM) that is almost void of ANFs (Table 2). The process of producing ESBM involves controlled conditions with gentle sterilization. This transformation converts inconsistent SBM to a protein-rich ingredient with a consistent crude protein content and consistent amino acid profile (Figure 1).

Table 2. *Range and mean anti-nutritional levels in SBM and enzymatically-process SBM (ESBM).*

	SBM		ESBM	
Anti-nutritional factor	Range[a]	Mean[a]	Range[a]	Mean[a]
TIA[b], mg/g	1.23-4.84	2.7	1.0-1.8	1.3
Stachyose and raffinose, %	4.55-10.12	7.11	0.5-1.5	1.0
Beta-conglycinin, ppm	15,000-154,501	15,000	1.0-3.0	2.0

[a]Internal Hamlet Protein data. [b]TIA=trypsin inhibitor activity.

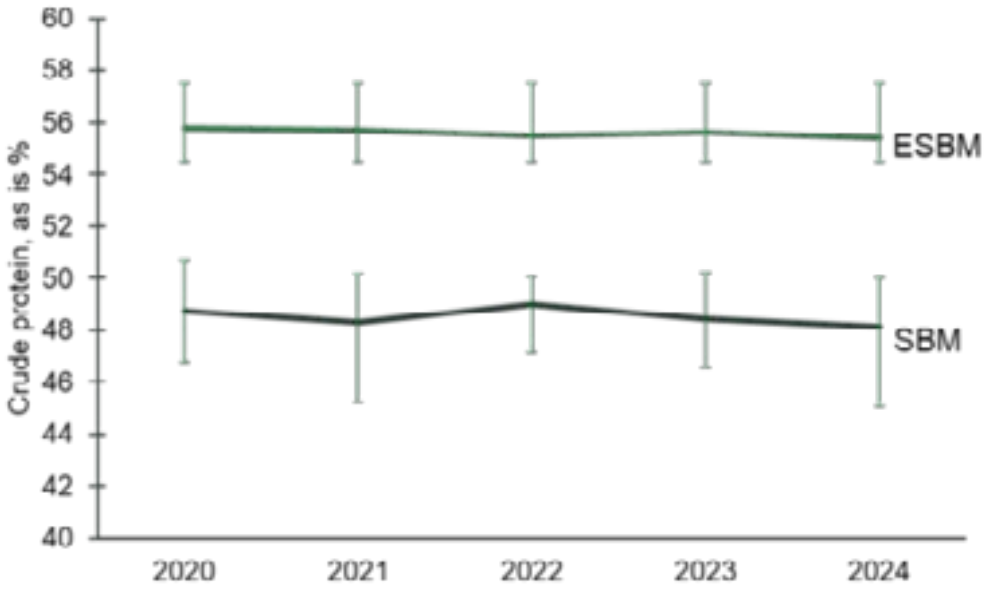

Figure 1. *Consistent crude protein levels of enzymatically-processed SBM (ESBM) vs. SBM from 2020 to 2024 (internal Hamlet Protein data).*

Growth Performance

A study conducted by Brown et al. (2021), evaluated the use of increasing levels of ESBM in poult diets. There were 480 one-day-old female Nicholas Select poults placed in battery pens for a total of 42 days. The study consisted of four dietary treatments: corn-SBM (0% ESBM); corn-SBM + 5% ESBM (5 % ESBM); corn-SBM + 10% ESBM (10% ESBM); corn-SBM + 20% ESBM (20% ESBM). The ESBM was added at the expense of SBM. Dietary treatments were isocaloric and had a similar amino acid profile. Poults were fed a two-phase feeding program consisting of a starter (0-21 days) and grower (21-42 days). Diets contained xylanase and phytase. Young turkeys were vaccinated orally with a coccidiosis vaccine at 10x the recommended manufacturer's dose to initiate a mild challenge.

The inclusion of ESBM had a positive effect on young turkey growth performance during the 42-day study. For day 21, poults fed 10% ESBM had a 1.5 point numerical improvement in FCR when compared to poults fed 0% ESBM (Figure 2). No difference was observed for day 21 BW gain. There was a significant, 5 point, improvement (P≤0.05) in FCR in the grower phase (day 42) for turkeys fed 10% ESBM in contrast with no ESBM in the diet (Figure 3). When evaluating regression analysis for BW during the starter phase, there was a trend (P=0.06) with the quadratic polynomial model for an estimate of optimal dose to be 9.9% of ESBM for the starter. There was a trend (P=0.09) with the quadratic broken line model for an estimate of optimal dose for ESBM to be 5.9% when evaluating the FCR for the grower phase.

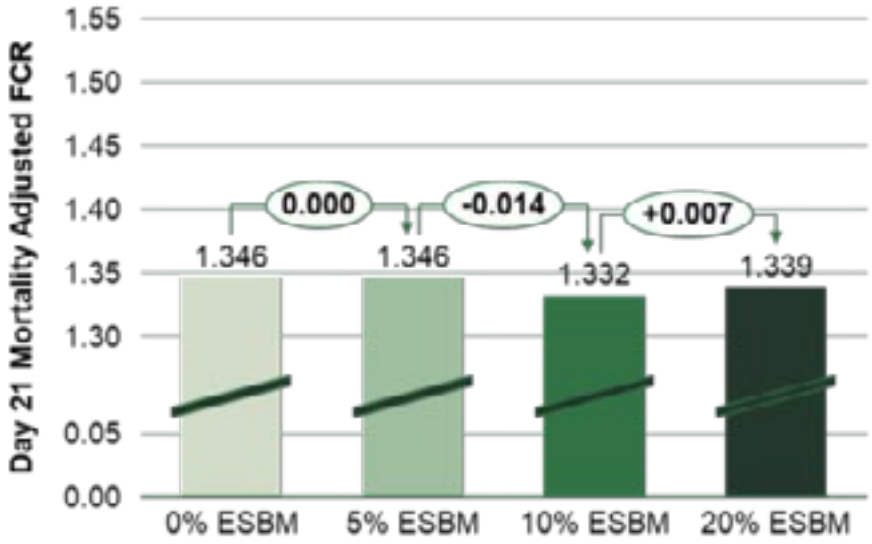

Figure 2. *Mortality adjusted FCR for poults fed increasing levels of ESBM for d 21 (Brown et al., 2021).*

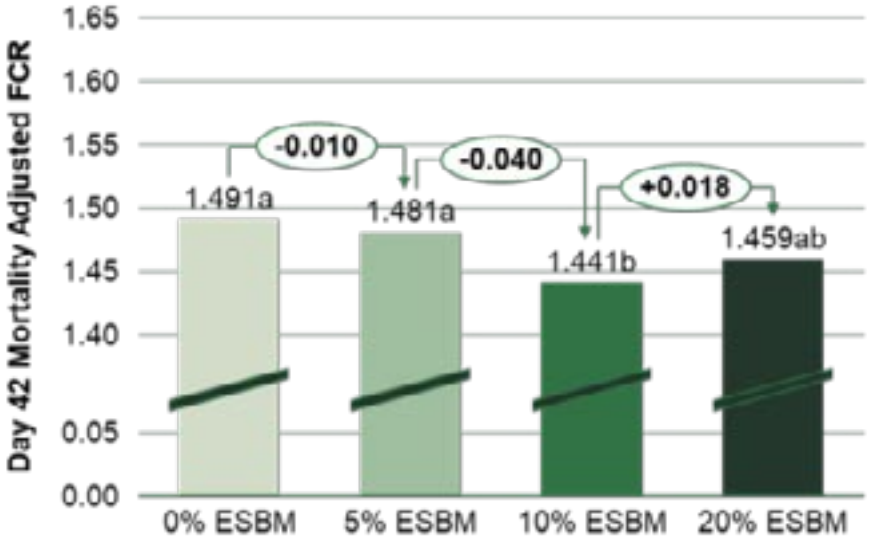

Figure 3. *Mortality adjusted FCR for poults fed increasing levels of ESBM for d 21. a,b Superscripts indicate significant difference between treatments P≤0.05 (Brown et al., 2021).*

Thus, it was concluded based on the performance of the experiment, the optimal levels of ESBM are 10% ESBM for the first 21 days and 6% during days 21 to 42. In conclusion, the study demonstrated that poult diets should contain less than 40% SBM to mitigate the negative effects of ANFs.

Discussion

Although SBM is an excellent source of amino acids, the variability of ANFs, performance decrease associated with ANFs, and inconsistency of crude protein is something that should be considered. The addition of ESBM will aid in improving the consistency of amino acids and decreasing ANF levels poult starter diets. Further research is to be conducted to evaluate a step-down approach of feeding ESBM in young turkey diets.

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Does flickering light have an impact on turkey welfare or productivity?

Karen Schwean-Lardner and April Hammond
University of Saskatchewan, Canada

Flickering light in turkey houses can arise from a number of reasons, but it appears the most common is because of incompatible controller and dimmer boxes. But does this flickering light have an impact on turkeys? The objective of the work conducted at the University of Saskatchewan was to examine the effect that flickering light has on a wide degree of outcome measures, including productivity, welfare and health.

The study utilized light-emitting-diode lamps, and examined impacts on Nicholas Select turkey hens (n=5824 over two repeated trials) from 0-11 weeks of age. Purpose built controller boxes for producing flicker were designed by Greengage Lighting Ltd. Birds were placed in individually controllable rooms (364 poultry per room), with a total of 9 rooms (replicate) per trial, with the trial conducted twice.

Three flicker frequencies were tested: 30 Hertz (both humans and birds can see; 30 Hz), 90 Hz (humans cannot see but birds may be able to) and 195 Hz (neither humans nor birds can see). Measures included all performance data (body weight at placement and at 4, 8 and 11 wk of age), feed intake over those periods, mortality and cause of mortality. Behavioural output was quantified by watching scan samples of video recordings at 4, 8 and 10 wk of age during the photophase. Birds (20/room) were assessed for mobility via gait scoring, footpad lesions, feather condition and cleanliness at 10 wk of age. Ocular size was assessed on euthanized birds at 11 wk of age (4 birds/room). Stress was measured via heterophil to lymphocyte ratio at 4, 8 and 11 wk of age. Data were analyzed using Proc Mixed (SAS 9.4), with Tukey's range test to separate means when differences were found ($P<0.05$).

Flicker impacted birds early in life. Birds exposed to 30 Hz weighed less than those in the 195 Hz until 8 wk of age (Table 1), and feed intake was reduced in the 30 Hz treatment birds from 0-4 and 4-8 wk of age (Table 2). No differences were noted at the end of the trial.

Table 1. Effect of LED light flicker frequency on average body weight (kg) of turkey hens weighed at 0d, 4, 8, and 11 weeks of age

Age (wk)	Treatments (Hz)			SEM[1]	P-value
	30	90	195		
Average Body Weight					
0 d	0.06	0.06	0.06	0.001	0.592
4*	0.75	0.75	0.78	0.010	0.200
8*	3.77[b]	3.84[ab]	3.86[a]	0.019	0.025
11*	7.28	7.16	7.28	0.032	0.100

* Block differed significantly: included as a random factor ($P<0.05$)
[a,b] Values with different letters within the same row differ significantly ($P<0.05$)
[1] Standard error of the mean

Table 2. *Effect of LED light flicker frequency on turkey hen feed consumption (kg) from 0d-4, 4-8, 8-11, and 0d-11 weeks of age*

Age (wk)	Treatments (Hz)			SEM[1]	P-value
	30	90	195		
0d-4*	0.84[b]	0.89[a]	0.88[a]	0.009	<0.001
4-8*	4.64[b]	4.75[a]	4.75[a]	0.019	0.001
8-11*	6.99	6.73	6.90	0.080	0.185
0d-11*	12.46	12.37	12.53	0.069	0.619

* Block differed significantly: included as a random factor (P <0.05)
[a,b] Values with different letters within the same row differ significantly (P <0.05)
[1] Standard error of the mean.

Total mortality was highest for birds exposed to the 30 Hz flicker (6.48%), and lowest for the birds in the 195 Hz treatment (5.22%) with the 90Hz birds being intermediate (5.36%) (P=0.024). No effects of flicker frequency were noted on gait scores (P=0.101 for average score), but average lesion scores were the lowest for birds in the 90 Hz compared to the 30 or 195 Hz (Table 3).

Table 3. Effect of LED light flicker on average and frequency (% in each category) of footpad lesion scores (scale 0-4[2]) in 10-week-old turkey hens

Score	Treatments (Hz)			SEM[3]	P-value
	30	90	195		
Footpad Lesion Scores					
0*	10.67[b]	24.44[a]	10.67[b]	3.855	0.011
1*	16.67	26.11	15.33	3.256	0.341
2	24.00	28.89	28.67	2.674	0.736
3*	27.33	14.44	25.33	3.347	0.147
4*	21.33	6.11	20.00	4.093	0.179
Average Score*	2.32[a]	1.52[b]	2.29[a]	0.262	0.021

* Block differed significantly: included as a random factor (P <0.05)
[a,b] Values with different letters within the same row differ significantly (P <0.05)
[1] Score of 0=no impairment, 5=complete lameness (adapted from Garner et al., 2002 by Vermette et al., 2016b)
[2] Score of 0=no external signs of a lesion, 1=harder and denser footpad than score 0, 2=swelling and less than ¼ area necrotic, 3=swelling and ½ area necrotic, 4=more than ½ area necrotic (Hocking et al., 2008)
[3] Standard error of the mean

Although feather condition was not impacted by flicker frequency, feather cleanliness was. Birds in the 195 Hz were dirtier than those in the 90 Hz treatments, with the 30 Hz birds being intermediate (Table 4).

Table 4. Effect of LED light flicker on average and frequency of feather cleanliness scores (scale 1-4[1]) of turkey hens at 10 weeks of age

% of scores in category	Treatments (Hz)			SEM[2]	P-value
	30	90	195		
1*	42.00[ab]	62.78[a]	26.67[b]	7.722	0.030
2*	54.00	35.56	56.67	5.828	0.088
3	4.00	1.67	16.00	2.780	0.071
4	0	0	0.01	0.208	0.357
Average Score*	1.62[ab]	1.39[b]	1.91[a]	0.100	0.021

* Block differed significantly: included as a random factor (P<0.05)
[a,b] Values with different letters within the same row differ significantly (P<0.05)
[1] Score of 1=very clean, 2=moderately clean, 3=moderately dirty, 4=very dirty (Forkman and Keeling (2009) as modified from Wilkins et al. (2003))
[2] Standard error of the mean

Eye shape did change with exposure to flicker. Birds in the 30 Hz had the widest dorso-ventral width compared to those in the 195 Hz, and had the widest anterior posterior size compared to those in the 90 Hz (Table 5).

Table 5. Effect of LED light flicker on eye weight (g), medio-lateral diameter (mm), dorso-ventral diameter (mm), and anterior posterior size (mm) of turkey hen eyeballs at 11 weeks of age

	Treatments (Hz)			SEM[1]	P-value
	30	90	195		
Eye weight	5.75	5.63	5.67	0.039	0.386
Medio-lateral diameter*	24.45	24.25	24.35	0.060	0.211
Dorso-ventral diameter*	24.56[a]	24.42[ab]	24.33[b]	0.069	0.046
Anterior posterior size	18.89[a]	18.60[b]	18.62[ab]	0.048	0.033

* Block differed significantly: included as a random factor (P <0.05)
[a,b] Values with different letters within the same row differ significantly (P <0.05)
[1] Standard error of the mean

Inconsistent results were noted in bird behaviour. However, it was interesting to see that the percentage of time birds spent flighting (P=0.049) and in aggressive pecking (P=0.022) at 8 wk was lowest in birds exposed to 30 Hz compared to those in the 90 Hz treatment, with those in the 195 Hz being intermediate (Table 6).

Table 6. Percentage (%) of time spent performing behaviours by 8-week-old turkey hens within field of view (20-minute intervals) during the photoperiod

Behaviours	Treatments (Hz)			SEM[1]	P-value
	30	90	195		
Aggressive					
Fighting	0.06[b]	0.17[a]	0.11[ab]	0.022	0.049
Posturing	0.06	0.13	0.07	0.023	0.216
Aggressive pecking	0.68[b]	1.20[a]	1.07[ab]	0.094	0.022
Total	0.80[b]	1.50[a]	1.24[ab]	0.112	0.013

[*] Block was included as a random factor because it had significance (P<0.05)
[a,b] Values with different letters within the same row differ significantly (P<0.05)
[1] Standard error of mean
[2] **Total Active:** Walking, frolicking, pacing, jumping, standing
[3] **Low Incidence/Other:** Eating novel object piece, tripping, accidental collision, tail twitching, frolicking, litter scratching, head scratching, perching, head rubbing, pacing, jumping

Conclusion

To conclude, visible flicker had significant impacts early in life of turkey hen poults. While production parameters did equalize by 11 wk of age, other parameters indicated a reduction in bird welfare and likely a reduction in economic return (higher mortality) when flicker was visibly present in the barn. In addition, data did indicate that flicker not visible to humans (90 Hz in this study) may have impacts on birds as well. Visual ability of turkeys and other poultry species is much more advanced than is our vision. Therefore, producers should measure flicker in their facilities, (with the use of a spectrometer) , keeping flicker frequencies above the 119 Hz that has been shown to be the upper limit of what birds can see.

These works have been published:

Hammond, A., K. Buchynski, T. Shynkaruk, J. Brown, T. Crowe and K. Schwean-Lardner. 2024. Do flickering lights impact turkey hen behavior, stress and fear? Poultry Science 103: https://doi.org/10.1016/j.psj.2024.103699

Hammond, A., K. Buchynski, T. Shynkaruk, T. Crowe and K. Schwean-Lardner. 2024. Are turkey hens affected by light flicker? Effects on performance and health. Poultry Science 103: https://doi.org/10.1016/j.psj.2024.103747

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Nutrition density in starting and fattening period and its impact on final performance

Valentin Theneau
Techna France Nutrition, France
valentin.theneau@groupe-techna.com

Introduction

The turkey, a demanding bird in terms of nutrition. Its rapid development and its production of lean meat make it a particularly sensitive to the quality and balance of diet formulation and composition.

Energy and protein nutrition play a vital role in the growth, health, welfare, meat quality and carbon footprint. Within the article we will explore in detail the energy and protein need of this bird and the impact of a balanced diet on its technical performance, economics, welfare and environmental aspects.

Key words; Male, Premium, Protein, Nutrition, Energy, Performance, Economics, Welfare, meat quality, breast meat yield, Carbon footprint, Environment, Growth.

Material and methods

The trial was carried out at the experimental research station in St Symphorien, France.

528 Aviagen Premium male turkeys were raised in 48 floor pens (2.6 m²) from 1 day to 112 days old and then split in 8 groups.

The feeding program contained 6 phases. Feed presentation was a crumble from d0 to D35, then pellets of 3.25-inch diameter. In order to give an accurate understanding of the protein and energy response at different development stages the 8 groups were divided into a starter/grower period and a finishing period.

- Starting/Growing period from 0 to 56 days – 4 levels of balanced protein (Digestible Lysine) with 2 levels of Metabolisable Energy (ME)
- Fattening period from 56 to 112 days – 4 levels of ME with 2 levels of balanced protein (Digestible lysine)

The nutritional levels were adjusted according to Techna France Nutrition specifications, matrix and energy system for commercial turkeys (Table 1) and contained xylanase and phytase. Feed and water were provided ad libitum.

Table 1 : *Nutritional value of diets*

Groups		1	2	3	4	5	6	7	8
Period 0-56 days		ME-Lys--	ME-Lys-	ME-Lys+	ME-Lys++	ME+Lys--	ME+Lys-	ME+Lys+	ME+Lys++
Starter	ME – kcal/kg			2685				2835	
0-21 days	Dig Lys. %	1,50	1,55	1,60	1,65	1,50	1,55	1,60	1,65
Grower 1	ME – kcal/kg			2785				2915	
21-35 days	Dig Lys. %	1,38	1,43	1,48	1,53	1,38	1,43	1,48	1,53
Grower 2	ME – kcal/kg			2835				2965	
35-56 days	Dig Lys. %	1,22	1,27	1,32	1,37	1,22	1,27	1,32	1,37
Period 56-112 days		ME--Lys+	ME-Lys+	ME+Lys+	ME++Lys+	ME--Lys-	ME-Lys-	ME+Lys-	ME++Lys-
Finisher 1	ME – kcal/kg	2975	3050	3125	3200	2975	3050	3125	3200
56-77 days	Dig Lys. %			1,18				1,08	
Finisher 2	ME – kcal/kg	3075	3150	3225	3300	3075	3150	3225	3300
77-91 days	Dig Lys. %			1,09				0,99	
Finisher 3	ME – kcal/kg	3125,00	3200	3275	3350	3125	3200	3275	3350
91-112 days	Dig Lys. %			1,06				0,96	

**According to TECHNA FRANCE NUTRITION matrix and energy system*

For all groups, synthetic amino acids were incorporated in feed to achieve TECHNA's specification for the ideal amino acid profile of turkey requirements.

From 0 to 56 days, groups 1 to 4 are groups with low energy level with different level of digestible lysine. Groups 5 to 8 are groups with energy level with different level of digestible lysine.

Then, from 56 to 112 days, groups 1 to 4 are groups with high digestible lysine with different level of ME. Groups 5 to 8 are groups with low digestible level with different level of ME.

Table 2 presents the nutritional specification.

Table 2 : *Nutrition specification*

Phase		0-21d	21-35d	35-56d	56-77d	77-91d	91-112d
*Nutritional value of the feed**							
METABOLISM ENERGY	Kcal/kg	2685-2835	2785-2915	2835-2965	2975-3200	3075-3300	3125-3350
CRUDE FAT	%	2,9-5,3	3,5-5,4	4,0-5,4	4,8-8,2	4,9-9,0	5,0-8,80
CRUDE PROTEIN	%	27,3-28,4	24,5-26,7	22,5-24,5	19,8-21,7	18,5-19,9	18,0-19,5
DIGESTIBLE LYSINE	%	1,50-1,65	1,38-1,53	1,22-1,37	1,08-1,18	0,99-1,09	0,96-1,06
CALCIUM	%	1,40	1,25	1,10	0,95	0,85	0,80
PHOSPHORUS Av.	%	0,75	0,63	0,57	0,46	0,41	0,38

**According to TECHNA FRANCE NUTRITION matrix and energy system*

For this trial, Calcium and phosphorus level are the same for all groups.

Table 3 : *Diets*

Phase		**0-21d**							
Groups		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Raw materials									
Corn	%	19,9	19,3	18,3	16,7	19,6	19,3	17,4	15,0
Wheat	%	20,0	20,0	20,0	20,1	20,0	19,9	20,1	20,0
Soybean meal	%	45,7	45,8	46,5	49,0	47,0	46,7	47,9	49,9
Sunflower meal	%	4,0	4,0	3,7	2,6	2,0	2,0	2,0	2,0
Corn DDGS	%	3,0	3,0	3,0	3,0	2,0	2,0	2,0	2,0
Soya oil	%	0,60	0,60	0,65	0,65	2,65	2,60	2,85	3,15
Amino acid, phosphate, c.	%	6,76	7,30	7,82	7,93	6,77	7,47	7,79	7,97

Phase		**21-35d**							
Groups		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Raw materials									
Corn	%	24,0	23,5	22,2	20,6	25,3	24,7	22,3	19,9
Wheat	%	20,1	20,1	20,1	19,9	20,0	20,0	20,0	20,1
Soybean meal	%	40,8	40,8	42,3	44,8	39,7	41,8	43,8	45,7
Sunflower meal	%	4,0	4,0	3,4	2,5	2,0	2,0	2,0	2,0
Corn DDGS	%	4,0	4,0	4,0	4,0	4,0	2,0	2,0	2,0
Soya oil	%	1,05	1,05	1,10	1,15	2,50	2,65	2,95	3,25
Amino acid, phosphate, c.	%	6,07	6,59	6,93	7,02	6,54	6,81	6,94	7,06

Phase		**35-56d**							
Groups		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Raw materials									
Corn	%	29,7	29,1	27,6	26,0	31,1	32,0	29,7	27,3
Wheat	%	20,0	20,0	20,0	20,0	20,0	19,9	20,0	20,0
Soybean meal	%	30,6	30,8	33,2	35,6	31,9	33,7	35,6	37,5
Rapeseed meal	%	3,0	2,9	2,0	2,0	2,0	2,0	2,0	2,0
Sunflower meal	%	5,0	5,0	4,8	3,9	2,0	2,0	2,0	2,0
Corn DDGS	%	5,0	5,0	5,0	5,0	5,0	2,0	2,0	2,0
Soya oil	%	1,35	1,35	1,40	1,40	2,45	2,55	2,80	3,10
Amino acid, phosphate, c.	%	5,40	5,84	6,00	6,07	5,53	5,82	5,95	6,08

Phase		**56-77d**							
Groups		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Raw materials									
Corn	%	34,0	35,2	33,7	32,2	37,3	40,0	38,4	36,8
Wheat	%	19,9	20,1	20,0	20,1	20,0	20,0	20,0	20,0
Soybean meal	%	29,3	30,2	30,4	30,5	24,4	26,4	26,7	26,9
Rapeseed meal	%	2,5	2,0	2,0	2,0	3,9	2,0	2,0	2,0
Sunflower meal	%	2,0	2,0	2,0	2,0	2,7	2,0	2,0	2,0
Corn DDGS	%	5,0	2,0	2,0	2,0	5,0	2,0	2,0	2,0
Soya oil	%	2,05	3,15	4,50	5,85	1,95	2,60	3,95	5,30
Amino acid, phosphate, c.	%	5,21	5,36	5,38	5,40	4,77	5,00	4,98	4,97

Phase		**77-91d**							
Groups		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Raw materials									
Corn	%	40,4	38,9	37,4	35,9	44,8	43,0	41,3	39,5
Wheat	%	20,1	20,0	20,0	19,9	20,0	20,0	19,9	20,0
Soybean meal	%	27,7	27,9	28,0	28,2	23,7	23,8	23,9	24,0
Sunflower meal	%	2,0	2,0	2,0	2,0	2,0	2,0	2,0	2,0
Corn DDGS	%	2,0	2,0	2,0	2,0	2,8	3,1	3,4	3,7
Soya oil	%	1,90	3,25	4,60	5,90	1,40	2,75	4,10	5,45
Palm oil	%	0,90	0,90	0,90	0,90	0,90	0,90	0,90	0,90
Amino acid, phosphate, c.	%	5,04	5,08	5,10	5,16	4,45	4,47	4,49	4,49

Phase		91-112d							
Groups		1	2	3	4	5	6	7	8
Raw materials									
Corn	%	37,1	35,5	35,5	34,0	41,0	40,1	40,2	38,4
Wheat	%	25,0	25,0	25,0	24,9	25,0	25,0	25,0	25,1
Soybean meal	%	26,3	26,5	27,7	27,9	22,3	22,6	23,9	23,9
Sunflower meal	%	2,0	2,0	0,0	0,0	2,0	2,0	0,0	0,0
Corn DDGS	%	2,0	2,0	2,0	2,0	3,0	2,2	2,0	2,4
Soya oil	%	2,05	3,40	3,30	4,65	1,50	2,85	2,75	4,10
Palm oil	%	1,00	1,00	2,00	2,00	1,00	1,00	2,00	2,00
Amino acid, phosphate, c	%	4,55	4,58	4,50	4,52	4,20	4,22	4,15	4,15

Raw material quality and origin are the same for all groups. According to the level of ME or digestible lysine required in the formulas, quantity of raw materials might differ from each group. In order to achieve the durability and hardness specification, a minimum inclusion of wheat was used for all groups during the complete trial period.

Economic criteria calculation;

Feed price (€ per ton of feed) = Σ for each phase (feed quantity of the phase x feed price of the phase)

Feed price (€ per ton of live birds) = Feed price x FCR

Cost of production (€ per ton of live birds) = Feed cost + Farmer remuneration + cost of 1 day old poults

Breast Feed cost (€ per ton of breast) = Feed cost + Breast FCR = Feed price x FCR x $\frac{\text{Mean weight}}{\text{Mean breast yield}}$

Climate change calculation;

Climate change (kgs eq CO2 per ton of feed)
= Σ for each phase (feed quantity of the phase x climate change of the phase)

Climate change per kgs of live bird (kgs eq CO_2 per kgs of live birds) = Climate change x FCR

Climate change per kgs of carcass (kgs eq CO_2 per kgs of carcass)
= Climate change per ton of live bird ÷ Carcass percentage

Climate change per kgs of breast meat (kgs eq CO_2 per kgs of breast meat)
= Climate change per ton of live bird ÷ Breast meat percentage

Results

At day 21 (table 4), the ADFI was significantly lower for group 5 to 8 with the highest energy level compared to groups 1 to 4 (p=0.039). During this phase, it seems that better body weight would be given by the highest digestible lysine and the lowest energy level (p=0.023).

At day 56 (table 4), the ADG, ADFI and FCR were significantly lower for the groups received a feed with energy boost (+150 kcal/kg; p=0.000). The increase of energy in the feed will decrease ADFI and have an impact on ADG. On this period, an increasing of digestible lysine will significantly increase the ADG (p=0.000). The interaction between digestible lysine and energy isn't significative for ADG, ADFI and FCR.

Table 4 **:** *Weight, average daily weight gain* (ADG), *daily feed intake* (DFI) *and feed conversion ratio* (FCR) – 0-56 *days*

	Groups																p-value			
	G1	G2	G3	G4	G5	G6	G7	G8												
	ME-				ME+				ME-	ME+	LYS-	LYS-	LYS+	LYS++	ETR	ME	LYS	ME*LYS	Groups
	LYS-	LYS-	LYS+	LYS++	LYS-	LYS-	LYS+	LYS++											
Weight 0d, g	61,1	60,7	60,7	60,8	60,8	60,8	60,9	60,7	61	61	61	61	61	61					
0-21d																			
Weight, g	664	679	694	723	663	640	657	650	690	653	664	660	675	686	27	0,069	**0,005**	**0,023**	**0,000**
ADG, g/d	28,7	29,5	30,1	31,5	28,7	27,6	28,4	28,0	30,0	28,2	28,7	28,5	29,3	29,8	1,3	0,068	**0,005**	**0,022**	**0,000**
ADFI, g/d	40,1	41,1	42,7	43,2	38,2	37,3	37,8	37,9	41,7	37,8	39,1	39,2	40,3	40,5	1,5	**0,039**	**0,003**	**0,041**	**0,000**
FCR	1,396	1,394	1,417	1,370	1,330	1,352	1,332	1,354	1,394	1,342	1,363	1,373	1,375	1,362	0,030	**0,000**	0,073	**0,043**	**0,000**
21-35d																			
Weight, g	1755	1792	1827	1896	1712	1672	1742	1766	1817	1723	1734	1732	1784	1831	50	**0,000**	**0,000**	NS	**0,000**
ADG, g/d	78,0	79,4	80,9	83,8	74,9	73,7	77,5	79,8	80,5	76,5	76,4	76,6	79,2	81,8	2,0	**0,000**	**0,000**	NS	**0,000**
ADFI, g/d	119,6	121,2	122,4	126,3	110,6	107,5	111,6	112,6	122,4	110,6	115,1	114,4	117,0	119,4	3,3	**0,000**	**0,002**	NS	**0,000**
FCR	1,534	1,526	1,513	1,507	1,476	1,458	1,441	1,412	1,520	1,447	1,505	1,492	1,477	1,459	0,024	**0,000**	**0,000**	NS	**0,000**
35-56d																			
Weight, g	4289	4406	4499	4635	4193	4125	4299	4419	4457	4259	4241	4265	4399	4527	124	**0,000**	**0,000**	NS	**0,000**
ADG, g/d	120,7	124,5	127,3	130,4	118,1	116,8	121,8	126,3	125,7	120,8	119,4	120,6	124,5	128,4	4,2	**0,000**	**0,000**	NS	**0,000**
ADFI, g/d	227,5	230,6	234,0	241,2	208,0	206,1	216,2	221,0	233,3	212,8	217,8	218,3	225,1	231,1	8,2	**0,000**	**0,001**	NS	**0,000**
FCR	1,886	1,855	1,839	1,850	1,760	1,764	1,776	1,751	1,857	1,763	1,823	1,809	1,807	1,800	0,048	**0,000**	0,706	NS	**0,000**
0-56d																			
ADG, g/d	75,5	77,6	79,3	81,7	73,8	72,6	75,7	77,8	78,5	75,0	74,7	75,1	77,5	79,8	2,2	**0,000**	**0,000**	NS	**0,000**
ADFI, g/d	130,2	132,2	134,4	138,2	119,9	118,1	123,2	125,3	133,7	121,6	125,1	125,1	128,8	131,7	4,0	**0,000**	**0,000**	NS	**0,000**
FCR	1,725	1,704	1,695	1,692	1,625	1,628	1,627	1,610	1,704	1,622	1,675	1,666	1,661	1,651	0,029	**0,000**	0,231	NS	**0,000**

From 56 to 112 (table5), energy have a significant impact on ADFI and FCR (p=0.000), but we could just see a trends for ADG. An increasing in the energy level will decrease the ADFI and the FCR. To the opposite, on this period, the increasing of digestible lysine will have an effect on the ADG and ADFI (p=0.000), but not the FCR (p=0.456). More digestible lysine will increase the ADG and increase the ADFI but no difference for the FCR. Overall, the interaction between energy and digestible lysine has an effect on ADG (p=0.047) and we see a trends for the ADFI (p=0.056). For the ADG, best performance would be obtained with Group 1; ME— & Lysine +. For the group 8 ADFI and FCR, best performance would be obtained with group 8; ME++ & Lysine -.

Table 5 **:** *Weight, average daily weight gain* (ADG), *daily feed intake* (DFI) *and feed conversion ratio* (FCR) – 56-112 *days*

	Groups																p-value			
	G1	G2	G3	G4	G5	G6	G7	G8												
	Lys+				Lys-				Lys+	Lys-	ME-	ME-	ME+	ME++	ETR	ME	LYS	ME*LYS	Groups
	ME-	ME-	ME+	ME++	ME-	ME-	ME+	ME++											
Weight 56d, g	4289	4406	4499	4635	4193	4125	4299	4419	4457	4259	4241	4265	4399	4527					
56-77j																			
Weight, g	8351	8272	8374	8427	7860	7723	7863	7955	8356	7850	8106	7997	8119	8191	131,14	**0,013**	**0,000**	NS	**0,000**
ADG, g/d	193,4	184,1	184,5	180,6	174,6	171,3	169,7	168,4	185,7	171,0	184,0	177,7	177,1	174,5	6,20	**0,004**	**0,000**	NS	**0,000**
ADFI, g/d	368,4	344,2	337,8	339,2	340,0	325,1	317,7	318,6	347,4	325,3	354,2	334,6	327,8	328,9	9,48	**0,000**	**0,000**	NS	**0,000**
FCR	1,905	1,870	1,832	1,879	1,947	1,898	1,874	1,893	1,871	1,903	1,926	1,884	1,853	1,886	0,04	**0,000**	**0,000**	NS	**0,000**
77-91j																			
Weight, g	11301	11316	11206	11340	10612	10315	10583	10621	11291	10533	10957	10816	10895	10980	196,42	**0,001**	**0,000**	NS	**0,000**
ADG, g/d	210,7	217,5	202,3	208,0	196,6	185,1	194,3	190,4	209,6	191,6	203,6	201,3	198,3	199,2	13,16	0,152	**0,000**	NS	**0,006**
ADFI, g/d	461,4	448,0	431,1	418,3	427,4	399,3	399,8	387,3	439,7	403,4	444,4	423,7	415,5	402,8	14,40	**0,000**	**0,000**	NS	**0,000**
FCR	2,193	2,064	2,135	2,017	2,179	2,161	2,062	2,035	2,102	2,109	2,186	2,113	2,099	2,026	0,10	**0,003**	0,810	NS	**0,012**
91-112j																			
Weight, g	15882	16027	15837	15836	15001	14953	15401	15380	15895	15192	15481	15490	15619	15608	316,32	0,065	**0,001**	**0,045**	**0,001**
ADG, g/d	218,1	224,3	220,5	214,1	212,9	220,9	229,4	226,7	219,3	222,9	215,7	222,6	225,0	220,4	11,24	0,272	0,307	NS	0,156
ADFI, g/d	564,9	557,6	535,4	511,4	540,0	529,8	543,8	520,5	542,3	533,2	553,6	543,7	539,6	515,9	19,75	**0,000**	**0,044**	**0,037**	**0,000**
FCR	2,594	2,489	2,433	2,391	2,540	2,399	2,371	2,297	2,477	2,395	2,569	2,444	2,402	2,344	0,08	**0,000**	0,002	NS	**0,000**
56-112j																			
ADG, g/d	207,0	207,5	202,5	200,0	193,2	193,4	198,3	195,7	204,3	195,2	200,7	200,4	200,4	197,9	5,65	0,078	**0,000**	**0,047**	**0,000**
ADFI, g/d	465,3	450,2	435,2	423,5	434,5	420,4	423,0	411,5	443,6	421,8	451,3	435,3	429,1	417,5	11,32	**0,000**	**0,000**	0,056	**0,000**
FCR	2,249	2,170	2,151	2,118	2,249	2,174	2,134	2,102	2,172	2,161	2,249	2,172	2,142	2,110	0,04	**0,000**	0,456	NS	**0,000**

From 0 to 112 days (table 6), highest final body weight was achieved within group 2, ME-Lys-/ME-Lys+ compared to the lowest body weight achieved with group 6, ME+Lys-/ME-Lys-. Group 1 to 4 have the highest body weight compared to groups 5 to 8. All groups are significantly different in terms of Body weight, ADG, ADFI and FCR (p=0.000). Best FCR for the period was seen in groups 4 and 8, where ME was high during the period 56 to 112 days.

Table 6 : *Weight, average daily weight gain* (ADG), *daily feed intake* (DFI) *and feed conversion ratio* (FCR) *– 0-112 days*

		Groups								ETR	p-value
		G1	G2	G3	G4	G5	G6	G7	G8		
		ME-Lys--/ME--Lys+	ME-Lys-/ME-Lys+	ME-Lys=/ME+Lys+	ME-Lys+=/ME++Lys+	ME+Lys--/ME--Lys-	ME+Lys-/ME-Lys-	ME+Lys=/ME+Lys-	ME+Lys+=/ME++Lys-		Groups
0-112d	Weight 0d, g	61,1	60,7	60,7	60,8	60,6	60,8	60,9	60,7		
	Weight, g	15682	16027	15837	15836	15001	14953	15401	15380	358	**0,000**
	ADG, g/j	141,3	142,6	140,9	140,8	133,4	133,0	137,0	136,8	3,2	**0,000**
	ADFI, g/d	297,8	291,2	284,8	280,9	276,9	269,3	273,1	268,4	6,6	**0,000**
	FCR	2,109	2,043	2,022	1,995	2,076	2,025	1,994	1,962	0,026	**0,000**

Live bird weight, carcass weight, breast weight and leg weight (Table 7) are significantly different between groups (p=0.000). Fat weight is not different (p=0.436). In terms of yield, only breast meat yield is different between groups (p=0.000). An increasing level of energy level during the period 56 to 112 days appears to decrease breast percentage. An increasing level of digestible lysine during the period 56 to 112 days has an impact on breast meat yield.

Table 7 : *Carcass weight and yields at 112d.*

	Groups								ETR	p-value
	G1	G2	G3	G4	G5	G6	G7	G8		
	ME-Lys--/ME--Lys+	ME-Lys-/ME-Lys+	ME-Lys=/ME+Lys+	ME-Lys+=/ME++Lys+	ME+Lys--/ME--Lys-	ME+Lys-/ME-Lys-	ME+Lys=/ME+Lys-	ME+Lys+=/ME++Lys-		Groups
113d	15783	15891	15748	15664	15262	14749	15396	15326	650	**0,000**
Carcass weight, g	12096	12152	12003	11979	11661	11222	11674	11674	599	**0,000**
Fat weight, g	111,1	109,6	127,7	124,5	104,5	100,0	116,9	114,9	40,0	0,436
Breast weight, g	3686	3774	3621	3551	3460	3259	3436	3343	280	**0,000**
Leg weight, g	3770	3854	3758	3813	3692	3602	3710	3787	192	**0,006**
Carcass yield, %	76,6	76,5	76,2	76,5	76,3	76,1	75,8	76,1	1,20	0,591
Fat yield, %	0,70	0,69	0,81	0,79	0,68	0,67	0,76	0,75	0,25	0,625
breast yield, %	23,3	23,7	23,0	22,7	22,6	22,1	22,3	21,8	1,33	**0,000**
Leg yield, %	23,9	24,3	23,9	24,3	24,2	24,4	24,1	24,7	0,32	0,127

Feed costs/T Liveweight (Table 8) is lower in group 5 (608€/T), mainly due to the lower feed cost (293€/T). The highest feed cost/T Liveweight was seen in Group 4 (654€/T). Even if the FCR is low (FCR = 1.995), the feed price to obtain this performance is too high compared to other strategies.

Feed cost / T Breast (Table 8) were lower within group 2 due to a good combination between feed price, FCR and breast meat yield (BMY= 23.7%). Conversely, group 8 obtained the highest feed cost / T Breast (2905€/T), due to the high feed price, good FCR, but the lowest breast meat yield compared to other groups.

Table 8 : *Economical approach*

	Groups							
	G1	G2	G3	G4	G5	G6	G7	G8
	ME-Lys--/ME--Lys+	ME-Lys-/ME-Lys+	ME-Lys=/ME+Lys+	ME-Lys+=/ME++Lys+	ME+Lys--/ME--Lys-	ME+Lys-/ME-Lys-	ME+Lys=/ME+Lys-	ME+Lys+=/ME++Lys-
Average feed price, €/T	299	308	318	328	293	301	312	323
Final body weight at slaughterhouse, kgs	15,88	16,03	15,84	15,84	15	14,95	15,4	15,38
FCR	2,109	2,043	2,022	1,995	2,076	2,025	1,994	1,962
Feed cost, €/ton of live bird	630	629	643	654	608	610	622	633
Breast yield, %	23,3	23,7	23	22,7	22,6	22,1	22,3	21,8
Feed cost, €/ton of breast	2705	2656	2795	2880	2691	2762	2789	2905

In terms of climate change criteria Table 9, the best strategy to decrease carbon footprint was seen within group 5 (14.76kgs of CO2/kg of breast). Group 5 achieved the result because of the lowest digestible lysine, and balanced protein, and the lowest energy feed concentration. Group 4 obtained the highest carbon footprint result with a strategy of high digestible lysine & high ME (17.05kgs of CO2/hg of breast)

Table 9 : *Environmental criteria*

	Groups							
	G1	G2	G3	G4	G5	G6	G7	G8
	ME-Lys--/ME--Lys+	ME-Lys-/ME-Lys+	ME-Lys+/ME+Lys+	ME-Lys++/ME++Lys+	ME+Lys--/ME--Lys-	ME+Lys-/ME-Lys-	ME+Lys+/ME+Lys-	ME+Lys++/ME++Lys-
Average feed price, €/T	299	308	318	328	293	301	312	323
Final body weight at slaughterhouse, kgs	15,88	16,03	15,84	15,84	15	14,95	15,4	15,38
FCR	2,109	2,043	2,022	1,995	2,076	2,025	1,994	1,962
Feed cost, €/ton of live bird	630	629	643	654	608	610	622	633
Breast yield, %	23,3	23,7	23	22,7	22,6	22,1	22,3	21,8
Feed cost, €/ton of breast	2705	2656	2795	2880	2691	2762	2789	2905

Discussion

The result of this trial confirms most of the theorical scenarios used by authors for the estimation of poultry production.

For the starting period, from 0 to 56 days, as mentioned by Boling (1998), Dorigam (2016), Thompson (2004), Noy (2004) and Firman (2004), digestible lysine is important. More digestible lysine, balanced protein, would increase the ADG and the body weight. From 0 to 56 days, as mentioned by Noy (2004) and Veldkamp (2005), an increasing of the energy level would decrease the feed intake and the body weight. Interaction between energy and protein is also described in studies by Velkamp (2005), we could see the effect of the interaction between ME and Digestible Lysine for this period.

For the second period, from 56 to 112 days, as mentioned Baker (2003), Thompson (2005) and Firman (2010), an increase of digestible lysine will increase the ADG. In this period, for the ME, our trial shows very similar results as Noy (2004) regarding the consequence of increasing the ME. An increase of ME will decrease the feed intake and might have some consequence on the ADG. In a previous trial by Messaoud (2020), we confirmed the impact of ME on feed intake and then on the FCR.

For the total period, interaction between ME and digestible lysine has been observed. We also see that a higher body weight during the starting period might have an impact on the final body. Group 1 to 4 are heavier compared to group 5 to 8.

Overall, it depends on the strategy the turkey industry would like to achieve. In order to achieve the best body weight during the period 0 to 56 days, we should favour the strategy of the group 4 (Low ME and Lysine ++). If we want to target FCR on this period then Group 8 (ME+ & Lysin ++) would be more likely to achieve this goal.

Economic performance in this trial shows us that a different strategy will have an economic impact. Best performance (low FCR for example) does not necessarily result in the best economic performance, for example; feed cost per ton of breast meat.

According to the latest calculation methods and raw materials database (Wilfart – 2016, ECOALIM, GFLI), on environmental performance the best production performance does not necessarily equate to a lower carbon footprint. Carbon footprint remained lower for a strategy where a lower ME and lower balanced protein formulation was used. Even if the final performance were lower compared to others. Group 5 had a lower carbon footprint than other groups. And, Group 4, most concentrated feed, had the highest carbon footprint.

Conclusion

The results of this study highlight the importance of an energy and protein intake adapted to the specific needs of turkeys at each growth stage. A protein deficit either by lack of balanced protein or lack of feed intake will decrease growth during the whole life of the birds. An excess of Energy, especially during the starting period, might decrease growth. Different nutritional strategies detailed in this trial and attained different performance, in terms of economics and/or environment. Although this study has provided a better understanding of the nutritional needs of turkeys. An integrated approach, combining nutritional, physiological and genetic data, will allow the development of increasingly precise and sustainable feeding strategies.

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List of abbreviations

ADFI: Average Daily Feed Intake
ADG: Average Daily Gain
BMY = Breast meat yield
CO2: Carbon Dioxide
D: Days
FCR: Feed Conversion Ratio
LYS: Digestible Lysine
ME: Metabolism Energy

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PMV vaccination associated with Ornithobacterium rhinotracheale autogenous vaccines as a mean of antibiotic demedication in broiler turkeys in France

***Dr. Alexandre* VOVE**
Amont LDC, France
alexandre.vove@ldc.fr

Context :
In 2012, a national CIDEF's study (The French Turkey Interprofessional committee) showed that 80% of turkey flocks were treated with at least one antibiotic, with an average of 3.1 treatments per flock. At the same time, the Ecoantibio plan (French national project to reduce the use of antibiotics in veterinary medicine, in regards to the One Health concept) was led. The aim of this plan was a 25% reduction of antibiotic use over 5 years.

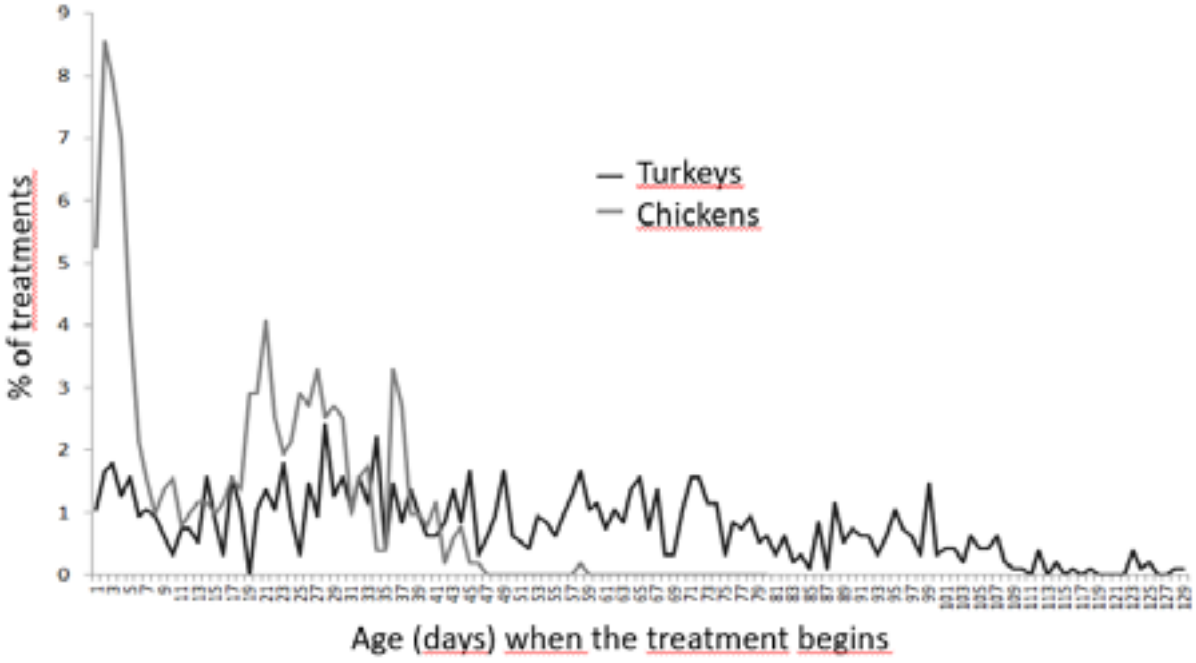

***Figure 1** : Percentage of treatments according to the starting age of treatment*

In turkey production, the 3 major diseases requiring an antibiotic treatment are : enteritis (ex: Clostridiosis), respiratory diseases (ex : Ornithobacteriosis) and bacterial septicemia (ex : Escherichia coli). Moreover, some of those diseases have a viral origin and bacterial complications only, thus making vaccines useful to prevent the need for antibiotics.

Four pillars have been defined to reduce antimicrobial prescriptions in turkey flocks :
- Good breeding practices to avoid diseases caused by malpractices
- Vaccination to limit consequences of a viral or a bacterial environmental pressure
- Good hygiene practices
- Digestive stability for the animals (which limits the risk of enteritis)

When considering viral pressure, two viruses are under strict surveillance in turkey production: PMV-1 (Newcastle disease) and aMPV (avian metapneumovirus).

A study performed by MERIAL in 2014 showed several positive detections in LDC's turkey farms which means wild virus strains were circulating.

Based on these elements, a new vaccination prophylaxis plan had been led in 2014, as following plan : 3 aMPV vaccinations (one hatchery shot included) and 3 PMV-1 vaccinations.

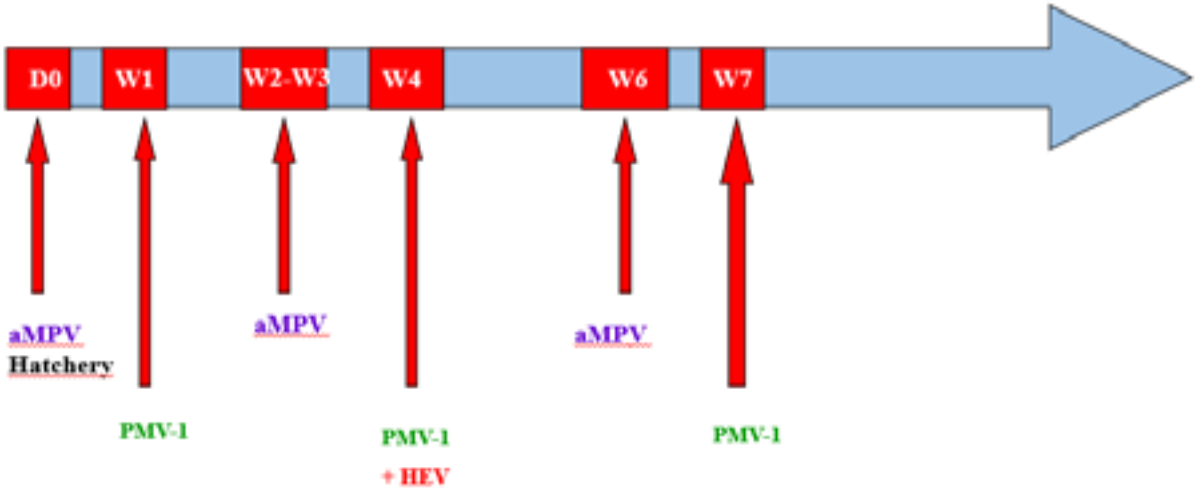

***Figure 2** : First prophylaxis plan including aMPV and PMV-1 protection*

Despite this protocol, in 2017, another study by CEVA including 64 turkey farms revealed a PMV-1 persistency in 50% of the farms.

This result raises several questions: have the farmers completed the whole vaccination plan? Is the vaccine used correctly? Is the prophylaxis plan really efficient to start of with?

As a result, in 2017, we did a trial where all of our turkey flocks were vaccinated against PMV-1 with a single shot at the hatchery (VECTORMUNE ND®). The protection is effective at 3 weeks of age and do not require another shot. Prophylaxis plan against aMPV remained identical.

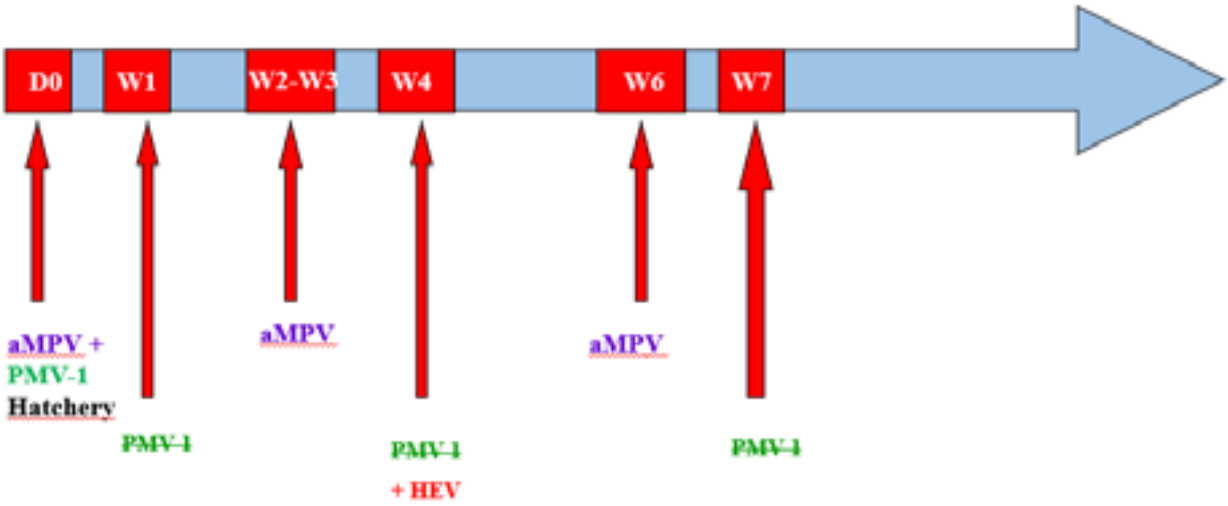

***Figure 3** : Second prophylaxis plan with the hatchery vaccination against PMV-1*

Finally, between 2020 and 2022, the consequences of autogenous vaccines use against Ornithobacterium rhinotracheale and Escherichia coli was studied.

Results:

An important decrease of the number of antibiotic treatments was observed: -41%. This good result needs to be related with the prophylaxis plan but also with our three previous pillars (breeding practices, hygiene practices and digestive stability).

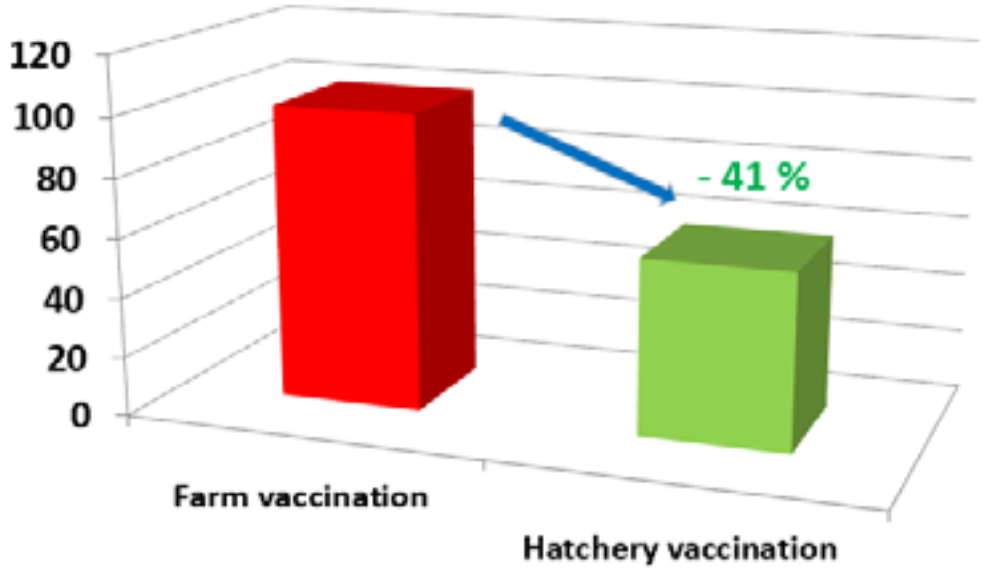

***Figure 4** : Evolution of percentage of treatments according to PMV-1 vaccination*

Among 100% of hatchery-vaccinated flocks, the daily weight gain was increased by 2.96% (+310 g per animal, males and females). This improvement is more significant among farms with initial PMV-1 pressure: +4.2% (+460 g per animal, males and females).

The last result concerned performances of 1937 turkey flocks vaccinated with VECTORMUNE ND® (including 1601 auto-vaccinated flocks). In flocks vaccinated with VECTORMUNE ND® and ORT/E.coli autogenous vaccines, we observed an increase of the performance index and of daily weigh gain: 4.2% and 5.2% respectively compared to flocks without autogenous vaccines.

Performances improvement with the use of ORT and E.coli autogenous vaccines had also been studied by another French company among 2117 turkey flocks. An increase of the performance index was observed (382 to 406), along with a decrease in mortality rates after 10 days of age (5.02% to 4.22%) and a decrease in culls (1.62% to 1.45%). Furthermore, for ORT-autovaccinated flocks, a significant decrease of the ALEA (Animal Level Exposure to Antibiotics) was observed (0.36 to 0.03).

Conclusion:

Those retrospectives studies over 12 years have had a true positive impact concerning prophylaxis plans in turkey flocks. Performances and carcass qualities were improved.

Nowadays, in France, the third Ecoantibio Plan is about to start and the percentage of non-treated turkey flocks is 68% against 19% in 2012 which is a very hopeful result.

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Overview of phytobiotic use on turkey farms: From in vitro tests on Histomonas and Eimeria to field results

J. M. Watier, A. Mahieu, A. Canin, R. Domitille, M. Aoun, A. Greuter
IDENA, 21, rue du moulin, F-44880, Sautron, France

Abstract:
Since the ban on antihistomonosis molecules, histomonosis has continued to pose a significant threat to turkey production. At that time, a phytobiotic solution was developed and tested as an alternative, demonstrating efficacy against Histomonas meleagridis, but also focusing its effects on Eimeria tenella, and bacterial pathogens. In vitro studies showed that specific compounds inhibited parasites development and bacterial growth while sparing beneficial gut flora. Field tests of a powder form indicated improved feed conversion ratio (FCR), average daily gain (ADG), due to a better gut health. A liquid form was later developed to more specifically address stress-related dysbiosis and enhance gut health disorders prevention. Long-term field results confirmed the phytobiotic's effectiveness in maintaining turkey health without histomonosis outbreaks.

Introduction
Since the stop of specific antihistomonosis substances in 2003, histomonosis has posed a considerable threat to all turkey producers, as no effective drugs or treatments have been designed and developed since then.

Furthermore, coccidiosis and non-specific enteritis, which can exacerbate the risk of blackhead disease outcome, due to their impact on intestinal health, are also causes for concern.

Consequently, the development of a comprehensive solution to address these challenges is of paramount importance. In the field of phytobiotics, a solution has been tested in vitro as an alternative to specific drugs that are no longer available. That was the subject of a poster in 2024 (see Proceedings of the 15th Turkey Science and Production Conference). Since then, field applications have been carried out using a powder form, added to turkey feed and a liquid form of the solution, and has also been tested and used for a long time.

This document aims to provide a brief summary of the main in vitro results and to present some field applications that have produced good results.

The main features enabling a phytobiotic product to act on Histomonas meleagridis and Eimeria

Description of the study process:
The study focused on the development of bioactive substances targeting histomonads, coccidia and bacterial pathogens. To test the activity of the histomonads, ten aromatic and synthetic substances were evaluated at two concentrations in vitro at the University of Veterinary Medicine Vienna. For coccidiosis, in collaboration with INRAE, the study evaluated the bioactives' ability to inhibit the invasion and replication of Eimeria parasites in intestinal cells, as well as the sporulation step. The findings suggest that the mechanism of action is shared across the Apicomplexa phylum, making the results applicable to Eimeria species in turkeys.

For the purpose of bacterial control, an extensive screening of active ingredients was conducted in collaboration with the University of Lille, encompassing gel diffusion tests and Minimum Inhibitory Concentrations (MICs) evaluation. The incorporation of anti-inflammatory and antioxidant plant extracts was undertaken to address inflammation during infections. A specialised emulsification process was employed to enhance the bioavailability and stability of the active ingredients, thereby improving their efficacy against pathogens while minimising the drawbacks associated with essential oils.

In vitro results obtained:
The study evaluated the efficacy of bioactive compounds against Histomonas meleagridis, Eimeria tenella, and bacterial pathogens. The Minimum Lethal Concentration (MLC) test demonstrated that FORKEY and substance #6, at 500 and 50 ppm respectively, significantly reduced live H. meleagridis cells without re-growth in fresh medium.

Tableau 1: *Bioactives sensitivity tests on Histomonads cultures*

Example of results obtained in vitro on histomonas compared with the positive control (DMZ**)

Mixtures tested	Bioactives concentration	Average number of H. meleagridis before and after 24, 48 and 72 h of incubation x 10⁵ histomonas: 0 h	24 h	48 h	72 h	Mann-Whitney U	Regrowth
FORKEY LS I.O.N*	1/5 normal dose of use	100	44	132	379		
	normal dose of use	100	0	0	0	p<0,05	NO
FORKEY LS	1/5 normal dose of use	100	32	103	241		
	normal dose of use	100	0	0	0	p<0,05	NO
DMZ**	0,4 ppm	100	0	0	0	p<0,05	
Negative control		100	154	163	301		

* I.O.N: IDENA Organic Nutrition
** DMZ: DiMetridaZole

At the FORKEY LS using dose, in vitro tests showed equivalent activity to DMZ on Histomonas meleagridis.

For coccidia, 150 compounds were screened against E. tenella stages, with six compounds inhibiting oocyst sporulation by over 90%, three limiting invasion by at least 50% with a Selectivity Index (S.I.) up to 3.5, and 15 inhibiting parasite development in epithelial cells with efficacy ranging from 50% to 100% and a S.I. as high as 8 (the Selectivity Index is the ratio: efficacy/toxicity). Antibacterial tests at the University of Lille identified a blend (#4) effective against Clostridium spp. (average MIC: 6.2 ppm) while sparing beneficial bacteria, with minimal effects on positive strains like lactobacilli (MIC > 512 ppm). This blend was optimized for strong antibacterial activity and minimal impact on beneficial gut flora.

Tableau 2: *MIC tests of bioactives on selected bacteria*

ANTIBACTERIAL EFFECTS: CHOOSING THE RIGHT COMPOUNDS

- Screening of ingredients as part of a study conducted in partnership with a pharmacy university (Lille, France)

Université de Lille

- Selection of active ingredients with efficacy on *Clostridium perfringens* with no effect on beneficial flora

→ Minimum Inhibitory Concentration (MIC) Measurements with agar diffusion technique

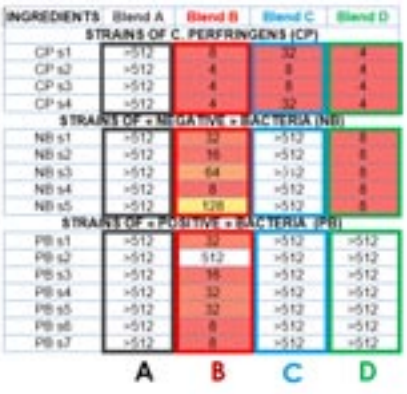
EXAMPLE OF AGAR DIFFUSION RESULTS
(MIC results- µ/ml)

A- No or little antibacterial effect = high MIC

B – Antibacterial against *Clostridium perfringens* but also against positive microflora

C – Antibacterial on *Clostridium* spp. and no action on positive microflora (e.g. Lactobacillus spp.)

D – Activity against Clostridium + negative bacteria (e.g. *Enterococcus spp.*)
VERY GOOD POTENTIAL FOR THESE ASSETS



Overview of some field results:

The long-lasting results obtained with the in-feed form
The powder form of this product had been used for a long time in a turkey breeding country since the ban of the last anti-histomonosis molecule, nifursol. Replacing the historic molecule with Forkey had led to the same technical results and even to an improvement in FCR and ADG thanks to other properties of this phytobiotics mixture. No histomonosis outbreaks were seen during this period.

Moreover, in collaboration with IRTA (Spain) in 2005, we showed an improvement in mortality rate, in homogeneity and in the harvest of Lactobacilli in the turkey guts. The experimental protocol was as follow: 6 groups of 224 turkeys per group, 4 replications per group and the experimentation lasted 12 weeks.

In France, over the last 10 years, many flocks of free-range turkey and guinea have been supplemented with Forkey and the technical results were at least as good as the national average, with no clinical symptoms of histomonosis.

The recent results on turkey flocks using the liquid form
With the aim of being more flexible in the prevention during critical periods in the life of turkeys and to provide a solution for other species (game birds in particular), we have developed a liquid version of the product.

It has been developed with the same criteria as the other liquid products: the same choice of active compounds as those designed for the powder form with an increased bioavailability and a better efficiency thanks to our specific emulsification process.

To position the application protocol, we know that anything that can be done to limit the development of certain bacteria in the intestinal tract, such as E. coli, is a good way in preventing the risk of Histomonosis. In addition, these specially designed bioactives have a direct effect on histomonads as previously demonstrated.

Knowing the specific sensitivity of turkeys to the taste of some liquids, the ability of turkeys to easily ingest the liquid product was tested (figures 1 and 2 below):

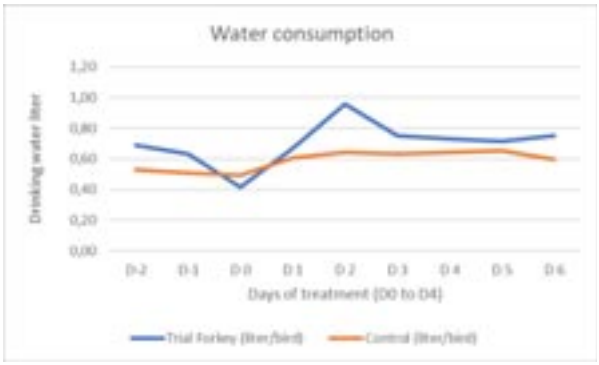


Figure 1: *Water consumption during Forkey liquid application*

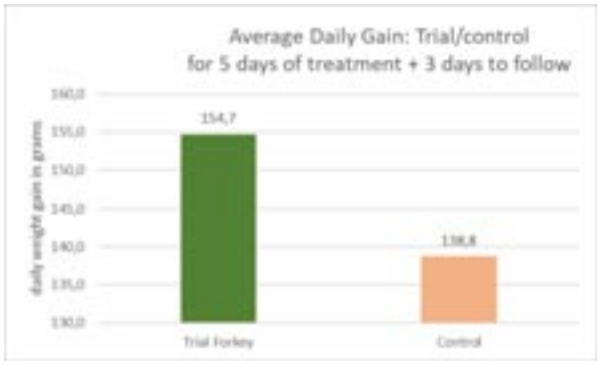


Figure 2: *Daily weight gain during treatment*

The first applications in meat and breeder turkeys have yielded positive results, indicating the product's potential for success. Its use during periods of stress helps to prevent the process of dysbiosis, which represents a particular risk of triggering histomonosis symptoms. Its use for 5 days at around 28 days of age, in meat turkey, has, in most of the cases, led to an improvement in faecal quality and helped to reduce the use of antibiotics.

The use in some turkey flocks helped to reduce the increase in mortality, which is a good result knowing that it is quite impossible to cure the symptomatic birds.

Conclusion:

We demonstrated that it is possible to formulate a viable phytobiotic product able to tackle at the same time the main threats that is facing the turkey production (detrimental bacteria, coccidial and histomonads risks).

Over several years, some turkey production companies have fed turkeys with this alternative in-feed solution replacing the old anti-histomonosis molecules. They have achieved good results and encountered no clinical cases of histomonosis.

The development of a liquid form that acts at the same level but with a different way of application, showed us some promising results in preventing these symptoms by using a phytobiotic product.

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The Turkey Microbiome

Laura Hoving, *MSc, Novonsis, Denmark*
Guilherme Borchardt, *DMV MSc PhD*

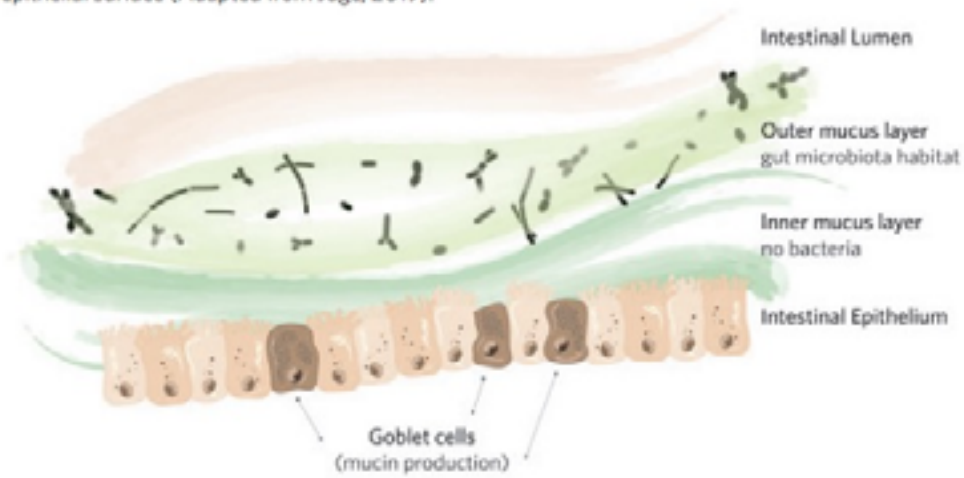
In Turkey production, like in the production of other animal species, the term microbiome is heard more and more frequently.

The microbiome is a term describing the collection of trillions of microorganisms, including bacteria, in- and outside of the body. The most referred to microbiome is the one inside of the gut, the gastrointestinal microbiome. This microbiome plays a key role in the operation of the human and animal body, although it is not the only one.

The body of a human or animal exists of more bacteria than specie specific cells. Making the microbiome very important, as we have observed that it has a significant impact on animal health. The relationship between microbiome composition robustness and the growth of animals and their resistance to pathogens is something we learn more and more about every day.

To understand a bit more about the microbiome, we must take a closer look at the intestine itself, and more specifically, to the mucus layer structure. The intestinal mucus layer is a very complex system with different functions: in the past, it was primarily known as a defensive barrier of the gut, belonging to the innate immune system. Various scientific studies over the last couple of years, have demonstrated that the same layer that provides a protective barrier against microorganisms, is the habitat of hundreds or even thousands of species of bacteria, viruses, protozoa, and fungi, which can be defined as the gut microbiota (Juge, 2019). To develop its full functionality, the intestinal mucus layer requires the presence of gut microbiota (Cornfield, 2018). Several bacteria can induce the activation of MUC2 gen by the goblet cells, triggering the production of mucus.

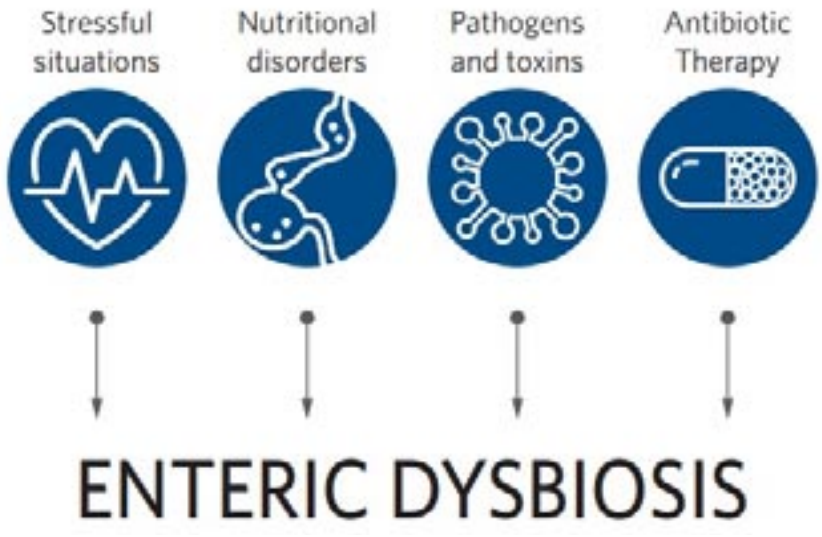
Figure 2. Mucus Layer structure in healthy large intestine. The outer layer is the habitat for gut microbiota, whereas the inner layer maintains them at a safe distance from the epithelial surface (Adapted from Juge, 2019).



Most of the time, the normal gut microbiota in the outer mucus layer forms a stable community, which is naturally able to resist the excessive growth, development, and colonization of pathogenic bacteria. This phenomenon has been known for decades and is called "pathogen colonization resistance". Gut dysbiosis is a term referring to disruption of the balanced or normal gut microbiota composition, followed by intestinal inflammation. In animal production, several factors can trigger gut dysbiosis, such as: stressful situations, nutritional disorders, high pressure of pathogens and antibiotic therapy, among others (Figure 3).

During the turkey production cycle, all factors that impact the gut microbiota balance, can lead to an open door for opportunistic pathogenic bacteria, like *Salmonella spp.*, *Clostridium*, *E. Coli* etc. In addition, vaccine program effectiveness could also be depressed by an unbalanced gut microbiota composition (Alberton, G. 2019). Following this paradigm, most farm programs designed to improve gut health recommend measures to reduce risk factors for dysbiosis. New tools start to be used in the field to evaluate poultry digestive microbiome during production cycle. Improved zootechnical performances can be observed when the microbiome is more resilient or agile.

Figure 3. Main risk factors for enteric dysbiosis in poultry production.



When taking measures to control gut dysbiosis, like an adapted feed strategy, including specific probiotic strains could be an effective measure. These strains demonstrate their ability to improve microbiome agility or resilience for microbiota imbalance, preventing a deeper and/or a longer period of gut dysbiosis. As the colonization resistance is negatively affected by gut dysbiosis, reducing the gut dysbiosis severity or time will naturally improve the ability of the host to control opportunistic pathogens. Also, as a rule, gut microbiota diversity is assumed to be positively correlated with the ability to keep potential pathogens under control (Ballou, et al. 2016).

All production animals including turkeys are susceptible to gut microbiota imbalance. However, turkeys, due to their relative long production cycle, and their sensitiveness to stressful conditions compared to broilers, are particularly affected by negative effects of gut dysbiosis. In addition to their action on gut microbiota balance, some specific probiotic strains have direct inhibitory effect on the growth of pathogens, such as: *Clostridium*, *E.coli* and *Salmonella spp*. This mode of action is known as "direct bacterial inhibition". The ability to produce specific antimicrobial peptides - so called bacteriocins and other metabolites, could explain this other important mode of action. It is important to notice that, for a more effective action, the chosen probiotic strains should be able to attach to the outer mucus layer, near the intestinal epithelium and form permanent or transient micro colonies.

To conclude, the microbiome inside the gut of a turkey, plays a huge role on the growth and wellbeing of the birds. It is, however, susceptible to dysbiosis, due to various stress factors that occur during the lifespan of the birds. One thing that could aid the robustness of the microbiome, keeping dysbiosis and pathogens at bay, are specifically selected probiotics.

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R&D Innovations and Focus on the Future

Charlie de Hollander,
Director, R&D, Hendrix Genetics Turkeys, USA

Introduction

Our goal as a turkey breeding company is to continuously improve the genetic potential of our product's performance. These genetic improvements are driven by selection procedures that deliver sustainable progress in a variety of traits, including egg production, livability, breast meat yield, feed efficiency, and live weight. The direction of selections is designed to fit our customer's current and future needs.

Selections are performed by geneticists and are based on Estimated Breeding Values (EBVs), these values are calculated by sophisticated models that combine information on pedigree, genotypes, and phenotypes. An EBV reflects the genetic potential of an individual for a specific trait, thus it is crucial that these values are as accurate as possible. This is where innovation and new technologies come in. Not only can we develop new phenotypes that are required for future needs, but we can also improve our current phenotyping. By automating data collection, we do not only increase operational efficiency, but we also increase the number of data points and reduce the risks for errors, both significantly improving accuracy. To be prepared for the future needs of the industry, we are always evaluating new technologies to explore new traits or redefine old ones.

Ongoing implementation of RFID technology

RFID technology is one area of focus that enhances the speed and accuracy of the genetic engine while increasing operational efficiency. We've connected this technology to collect data on the following traits.

- Feed efficiency: Using RFID technology, we can collect individual data on feeding behavior used to identify families that are more efficient than others in an environment where competition plays a role.

- Growth curves: RFID technology is the basis of our automated weighing system. This technology captures daily body weights on individual birds without any human interference, which benefits the animal and reduces our workload. The increase in weight data points helps us to map out a variety of growth curves and how they affect other traits.

- Egg production: The RFID tags help us automate egg data collection through the automated nest system installed in our pedigree facility. This reduces labor-intensive tasks and improves the quantity and quality of our data.

Enhancing selection methods for improved livability

Livability continues to be one of the most important but complex traits in the turkey breeding program. Livability is defined by a number of elements, such as leg health, metabolic health, and immune response. These elements in their turn are affected by many different factors, some of them genetic and some of them environmental. Due to its complexity, a range of phenotypic data is needed to select for this one trait. **CT scanning and CCPS testing** (Combined Crossbred & Purebred Selection) are two methods of expanding the genetic engine to select for livability.

Leg Health

Walking scores are highly correlated with leg health, but in a time where technology evolves rapidly, we need to be sure if there are other ways to assess leg health. We are currently evaluating new methods of selecting for leg health through the use of video recordings, artificial intelligence, and CT scanning.

Metabolic Health

Another important element of livability is metabolic health. With a growing body weight, we need to make sure the hearts and lungs grow to the same extend to support our healthy turkey. These phenotypes are now easy to collect but harder to understand. Currently, we are studying what is the optimal lung and heart size ratio using CT scanning to evaluate how this information can help to breed for metabolic health in our turkeys.

Immune response

The immune system might be the most complex element of livability and consists of specific and nonspecific immunity. To address both, commercial crossbred testing is utilized. We measure the performance of our crossbreds under commercial conditions and evaluate their performance. This data is then fed back into our breeding program which allows us to select for healthy and robust turkeys that perform well in our customer's operations.

Conclusion

Hendrix Genetics is always on the lookout for innovative and sustainable solutions. New technologies are evolving at a fast pace, and our company is tracking these developments closely to evaluate which one could make a difference in our breeding programs. The opportunity for new phenotyping tools is endless, so we need to remain critical and focused on the needs of our customers and future needs of the industry.

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Pre-harvest pathogen control – the role of prebiotics as a front-line defence measure

***Dr. Richard Murphy**, Ph.D.*
Director of Research, Alltech, Ireland

Introduction

Gut health and its management is an intricate and complex area governed by numerous factors including nutrition, microbiology, immunology and physiology. When gastrointestinal health is compromised, nutrient digestion and absorption are affected, feed conversion becomes reduced, susceptibility to disease is heightened and ultimately these result in a negative economic impact.

In general, the development of the adult gut microbiota begins on hatching or on birth whereby microbes are picked up predominantly from the post hatch/delivery environment and from the feed or drinking water. From a management point of view care needs to be taken not to inadvertently introduce pathogenic species. The time taken for the establishment of the stable adult microbiota will depend on many factors including the size of species, type of feeding regime and management practices in place.

Pathogen attachment is key to intestinal colonisation

Bacterial adherence to host tissue is an important initial step enabling gastrointestinal tract colonization and infection. Adherence typically involves the interaction of complementary molecules on the surface of a bacteria with those of the host epithelium. Historically, the first adherence specificity recognized in intestinal bacteria involved binding via mannose-selective receptors (Firon *et al*, 1983). Almost all isolates of E. coli, as well as other members of the Enterobacteriaceae, such as *Enterobacter*, *Klebsiella*, *Shigella*, and *Salmonella*, attach to mannose receptors by means of type 1 fimbriae (Becker, 2008).

Attachment of type 1 fimbriae to D-mannose receptors can be blocked by means of mannose-containing receptor analogues. From a nutritional standpoint, there are many feed supplements focused on pathogen adhesion and GI tract exclusion, with the most commonly used being yeast cell wall mannan oligosaccharides (MOS). These are complex mannose containing preparations that are linked to a protein group.

The use of MOS to protect and enhance gastrointestinal health stemmed from research which focussed on the ability of mannose, the pure single unit of the complex sugar in MOS, to control and prevent the risk of Salmonella colonisation in the intestinal tract. Subsequently, distinct forms of mannose-type sugars were found to interact differently with type-1-fimbriae and it was noted that the □-1,3 and □-1,6 branched mannans present in the cell wall of *Saccharomyces cerevisiae* were particularly effective. Based on the *in vitro* findings, applied research trials determined that the inhibition and reduction of Salmonella colonisation resulted in improved in vivo performance (Oyofo et al., 1989a, b, c).

Within the cell wall of *Saccharomyces cerevisiae* there are two main locations where MOS is found; attached to cell wall proteins as part of –O and –N glycosyl groups or as components of larger □-D-mannose polysaccharides (Kath et al., 1999). These larger mannose containing polysaccharides consist of □-(1,2)- and □-(1,3)- D-mannose branches, which are attached to extended □-(1,6)-D-mannose chains (Vinogradov et al., 1998). Yeast MOS, given their capability to bind type 1 fimbriae on the surface of bacterial membranes act as a front-line defence mechanism through their pathogen adhesion capabilities (Figure 1).

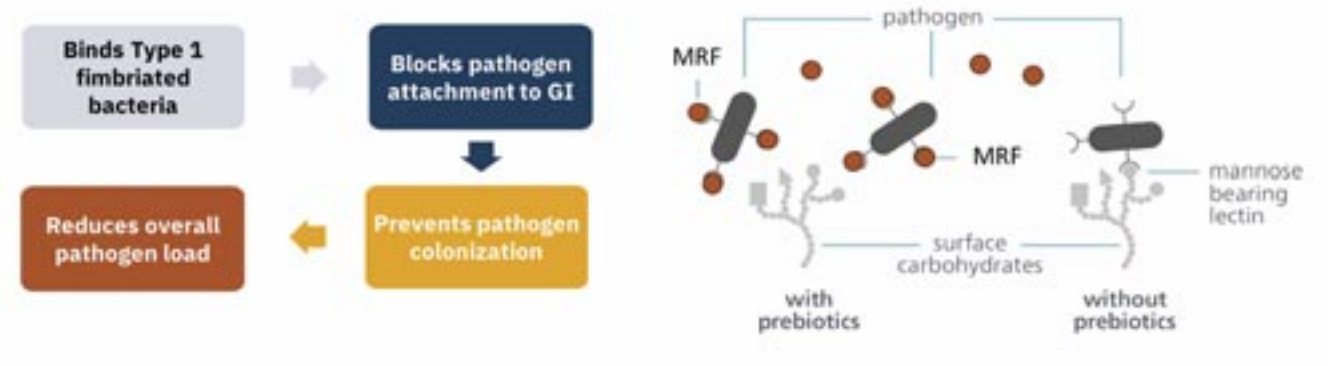

Figure 1 *– Yeast mannan oligosaccharides adsorb pathogenic bacteria*

Mannan prebiotics

MOS are widely used in animal nutrition given their well-documented ability to bind and limit the colonisation of gut pathogens. They have proven to be an effective solution for antibiotic-free diets, as well as providing support for immunity and digestion leading to notable improvements in performance and wellbeing.

As first-generation variants, most commercially available MOS products are derived from the cell wall of the yeast, *Saccharomyces cerevisiae*. Further research into yeast mannans focussed on fractionation of the yeast cell wall and the isolation of a mannose-rich fraction (MRF). This 'second generation' product can best be described as an enhanced MOS-type product and has been demonstrated to have capabilities beyond simple bacterial adherence and agglutination, some of which are summarised in Table 1.. In particular, MRF has been documented to have enhanced microbiome modulating capabilities.

A comparison of first- and second-generation MOS and MRF products are highlighted in table 1.

Table 1 *Comparison of MOS and MRF capabilities*

	MOS	MRF
Developed through Nutrigenomic studies	X	√
Branched mannan structure	√ (Low)	√ (High)
Enhances diversity of microbiome	X	√√
Agglutinates E. coli and Salmonella	√	√√
Broad spectrum Salmonella control	√	v√√
Reduces Campylobacter	X	√√
Increases weight gain	?	√
Improves FCR	√	√√
Decreases mortality	√	√√
Modulates immune response	√	√
Protects the gastrointestinal tract • Enhances protective mucin barrier • Improves gut structure Improves villus height to crypt depth ratio	√	√√
Increases goblet cell size	X	√
Protects against leaky gut (Improves barrier function)	X	√
Enhances digestive enzyme production	√	√√
Enhances energy production	√	√√
Reduces foot pad lesions	X	√

The variable nature of Salmonella serotype prevalence in turkeys

Salmonellosis as a disease, requires an efficient control system, including dietary measures, and its control is critical to produce safe food for human consumers. The variable nature of Salmonella serotype prevalence in turkeys is well documented and in the highly regulated environment of food production, information on the occurrence of individual Salmonella serotypes is readily available. Examples of sources for serotype occurrence include the USDA's Food Safety and Inspection Service (FSIS) and the UK's Department for Environment, Food and Rural Affairs (Defra). Figure 2, in the form of a heat map, presents accumulated data with respect to Salmonella isolated from turkey samples in both jurisdictions, with green representing low prevalence and red indicating high prevalence.

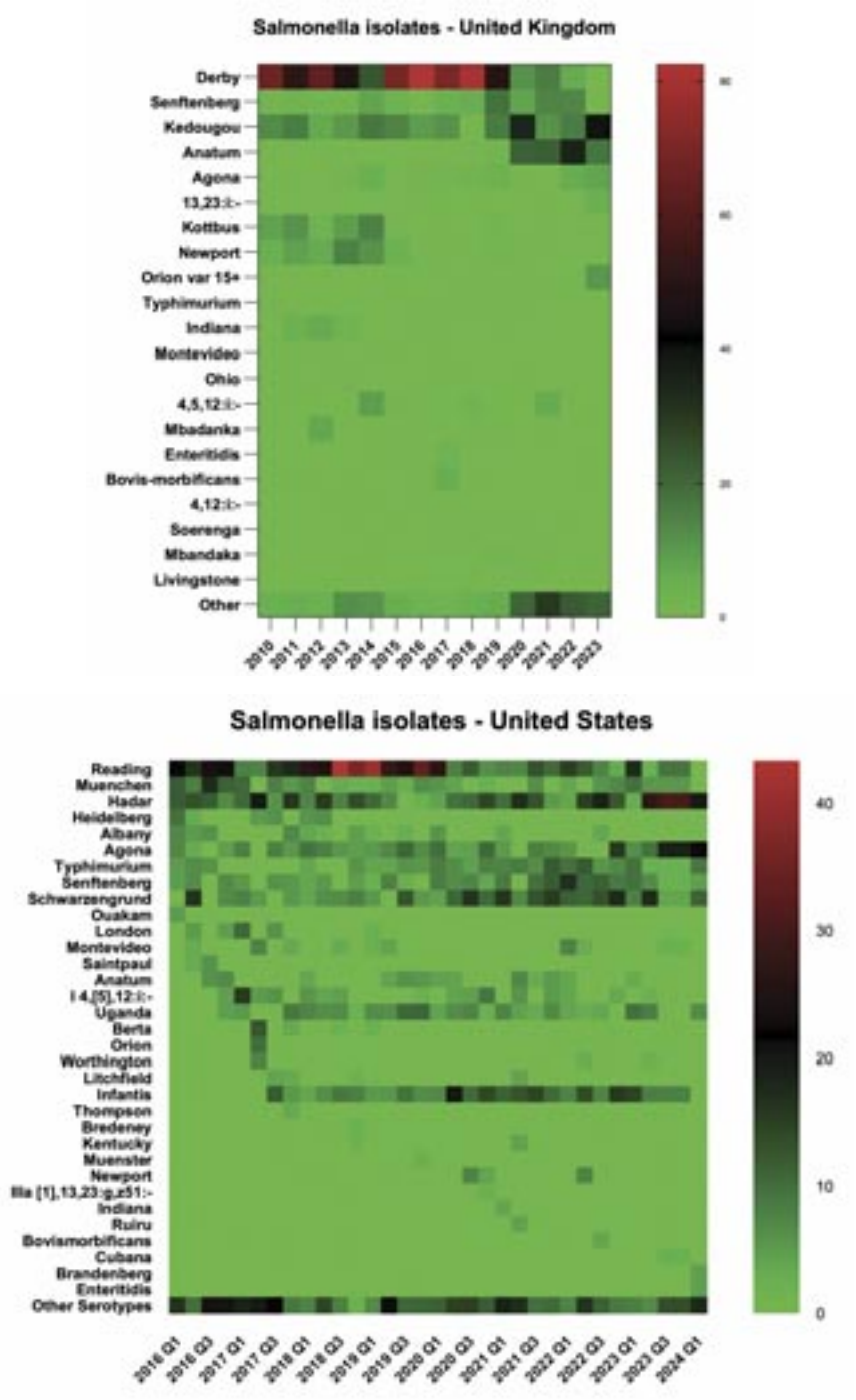

Figure 2. *Salmonella prevalence in turkey samples over time* (DEFRA 2010-2023; FSIS 2016-2024)

These heat maps highlight some interesting features associated with Salmonella occurrence in the preceding number of years. Firstly, one can appreciate the highly variable nature of serotype recovery in turkey samples from 2010-2023. Of more interest however are the quite striking temporal changes in Salmonella serotype prevalence. In the United States, whilst *Salmonella* Reading was the predominant serotype isolated in 2016, by 2024 *Salmonella* Hadar and *Salmonella* Agona had become the dominant serovars, with additional changes in dominance noted in the intervening time period. The situation in the UK shows similar shifts in overall serovar dominance, with *Salmonella* Derby being the most prevalent serotype isolated between 2010 and 2019, following which *Salmonella* Kedougou and *Salmonella* Anatum were detected with greater frequency. From a pathogen control viewpoint this presents a challenge for turkey and indeed other poultry producers in that any Salmonella control mechanism needs to be 'broad spectrum' to account for not only the variable nature but also the temporal changes in Salmonella abundance.

Whilst Salmonella proliferation in the GI tract is facilitated by attachment to the intestinal villi, the use of mannose-containing receptor analogues such as MRF can significantly reduce attachment by interacting with FimH of type-1 fimbriae thereby reducing pathogen colonization and proliferation (Fernandez et al., 2002; Hooge et al., 2003; Baurhoo et al., 2009b

Broad spectrum Salmonella agglutination with MRF

Commercially focussed *in vitro* agglutination studies can provide valuable insights into the relative control capabilities associated with individual gut health products. An example of this is highlighted in Figure 3 which illustrates findings from a long-term Salmonella monitoring program. The data highlights the broad-spectrum agglutination capabilities of MRF towards Salmonella isolates from poultry at serogroup level (panel a), in addition to a smaller sub-group associated with food borne illness that were isolated from an integrated Turkey production facility (panel b).

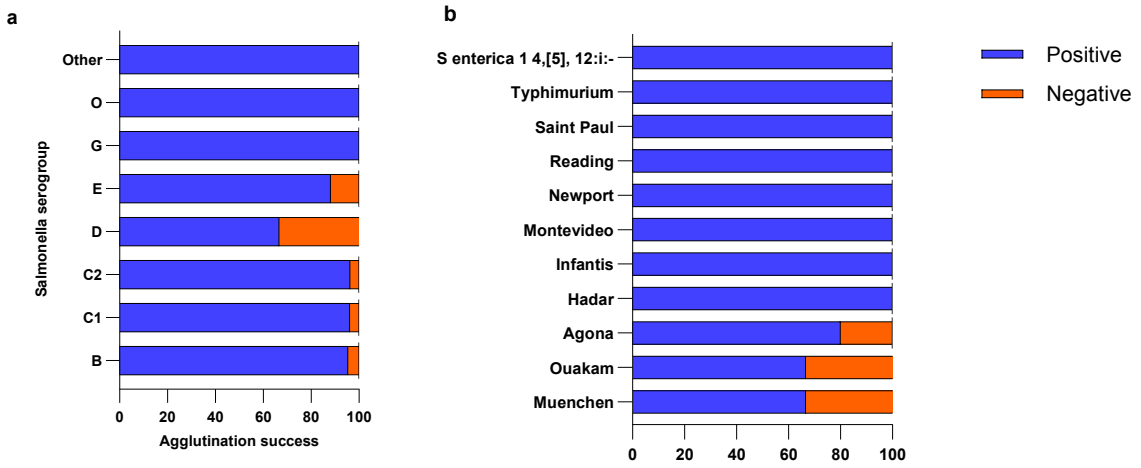

Figure 3. *In vitro Salmonella agglutination by MRF. (a) Serogroup level, (b) Salmonella isolates from Turkeys. Agglutination was assessed by the method of Spring et al, 2000*

Overall, at serogroup level the agglutination success rate with MRF was close to 90% while the agglutination of turkey specific isolates was closer to 95%. In essence, the use of MRF represents an exceptional front line or first line of defence with respect to Salmonella control and its importance in doing so cannot be understated.

Conclusion

Adherence and agglutination studies have demonstrated the ability of MRF to adhere to a wide range or broad spectrum of Salmonella isolates. In controlled studies with poultry, a reduction in the prevalence and abundance of Salmonella spp. as well as other pathogens such as E. *coli* and Campylobacter have been reported with the use of MRF (Girgis et al 2020; Spring et al, 2000; Spring et al 2015). As such, MRF represents an exceptional control mechanism for pathogens with food safety implications and provides a practical solution as a pre-harvest intervention strategy.

Reducing the prevalence and load of Salmonella in raw poultry products ultimately leads to safer food products and better public health outcomes. Given the increasing restrictions on the use of antibiotic gut microflora modifiers in animals, yeast MRF represents a technology that has become a critical part of the arsenal for veterinarians and animal producers.

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Sustaining the superfood industry: consumer values, brand positioning, and regional development of the turkey sector

Naum Babaev
Damate, Russia

We suggest a new, comprehensive approach to brand positioning strategies of turkey products on meat markets. In this presentation, we will review the potential of turkey meat as a new superfood and speculate on the future development of the turkey industry.

First of all, we address the global meat market agenda, outlining the current trends, challenges, and opportunities that turkey meat manufacturers are facing, as well as the future prospects for the industry in light of these dynamics.

The central section delves into the tools and practices that were used in order to enhance consumer experience and facilitate business-related procedures. These efforts have led to a significant shift in turkey meat consumption in Russia over the past decade. We'll show the strategy we used to establish Russia's premier turkey brand, which could be used in other markets through different marketing tools to highlight turkey's flawless taste and nutritional qualities.

Finally, we'll take a look at modern regional growth and development opportunities for turkey manufacturers worldwide and suggest a plan for all turkey producers.

Keywords: *regional growth and development*, ***superfood***, *brand positioning and marketing communications, turkey consumption, global market agenda.*

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Strain typing of Pasteurella multocida outbreak isolates and biosecurity implications

Dr. Kenton Hazel, *Aviagen Turkeys Ltd, UK*
Dr. Gerard Leveque, *Hendrix Genetics Turkeys, France*

Fowl cholera is a commonly occurring avian disease that in its peracute form is one of the **most virulent and infectious diseases** of poultry.

- Fowl Cholera is caused by *Pasteurella multocida* which is readily identified by culture from affected birds – grows on blood agar but generally not on MacConkey agar.
  - Gram negative, non-motile, non-spore forming rods. Grows in both aerobic and anaerobic conditions.
- Fowl Cholera usually occurs in older turkeys with the first sign frequently being mortality – turkeys tend to be very severely affected. Typically presents as dead birds with blood in their mouths.
- *Pasteurella multocida* infections occur in a wide range of species including humans but most mammalian isolates will not infect poultry and fortunately avian strains will generally not infect humans. Humans tend to get infected from animal bites.

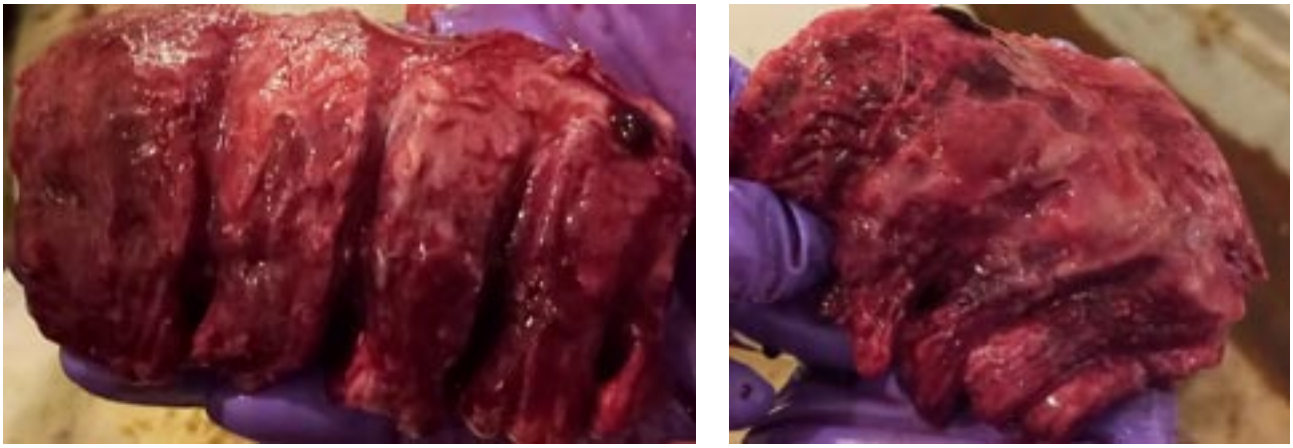


Figure 1 *Fowl cholera lungs - Acute pneumonia*

Fowl Cholera - disease situation (turkey breeder perspective)

Europe

- Historically, sporadic cases of Fowl Cholera have occurred in most countries but it is not a commonly seen disease issue in turkey breeding flocks in Europe.
- Generally, well controlled through the use of autogenous vaccines or inactivated commercial vaccines although this is becoming more unusual as there are currently very few commercial
- Fowl Cholera vaccines available in Europe.
- Some European turkey breeding companies don't vaccinate for Fowl Cholera reflecting the lack of challenge in their region.

USA

- Historically extremely problematic in turkey parent stock companies.
- Control has required the use of multiple doses of vaccine (autogenous and commercial) including live vaccines which are not available in Europe.
- Currently being better controlled across much of the turkey breeder sector due to a major focus on rodent control and intensive vaccination.

Current situation: Fowl Cholera in the last 2 years in France

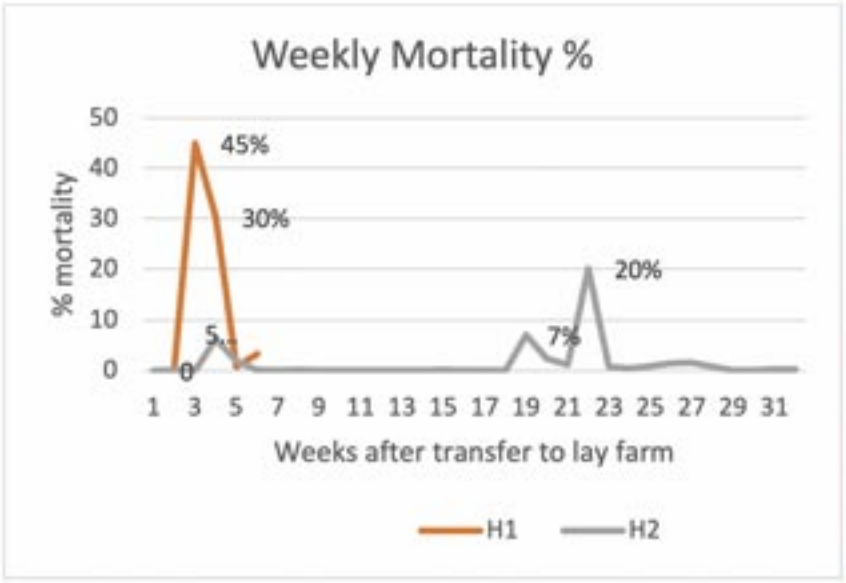
A major Fowl Cholera outbreak occurred across Brittany starting in late 2022 and continuing until late 2024.

- Turkey breeder flocks in all 4 different turkey breeder companies affected.
- 80 -100 cases in the past 2 years.
  - Only a few cases in commercial turkeys.
  - French breeder flocks historically vaccinated with autogenous vaccines or with Landavax as sporadic cases of Fowl Cholera were experienced.
- These vaccines proved to be completely ineffective at the outset of the current outbreak and even the new autogenous vaccines made with the current outbreak strains proved to be much less effective than expected.
- The vast majority of cases have occurred in laying flocks with only a very few cases in rear.

Figure 2 *Map showing flocks affected during the first 6 months of the outbreak*

Example of Fowl Cholera case in early 2023 showing the potential severity of the disease

- Breeder flock of 2600 hens transferred into House 1.
- 19 days after transfer mortality commenced.
- Flock treated with antibiotic in the drinking water.
- Flock was depleted as 75% mortality occurred within a week.
- There was also a second flock on the farm (2566 hens in House 2) reared in the same airspace as the house 1. Mortality commenced 2-3 days later but was much lower.
- Treatment with antibiotic by injection and in the drinking water. A significant second wave of mortality affected this flock at 19-22 weeks of lay.



Date	Mortality	% Mort	Cumulative mortality
06-Feb	2	0.08%	0.08%
07-Feb	1	0.04%	0.12%
08-Feb	0	0.00%	0.12%
09-Feb	0	0.00%	0.12%
10-Feb	26	1.00%	1.12%
11-Feb	139	5.35%	6.46%
12-Feb	1001	38.50%	44.96%
13-Feb	100	3.85%	48.81%
14-Feb	200	7.69%	56.50%
15-Feb	440	16.92%	73.42%
16-Feb	38	1.46%	74.88%
17-Feb	9	0.35%	75.23%

Figure 3 *Graph showing pattern of weekly mortality in Houses 1 & 2 and table showing daily mortality in House 1*

The control of all of these Fowl Cholera cases has required repeated antibiotic treatment as recrudescence of infection is a major feature of Fowl Cholera.

Current situation continued: Fowl Cholera in the last 2 years in the UK

Late in 2023 and early 2024 a number of cases of Fowl Cholera occurred in the UK in a number of companies and regions.

- Landavax vaccine and/or historical autogenous vaccines were largely ineffective.
- Identified as Serotype 3.
- Severe mortality – breeder flocks and commercials.
- Concern we were starting to see something similar to what was occurring in France.

A reliable strain typing approach was required that could assist with investigating the epidemiology of these outbreaks and help to identify which strains to use in future autogenous vaccines.

Strain typing methods used

The outbreaks have been investigated by the various veterinary teams involved in France and the UK using a variety of strain typing methods. A selection of the results of these different methods will be presented. The methods include:

- **Classical strain typing**
  - Pasteurella multocida strains are classified into serogroups (A, B, D, E and F) based on capsule antigens (Carter scheme).
  - Further classified into 16 serotypes(1–16) based primarily on somatic lipopolysaccharide antigens using the Heddleston scheme.
  - E.g. F3; A3.

- **PFGE – Pulse field gel electrophoresis**
  - 3 different enzymes were used to determine if these could help differentiate between the isolates from the current outbreak cluster.

- **MALDI-TOF mass spectrometry**
  - Matrix-assisted laser desorption/ionization – MALDI.
  - Time of Flight – TOF.
  - A phenotypic method of identifying bacteria.

- **Whole Genome Sequencing – WGS**
  - WGS provides a number of potential outputs including:
    - Predicted Serotype.
    - MLST.
    - SNP matrix.
    - Phylogenetic trees.
    - AMR genes.
    - Virulence genes.

Strain typing outcomes

- Serotyping to Heddleston LPS serotype only e.g. serotype 3 is not sufficient and even when we have both Carter capsule serogroup and LPS serotype, they can be very different.
  - E.g. UK F3 and French F3 – different MLST 179 vs 9 and differ by >9000 SNPs.

- All the strain typing methodologies (PFGE, MALDI-TOF and WGS) are able to readily distinguish between the main clusters.
  - Maldi-Tof has the advantage of being very quick and a lot cheaper, but WGS gives more outputs and comparable data.

- The aim of carrying out the WGS was to determine if we could identify “transmission clusters”. These are groups of cases where direct transmission would have occurred between the cases.
  - The SNP cut off to differentiate individual strains can be varied– the level used differs between bacterial species and the outbreak being investigated.
  - Increasing SNP cut-off may allow identification of transmission events that would otherwise be missed but will reduce specificity.

- The outcome of the WGS strain typing in the main French outbreak cluster generally shows as much variation within cases as there is between cases (generally less than 30 SNPs).
  - This suggests these are all the same strain and that there is direct transmission between these cases or in some instances potential carry over on the site at turnaround.
  - Given that these cases have occurred over a 2-year period and the bacteria would have been exposed to major selection pressure through intensive antibiotic usage and vaccination, it is remarkable how little genetic variation there is between the strains over a long time period and large geographical area.
  - Is this strain more transmissible? Is it better able to survive in the environment?

Epidemiology

- Four different companies with good biosecurity standards, competing with each other: Initial assumption that horizontal transmission from one flock to the other is not the main explanation.

- One region only, could lead to the conclusion that there is a high pressure from the environment, but no obvious increased concerns with Pasteurella in other species do not tend to confirm this hypothesis and should also exclude contamination coming from feed.

- Very few outbreaks in commercial turkeys, two years after the Pasteurella issues started in Breeders, which suggests there is no major vertical transmission risk.

- A likely hypothesis is that the easiest path of transmission for Pasteurella was the insemination crew, which is outsourced to the same company by the four hatcheries.

Why is artificial insemination outsourced?

- Historically, contract farmers were supported by their family in all aspects of running their farm. Today, this structure within the family farm is not common, and farm owners must outsource labor.

- General trend over the past years for many different types of activities, including industry and services, to outsource labor. It is even more common in agriculture.

- At the end it appeared that there was no other choice than outsourcing Insemination crew or stopping the activity.

Understanding the role of biosecurity in breeder flocks

- Biosecurity has permanently improved over the past decades by implementing the following protocols:
  - Shower in and out.
  - Heat treatment of the feed.
  - Dedicated logistics.
  - Cleaning and Disinfection procedures to eradicate salmonella.
  - Lab testing for early detection of pathogens in flocks and for assessing the risk coming from supplies or from the environment.
  - ...

- It is important to consider the impact of compliance as well as the procedures set in place.
  - Skilled laborers are difficult to find for all members of the agriculture industry, AI service providers included.
  - With labor shortages and higher turnover, there is risk of lowered biosecurity standards in order to accomplish the work needed.
  - Adequate focus must be put towards training, auditing, and supporting laborers.

Conclusion

- **Making improvements in biosecurity is a continuous effort, and all areas of the industry continue to learn from past mistakes.**

- **The first step is to invest in facilities that are well designed for biosecurity along with good procedures set in place; then the next critical point is to guarantee compliance of these procedures, by any person entering a farm, seven days a week, without exception.**

- **In the end, we should always remember that the most likely path of transmission for pathogens from one farm to the other is related to movement of animals, people or material. Limiting the movements as much as possible should always be the driver.**

- **A final point to consider is why the existing biosecurity systems prevented the spread of other pathogens such as Salmonella and Mycoplasma, but not Pasteurella. We need to have a better understanding on the pathogenicity and the resistance of this Pasteurella, which is where breeders will focus their investigation.**

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The Achilles' heel of Campylobacter in poultry?

K. Gibbs[1], A. Bello[1], L. Marchal[1], D. Reinhoudt[1]
[1]*Danisco Animal Nutrition & Health (IFF), 2342 BH Oegstgeest, The Netherlands*
kirsty.gibbs@iff.com

Introduction

Campylobacter jejuni is a major source of bacterial human gastroenteritis and poultry, including turkeys, are the primary reservoir. Handling or ingestion of undercooked, contaminated poultry meat poses a significant risk for *Campylobacter* infection in humans [1]. Poultry meat is commonly contaminated during processing via contact with infected intestinal contents. In a report published by the Food Standards Agency in 2023, the prevalence of *Campylobacter* spp. in retail turkey and broiler meat in the UK was 5% vs. 47.5%. In both meat types *Campylobacter jejuni* was the predominant species [2]. Although *Campylobacter* prevalence in turkey meat is lower compared to broilers, its presence remains a source of public health concern and of antimicrobial resistance.

Unlike in humans, *Campylobacter* is a commensal inhabitant of the distal poultry gastrointestinal tract, and bacterial numbers can increase rapidly following colonization. Certain minerals present in poultry diets, including turkey feeds, can promote *Campylobacter* colonization. As an example, iron (Fe) is sequestered by *Campylobacter* for growth and survival [3], whereas phosphorus (P) is critical for colonization processes [4]. Both minerals are typically supplemented in the diet with safety margins to ensure bird nutritional requirements are met.

Improving the availability of Fe and P from feed raw materials and reducing their direct supplementation could lower the likelihood of excess minerals reaching the distal gut thereby hampering *Campylobacter* colonization. Phosphorus availability can be increased by the targeted use of supplemental phytase, which is routinely added to broiler and turkey diets for this purpose. Phytase is also effective in increasing P availability in turkey diets [5]. Further, recent research has indicated that certain phytases are effective in increasing the availability of Fe and other trace minerals from feed raw materials, which may allow their supplementation to be reduced without a loss of performance [6]. This study tests the hypothesis that removal of added iron and inorganic phosphate from the diet, in combination with a phased dosing supplementation of phytase designed to increase nutrient and energy availability in the feed raw materials, would reduce *Campylobacter* loads in the cecum of broilers following challenge.

Materials and methods

The experiment was conducted as a completely randomized design with 2 dietary treatments, 10 replicates per treatment and 25 birds per pen. Ross 308 males (n = 500) were assigned to pens containing clean wood shavings in an environmentally controlled facility that conformed to breed recommendations. Diets were formulated in 3 phases (1 to 14, 15 to 28 and 29 to 42 d of age) and fed ad libitum from 1 to 42 d of age. Treatments comprised: 1) Control diet containing added Fe and inorganic phosphate (dicalcium phosphate), formulated to provide adequate nutrients for broilers with a nutrient and energy matrix applied for a commercial phytase (PhyG, Danisco Animal Nutrition & Health, IFF, Oegstgeest, the Netherlands) added at 1,000 phytase units (FTU)/kg during all phases; 2) Added Fe-free diet formulated without inorganic phosphate or added Fe and with a higher matrix applied for PhyG which was added at 3,000, 2,000 and 2,000 FTU/kg during starter, grower and finisher phase, respectively. Diet compositions are given in Table 1.

All birds were challenged orally with *C. jejuni* JB strain (0.1 ml of ~1.0 ´ 106 CFU/ml) at 7 d of age. Individual body weight (BW), per pen mortality-corrected BW gain, feed intake, mortality- and BW-corrected feed conversion ratio (FCRc) and liveability were determined per phase. Caecal digesta from 10 birds per pen was extracted and enumerated for *Campylobacter* using culture-dependent methods. Data were analysed by one-way ANOVA (performance measures) or Wilcoxon rank sum Added Fe-free (*Campylobacter* loads) in JMP version 16.0.

Table 1. *Ingredient and calculated nutrient composition of the treatment diets, % as fed, unless otherwise stated.*

	Starter (1 to 14 d of age)		Grower (15 to 28 d of age)		Finisher (29 to 42 d of age)	
	Control	Added Fe-free	Control	Added Fe-free	Control	Added Fe-free
Ingredients						
Corn	33.84	38.90	39.89	44.97	44.19	48.09
Wheat	10.00	10.00	10.00	10.00	10.00	10.00
Soybean meal	34.59	34.58	30.49	29.15	26.73	26.24
Rapeseed meal	5.00	5.00	5.00	5.00	5.00	5.00
Sunflower meal	2.26	2.18	1.83	2.01	2.56	2.56
Maize gluten meal	5.66	2.94	4.35	2.52	3.40	1.58
Soy oil	4.87	3.59	5.10	3.80	5.26	4.31
Limestone	0.27	1.46	0.52	1.24	0.64	1.01
Dicalcium phosphate	1.91	-	1.26	-	0.77	-
L-lysine HCL	0.34	0.29	0.29	0.27	0.27	0.24
DL-methionine	0.31	0.29	0.28	0.26	0.26	0.25
L-threonine	0.13	0.11	0.11	0.09	0.09	0.08
Mineral premix - with Fe[1]	0.17	-	0.12	-	0.12	-
Mineral premix - without Fe[2]	-	0.17	-	0.12	-	0.12
Vitamin premix[3]	0.05	0.05	0.05	0.05	0.05	0.05
Coccidiostat (amprolium)	0.01	0.01	0.05	0.05	0.05	0.05
Sodium bicarbonate	0.44	0.25	0.42	0.24	0.41	0.23
Sodium chloride	0.18	0.19	0.20	0.19	0.20	0.20
Phytase (PhyG), FTU/kg	1,000	3,000	1,000	2,000	1,000	2,000
Calculated nutrients						
Metabolizable energy, kcal/kg	2,945	2,896	3,020	2,977	3,070	3,036
Crude Fat	7.93	6.75	8.21	7.03	8.48	7.61
Starch	28.84	31.50	32.33	35.15	34.61	36.71
Calcium	0.80	0.71	0.70	0.61	0.60	0.52
Available phosphorus	0.35	0.14	0.27	0.13	0.21	0.12
Total phosphorus	0.81	0.42	0.65	0.40	0.55	0.39
Crude protein	25.07	23.71	22.75	21.44	20.98	19.91
Digestible Lys	1.32	1.27	1.18	1.13	1.08	1.04
Digestible Met+Cys	1.00	0.94	0.92	0.86	0.86	0.81
Digestible Thr	0.88	0.83	0.79	0.74	0.72	0.68
Digestible Trp	0.23	0.23	0.21	0.20	0.19	0.19
Digestible Arg	1.40	1.37	1.27	1.22	1.17	1.14
Digestible Val	1.00	0.95	0.91	0.86	0.84	0.80
Phytate phosphorus	0.31	0.31	0.30	0.30	0.29	0.29
Analyzed constituents						
Iron, mg/kg	208	134	163	106	146	80
Phytase, FTU/kg	979	3,219	1,243	2,049	907	2053

[1]Supplied per kilogram of diet (0.17% in starter diets at 0.12% in grower and finisher diets): 0.1% of the premix equates to Manganese (Mn), 120 mg; Zinc (Zn), 120 mg; Iron (Fe), 20 mg; Copper (Cu), 16 mg; Selenium (Se), 0.3 mg; and Iodine (I), 1.25 mg.

[2]*Supplied per kilogram of diet (0.17% in starter diets at 0.12% in grower and finisher diets): 0.1% of the premix equates to Manganese (Mn), 120 mg; Zinc (Zn), 120 mg; Iron (Fe), 0 mg; Copper (Cu), 16 mg; Selenium (Se), 0.3 mg; and Iodine (I), 1.25 mg.*

[3]*Vitamin premix per kilogram of finished feed (0.05%): Vitamin A, 3750 IU; Vitamin D3, 2000 IU; Vitamin E, 16 IU; vitamin B12 (cobalamin), 10 µg; Biotin, 0.08 mg; Menadione, 1.25 mg; Thiamine, 1.0 mg; Riboflavin, 3.5 mg; d-Pantothenic Acid, 6.0 mg; Vitamin B6, 1.5 mg; Niacin, 27.5 mg; Folic Acid, 0.5 mg.*

[4]*Calculated based on CVB feed table 2021.*

Results and discussion

Performance was not impacted by diet.

Descriptive statistics and statistical comparisons of the effect of treatment on cecal *Campylobacter* colonization are presented in Table 2. There was a numerical reduction (P=0.12) in average *Campylobacter* load in Added Fe-free vs Control treatments (4.90 x 107 CFU/g vs. 3.78 x 108 CFU/g). Additionally, the range in recorded loads of *Campylobacter* was wide in both treatments but, interestingly, the upper end of the range was 1 Log10 units lower in Added Fe-free vs. Control (2.97 x 109 vs. 2.45 x 1010, respectively) which is suggestive of a reduced upper limit of colonization and reduction in average *Campylobacter* levels.

It is widely believed that due to the complexity of the relationship of *Campylobacter* with its avian host, effective *Campylobacter* control strategies require a farm-to-fork approach. In the European Union (EU), surveillance programs are in place at processing plants to monitor and limit *Campylobacter* levels on broiler meat. EFSA, based on an extensive 2008/2011 report, defines *high* contamination loads, and increased public health risk, on broiler neck skins reporting > 1,000 CFU/gram [7]. Processors are obligated to monitor contamination levels and implement corrective measures in case the high limits are exceeded. The same EFSA publication also indicates that strategies curbing *Campylobacter* numbers during live bird production are expected to have bigger benefits for public health than actions taken later along the line [7].

Independent studies carried out in both England and Ireland, which evaluated the correlation between *Campylobacter* caecal levels and neck skin levels at processing reveal interesting results [8,9]. Both studies suggest that achieving levels <1,000 CFU/g neck skin requires a maximum level of ~1 x 108 CFU/g in the caeca coming into the processing plant. On average, Inorganic-Fe free birds demonstrated lower levels and achieved below the proposed ~1 x 108 CFU/g caeca.

Table 2. *Effect of treatment on caecal loads and categorized caecal loads of Campylobacter jejuni at 42 d of age.*

	Control	Added Fe-free
Cecal Campylobacter colonization, CFU/g		
Mean*	3.78×10^8	4.90×10^7
SD of all measurements (10 birds per pen)	2.26	2.29
SE of all measurements (10 birds per pen)	0.226	0.229
SD of pen average (10 pens per treatment)	0.72	0.61
SE of pen average (10 pens per treatment)	0.228	0.193
Average CV per pen (individual bird variation), %	45.0	50
Average CV of pens (between pen variation), %	14.1	13.1
Median	2.26×10^5	1.88×10^5
Range	0.00 to 2.45×10^{10}	0.00 to 2.97×10^9
Rate (no. of positive birds in 100)	88	84
>1×10^8 CFU/g	6	2

*Wilcoxon rank sum added Fe-free on pen averages: P value = 0.123

Conclusions

This study has provided suggestive evidence that small modifications to the diet composition to reduce nutritional virulence factors, in combination with phytase supplementation, can reduce C. jejuni colonization in the cecum of broilers. Based on the similar anatomy and function of the digestive tract in turkeys and broilers, high adaptation of Campylobacter to both poultry species and phytase efficacy in turkeys it would be valuable to evaluate if similar interventions in turkey feed could be the Achilles' heel of Campylobacter in turkey production.

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Critical control points in turkey egg incubation to achieve the best quality poults

Simona Gheorghitoiu **–** *Petersime, Belgium*
simona.gheorghitoiu@petersime.com

We, the "turkey "people, have always been more challenged in this hatching turkey's industry. Whether it is about producing the best quality eggs for the hatchery to incubate and hatch or being about how we make the most of each egg, or not the least challenge of giving the poults the proper house conditions in the barns and grow them with outmost efficiency , in order to optimize the overall costs , it is all a continuous and restless instigation for those who are driven by the inner vocation.

As a turkey incubation specialist -turkey hatchery manager for the past 11 years and turkey incubation consultant for Petersime for the past 5 years, I can definitely say the following: in order to achieve the best day-old poults, all the critical control points during the incubation and hatch of the turkey eggs must be met. Missing out on even one of these critical control points will "tax" the overall hatch results or affect the quality of the day-old poults. There hasn't been one single time that I – obviously accidentally – missed one the of the control points, and my results turned out great. One might get away with it when incubating chicken eggs, but "faults" in the incubation of turkey eggs will pay a toll in the outcome of the result.

In most of the actions we do in turkey incubation, we are guided by instincts, folklore, knowledge inherited from previous experienced passionate people that worked in the industry, but with less advanced technologies that the turkey hatcheries had decades ago.

I have never taken any batch of turkey eggs for granted, as a routine, I always considered the unexpected, I made a strict procedure of incubating the turkey eggs and came up with certain critical control points during the endothermic, exothermic and hatch. Luckily, every time the expected good hatch results failed, I was able to deliver to the egg supplier- first of all- and to the top management team of the company, the real cause of the failure. Since I started working for Petersime as their turkey incubation expert, I applied these principles of incubation, and they paid off with good results.

If I count 11 years of turkey incubation in STS Trading multiplied by an average of 1.8 million turkey eggs/ year, I could easily say that these critical control points watched over almost 20 million eggs.

With this edition of TSPC I will start sharing with you these critical control points that I use from egg receival until dispatch of the day-old poults. But not all will be revealed today, I want to make sure I have your ears here the years to come.

The main aspect that must be taken into consideration when incubating turkey eggs – why they are more difficult to comfort – is the fact that they come from very early stages of pre incubational cellular envelopment. This "translates "in the end, in hatch, in even 12 hours of hatch difference for some of the embryos, rooting back to a delayed start of the embryo development of even 12 hours.

Having this into constant consideration, I try to help the survival of as many embryos as possible by:

- At set of the eggs on setter trolleys: I always divide the total number of eggs to be set in a machine to the total number of trays of that setter. For the best possible air flow inside the cabinet of the setter, I leave no empty trays or trolleys, but each tray has a similar number of eggs on it.

- No batch of eggs goes into incubation process without having control trays, two control trays on front trolleys for each flock. These will be measured for weight loss during the entire incubation period, every 3-4 days, starting day 12-13 until transfer. No eggs will be rejected from these control trays until transfer.

- As a loading pattern: bigger eggs are set on the trolleys that will be placed near the pulsator, it always helps with achieving the weight loss. For hybrids that "breath" with more difficulty, that is a "must"

- Whenever time allows it– days left between egg receival until set- I apply ReStore treatment at 100.0 F eggshell temperature, for a minimum of 3 hours and maximum 5 hours. I consider the ReStore treatment complete once the Ovoscan reads below 80.0 F. This is meant to shift the embryos from the early weaker stages of pre incubational cellular development towards stronger stages that are more likely to start a better development and finish "the race" into a stronger poult.

- The preheating will never be shorter than 10-11 hours. The prewarming comforting temperatures need to reach the germinal disks, which are in the center of the eggs. Should one measure the temperature of the germinal disks after 3-4 hours of preheating, one will see that it is very close to the air temperature. So why not start the incubation program after a preheat of 3-4 hours? Because I believe it takes longer to properly awake from hibernation all the cells that make up the germinal disk, down to the last viable one that could add up to the initial reaction. The more cells take part in that first reaction, the stronger the embryo will be. And this strength will extend throughout its entire embryonic life, making a positive impact in each stage of development. Only 3-4 hours of preheating will most likely balance the air parameters inside the setter but will not do more.

Once I press the start button of the setter, the machine will follow during the endothermic phase, Petersime's default turkey incubation program, regardless of the hybrid, age category, egg age. Below, the detailed parameters:

Air temperature: in a setter filled with embryos with such a wide pre incubational development stage, it is most beneficial for the chance of a good start of development of as many embryos as possible, to create a very uniform environment. How the setter keeps the air temperature set point depends on the generation of machine, if technically it has all that is needed, and the setter is not faulty. The new generations of setters, from OX onwards, keep very steady air parameters inside the cabinet of the machine: air temperature is mostly on set point , due to their cabinet tightness, very efficient cooling with the help of the three way valve, much more efficient pulsator blades that mix the air very well, thus providing to all of the eggs very similar environmental conditions, regardless of the eggs' location inside the setter.

Older generations setters, like the BioStreamers, work with some air temperature deviations from set point-being less tight, not having the three-way valve, having a less performant pulsator. It is normal and reasonable to see deviations of +/- max 0.3 F. If the air temperature deviations are greater than+/- 0.3 F, then you need to start investigating the technical status of the machine, because that can be a cause of discomfort for the embryos.

Eggshell temperature: for the duration of the endothermic phase, I follow Petersime's default Ovoscan program. That has always given me good results. For all hybrids and flock ages, until day 12 I use the same eggshell temperature set points. The critical aspect that I constantly watch over is that Ovoscan readings stay on set point. Again, new generation machines provide a very uniform environment and keep the set point very steady, they rarely deviate from set point. Older generation machines normally deviate by +/- 0.3 F. The embryos will be more comfortable with less deviations or no deviations.

Be aware that the 12 eggs that are being monitored and recorded for eggshell temperature may send different signals to the infra-red sensors of the Ovoscans, because certainly those embryos have different metabolic activities as they come from different pre incubation development stages. A setter is filled with tens of thousands of embryos, coming from thousands of hens that were in a different stage of their laying cycle, thus laying a huge variety of pre incubational stages. Even more, each stage of pre incubation cellular development has more phenotypes, producing an even wider variability of the embryos inside the incubator. What the sensor displays is an average. Still, it is the best tool to control the heating and cooling functions of the setter, thus better comforting the embryos. Set the Ovoscan in control!

CO_2 will naturally build inside the setter during the endothermic phase, because of the metabolic activity of the developing embryos, and it must be captured, by setting the minimum ventilation to zero, and its benefits used. How we address this aspect will impact the developing of a strong heart and blood system of the embryos. It has been proven by several studies that high levels of CO_2 during the endothermic phase, up to approximately 0.7-1.5%, can promote embryo development and shorten hatching time. This also happens in nature, when the hen sits tightly on her nest for the first part of the incubation period, as she knows instinctively that it will be beneficial for her offsprings.

For every setter that starts incubation of a batch of turkey eggs, I monitor closely the buildup of CO_2 during the first 6-7 days of incubation, when set point should be reached. That is critical. I want the embryos to develop a strong heart and blood system, to be able to cope with the rapid growth phases that genetic improvement has selected in their DNA for the past decades, to be able to express their full genetic potential.

No body parts of a newly formed embryo will thrive without a strong heart and blood system: the "pomp" will push the blood with numerous -because of those high CO_2 levels - blood cells loaded with oxygen and nutrients, through the more developed - because of those high CO levels - circulatory tree. All the developing organs and muscles will have a better growth rate and will better support, overall, the metabolic activity of the embryo and future bird.

Deciding on the CO_2 set point during the endothermic phase is related to hatchery location and eggs specific details. Again, the generation of setter will also influence how fast the CO_2 will build up. Also, lower CO_2 set points for certain hybrids or flock ages, even 2nd lay flocks, will promote a better weight loss. I usually work with set points between 0.7-1.0 %, the younger the generation of setter, the lower the CO_2 set point.

Humidity usually builds up in the setter during the endothermic phase because of keeping the cabinet of the setter sealed, due to zero ventilation. In the new generation machines, it builds up to even above 97 F. In older generation setters, it can stay to a maximum of 92-93 F. Of course, the humidity during the endothermic phase will automatically and immediately be influenced by the ventilation rates and the tightness of the setter. Because the setters' ventilation works by CO_2 set points – the lower the CO_2 set point, the more frequent small ventilation rates of 1-2% that will bring inside the setter drier air, taking out the more humid air, thus promoting a bit more moisture loss of the eggs.

Humidity inside the setter is the factor that will directly affect the moisture loss of the eggs. As a value, expressed in F or %RH, no matter how high or low, will not harm the embryos directly during the endothermic phase. But it will influence how the eggs lose moisture and how they reach the correct % of moisture loss at transfer. And that is a critical point.

When I see humidity building up to values above 94 F, whether in new or older generations of setters, I know I need to work with lower CO_2 set points. By the time the eggs have completed the endothermic phase, I want the % moisture loss to be close to the weight loss target of that embryonic age.

Turning is of outmost importance. The embryos need it: for less early dead, less malposition, to prevent them from sticking to the inner membrane of the shell, to facilitate nutrient absorption, to stimulate membrane development, to improve the development of the air cell, for a good and proper air flow. Shortly said: it is essential for a proper embryo development.

So, checking the turning mechanism of the setters prior to load is critical. Maintaining the good technical condition of all the equipment involved in turning is critical. Finally, individual checks of the trolleys inside the setters are critical. Should one trolley slip out of the turning system and no alarm mechanism available to alert the hatchery, the price is high and in direct correlation with the duration the trolley spends outside of the turning system. I experienced this malfunction, with one trolley slipped out of turning. It only hatched 50%, the rest of the embryos were fully developed, alive but could not hatch because they were stuck to the inner membrane of the shell.

The default program of Petersime turns the eggs every 60 minutes.

When it comes to turning, Petersime has solid proof that more frequent turning is more beneficial for the embryos, as it increases the vascularized area of the chorioallantois membrane, facilitating the gas exchange of the embryo, during incubation. In my hatchery, turning is set to every 30 minutes the first 5 days of incubation.

The speed of the pulsator - how many RPM the pulsator performs – is also very important during the endothermic phase, when it comes to turkey incubation.

A certain speed of the fan will ensure a good mixing of the air and uniform air parameters in each zone inside the setter. How many RPM the setter performs will directly influence the % moisture loss the eggs will have at the start of the transition phase. I want the eggs to be close to 5% moisture loss by day 12 of incubation. I know that to achieve that, I need a certain CO2 set point to force small ventilation rates and refresh the air inside the setter. But unless the RPM isn't at least 200, even lower CO2 set points will not deliver the 5% moisture loss. You might say "there is plenty of time to achieve the % weight loss, from day 5 until transfer". I generally agree, but I always keep the unpredicted as a constant when incubating turkey eggs. What about hatcheries located in humid areas? What about hybrids that, due to shell properties, exchange gas with the environment more difficult? What about old flocks or second lay flocks? The speed of the air that "brushes off" the shell during incubation will make a big difference.

From own experience, I recommend a minimum of 200 RPM of the pulsator, regardless of the generation of machine. Lower RPM will not promote moisture loss, will not bring around the eggs a proper load of oxygen for the embryos to breath. The air cell will be small, their metabolism will be slower as the air available will be reduced, moisture will not be brushed off the surface of the shell and they won't be able to breath properly.

When all the above critical points are met, the first candle of the eggs done in day 13-14, deliver good results, according to the age category of the flocks.

If the numbers are as below – when it comes to % loss at candle:

- 4-5% rejected eggs for week of lay 5- 12
- 6-8% rejected eggs for week of lay 2-3 and 13-18
- 9-10 % rejected eggs for week of lay 1 and above 19 than I can trust the eggs were of good quality.

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Supporting gut function through dietary interventions

Gemma González-Ortiz and Rob ten Doeschate
AB Vista, Spain and UK

Introduction

Turkey meat consumption is ranked as the second preferred poultry meat by consumers behind broiler chicken consumption (MAPA, 2024). Genetic selection in turkeys has given us large animals with high meat yield which are more efficient in the production process. Despite these achievements, little has been progressed with regards to their sensitive behaviour in challenging conditions (Jennison, 2021). Turkey producers know that if the temperature in the barn is low, they can simply slow down activity leading to no consumption and starvation. Another feature of turkeys is their neophobic attitude to changes in taste, colour or particle size of their diets which may give negative responses. The same happens with the drinking water which must be considered if flavoured products are administered via the water. Drugs, acids or water sanitizers may be rejected to such extent as to cause dehydration. These peculiarities highlight how fussy turkeys are in comparison to the broiler chicken.

Turkeys are also known to be very susceptible to a wide range of diseases (Jennison, 2021). Most of the health issues are related to gastrointestinal disorders because of bacteria, viruses or parasitic infections that can appear at any age (Table 1). In the past, the common practice to use antibiotics as growth promoters (AGP) in the feed kept several of these infections under control in turkeys and masked the reality.

Table 1. *Most common health issues affecting turkeys by age (adapted from Jennison, 2021)*

Early brooding period (0-14 days)	Later rearing period (2-6 weeks)	Growing period (6-20 weeks)
• Poor feed intake/starvation • Colibacillosis • Avian metapneumovirus • Rotavirus • Other enteric viruses • Aspergillosis	• Rotavirus • Colibacillosis • Coccidiosis • Haemorrhagic enteritis • Wet litter • Poult enteritis and mortality syndrome (PEMS)	• Ornithobacterium rhinotracheale • Pasteurellosis • Erysipelas • Histomoniasis (blackhead) • Mycoplasmosis • Necrotic enteritis • Intestinal parasites (worms) • Neoplasic disease (Marek's) • Leg problems • Septic arthritis • Aortic rupture and renal haemorrhage

Underlined the gastrointestinal disorders

Since AGP's are no longer used and therapeutic use of antibiotics is also being reduced there is an ongoing interest in finding alternative ways to support the gut in its role of efficient digestion and absorption as well as being an effective barrier to infection (Gaggià et al., 2010; Thacker, 2013;). Certainly, the choice of alternatives is not an easy task for poultry nutritionists. The evolution of feeding for poultry productivity, health and welfare in an era of reduced antibiotic use, even for therapeutic purposes, requires an enhanced understanding of not only how an additive or an ingredient is utilised, but also how they influence the digestive physiology. This is of great importance in turkey production as it is believed that research in broiler chickens is directly applicable for turkeys, which is often not the case (Schwean-Lardner et al., 2016; Olukosi et al., 2020). In the field, turkeys are commonly reported to suffer digestive disorders which may be directly

related to an infectious origin, but it can also be indirectly the consequence of poor nutrient digestibility, although it is also possible these observations are the consequence of each other. What is very likely is that any digestive disorder can result in wet litter which may be seen quickly by the farmer.

This chapter aims to briefly describe a few of the particularities of the turkey's digestive tract with a focus on the main digestive disorders and discuss options for dietary intervention.

Upper gastrointestinal function and support

Feed intake is the start of the digestive process. To stimulate feed consumption and the development of a functional gastrointestinal tract, care must be taken in turkey diets, especially at young ages, as they do not accept fine particles (Jankowski et al., 2016). Some authors have evaluated the benefits of including whole wheat grain in the development of the gizzard (Zdunczyk et al., 2013; Jankowski et al., 2016) and the positive effects on performance are inconsistent (Ahmed et al., 2018), which maybe due to the lack of exogenous enzyme supplementation to hydrolyse the non-starch polysaccharides (NSP) present in wheat in an efficient manner (González-Ortiz et al., 2017). Another factor to consider in the feed consumption behaviour of turkeys is their sensitivity to very highly flavoured compounds which may lead to intake refusal (Milbradt et al., 2014).

Understanding the utilisation of protein, calcium and phosphorus is extremely important in turkeys as their requirements are higher than in other domestic avian species (FEDNA, 2018). With regards to the crude protein, the recommended levels at the starter phase range from 26 to 28% and decreasing to 16-18% crude protein in the finishing phase, thus the diets are formulated with very hight contents of vegetable protein sources and synthetic amino acids. Such high crude protein levels have consequences in the gastric pH, acting as a buffer which combined with normally higher pH of turkeys' proventriculus and gizzard compared to broilers (Farner, 1942), these birds are more prone to present difficulties for a proper protein digestion and absorption. Protein digestion needs to start in the stomach where at low pH pepsin is active. Any interference in this process results in higher undigested protein reaching the hindgut and being fermented into putrefactive compounds (Gonzalez-Ortiz et al., 2022). To compensate for the poor digestion of protein, turkeys tend to drink higher volumes of water in an attempt to excrete the excess of nitrogen, thus unbalancing the electrolytic status, damaging the gut epithelial integrity and consequently increasing litter moisture. Hence, the undigested protein leads into one of the main causes of digestive disorders in turkeys.

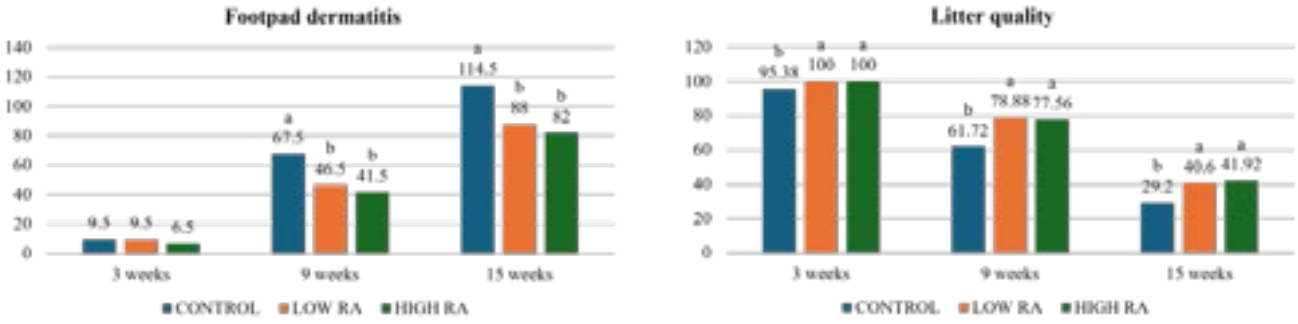
On the other hand, calcium and phosphorus requirements in turkeys are also high, however, improvements due to genetic selection, the use of phytase and improved understanding of the mineral metabolism have led to a considerable reduction of the calcium and phosphorus levels in turkey diets (Ingelmann et al., 2019; Novotny et al., 2023). The combination of bigger calcium particles to avoid quick solubility in the stomach, with an enhanced acidification strategy of the upper gastrointestinal tract with effective organic acids and the use of phytase (especially at high doses), allows for a better absorption of minerals and consequently a better digestion and absorption of proteins (FEDNA, 2018).

Small intestine function and support

The small intestine is the site where most of the digestion and nutrient absorption occurs. The success of the absorption processes is implicit for a healthy gut. Bischoff (2011) defined five major criteria that could form the foundation of an overarching definition of gut health, being: (1) effective digestion and absorption of food; (2) absence of gastrointestinal illness; (3) normal and stable intestinal microbiome; (4) effective immune status; and (5) status of well-being. As represented in Table 1, turkeys are very susceptible to a few enteric infectious diseases disturbing the epithelial cell line integrity, especially in the small intestine, jeopardising the nutrient absorption process. The intestinal epithelium is composed of a layer of cells, which are attached to the collagen-rich basal membrane. Inflammation induces the expression of specific matrix metalloproteinase (MMP) enzymes which degrade collagen and other

structural molecules of the extracellular matrix. This process damages the epithelial cell integrity and leads to the "leaky gut syndrome" which is a common issue of enteric disorders affecting turkeys. Research has demonstrated that diet-derived resin acids inhibit the expression and collagenolytic activity of inflammation-associated MMPs and promotes a reduction in the number of inflammatory T-lymphocytes in the small intestinal tissue (Aguirre et al., 2019). By these mechanisms, resin acids may reduce the impact of inflammation to the epithelial integrity supporting the restoration of the tissue. Moreover, resin acids have also demonstrated to interact directly with the intestinal microbiota in an *in vitro* model (Kettunen et al., 2014). In fact, the same authors reported a lower frequency of *Clostridium perfringens* isolated from ileal digesta samples when supplemented with a resin acid. The benefits of resin acid supplementation in turkey diets were also observed by Lipiński et al. (2020) on performance, but also on the incidence of foot pad lesions and litter quality which suggest an enhanced nutrient utilisation with lower moisture in the manure (Figure 1). It seems plausible that the benefits associated with resin acids can be extrapolated to other enteric disorders in the support of the epithelial cell integrity especially in a sensitive species like turkeys.

Figure 1. *Footpad dermatitis and litter quality of female turkeys supplemented with a resin acid (RA) product at low (orange bars) and high (green bars) concentrations against a control group (blue bars) for 105 days. Data is from 10 replicates per treatment and 20 birds per replicate. Different superscripts indicate P < 0.05 (adapted from Lipiński et al., 2020)*



Hindgut function and support

All nutrients not absorbed in the small intestine enter the caeca to be hydrolysed by the microbial populations. It is generally accepted that the caeca are the primary site of fermentation, and only small and/or soluble particles will enter the caeca (Vanderghinste et al., 2024). Therefore, the degree of caecal fill will be determined by characteristics of the diet and resulting digesta. Although the caeca do not make a huge contribution to the energy requirements in poultry, the caeca are considered to play an important role in bird health but may also be the predominant reservoir for *Salmonella* spp. and other zoonotic organisms (Classen et al., 2017). The nature of fermentation in the caeca and elsewhere can affect the bacterial profile and activity in a manner which could reduce pathogen colonisation. Considering the nutrient composition of a regular feed and the normal ileal digestibility coefficients for nutrients, it can be estimated that the main nutrients entering the caeca are undigested protein and dietary fibre. If high undigested protein concentrations arrive in the caeca, it results in detrimental changes due to its fermentation by putrefactive organisms. Bacterial metabolism of amino acids yields several fermentation products (e.g. ammonia, amines, phenolic and indolic compounds, H_2S, dimethyltrisulphide), these being associated with toxins, inflammation and reduced barrier function. On the other hand, dietary fibre fermentation results in the colonisation of beneficial species with the potential to increase the concentration of volatile fatty acids in the lumen, especially butyric acid which contributes to lower pH, inhibits the growth of *Salmonella* spp. and other *Enterobacteriaceae*, but also moderates the gene expression of virulence factors and promotes the mucosal immunity. Therefore, the benefits of establishing a fibre degrading environment are not questionable.

Stimbiotics were first introduced in the scientific literature in 2019 and are defined as a new category of functional additives which promote the development of a fibrolytic environment in the gut through a dual mode of action relating to the fibre dissolution capability of a xylanase and the direct effect of a small dose of xylo-oligosaccharide (XOS) (González-Ortiz et al., 2019). Today, there are more than 50 trials in poultry which have supported the efficacy of the stimbiotic product under different production and husbandry circumstances. The benefits associated with greater dietary fibre utilisation result in improved performance but also in other physiological effects. Stimbiotic supplementation has been shown to increase fermentation activity in the gut and thus lower pH of the intestinal lumen, reducing the chance of pathogens to proliferate (Davies et al., 2023). In fact, once infection is established in experimentally-challenged animals, stimbiotic supplementation has been shown to modulate the immune system and act as an effective anti-inflammatory, diminishing the release of proinflammatory cytokines such as TNF-α or IL-6 and increasing release of immunoglobulins, which in turn limit damage to the epithelium (Cho et al., 2020; Song et al., 2022; Lee et al., 2022; Rousseau et al., 2023). The ability of a stimbiotic to promote carbohydrate fermentation in preference to protein fermentation (Cho et al., 2020; Davies et al., 2024) results in increased concentrations of butyric acid which aids in maintenance of intestinal cell health and thus the functionality of the gut (Lee et al., 2022; Song et al., 2022). However, in the absence of disease, XOS supplementation has also been shown to improve growth efficiency by reducing gut epithelial cell turnover and consequently lowering the energy requirements for maintenance of the epithelial cells in broiler chickens (Castro et al., 2024). Stimbiotic supplementation is associated with higher broiler chickens' survivability (Gonzalez-Ortiz and Bedford, 2024), which has been also observed in an experimental turkey study (Table 2) and highlights the importance of the microbiota composition and the metabolites produced by the fibrolytic bacteria determining the productivity and health status of birds.

Table 2. *Response of turkey's survivability to stimbiotic supplementation**

	Period days				
	0-28	28-42	42-56	56-84	0-84
Control	98.3%	99.4%	100%	100%	97.8%
Stimbiotic	99.4%	100%	100%	100%	99.4%
Δ Survivability	+1.1	+0.6			+1.6

*Study performed in 360 females for 12 weeks (until 84 days of age) fed corn-SBM diets in crumble and pellet form for 4 feeding phases. Birds were distributed into a a control and a stimbiotic treatments (9 pens/treatment, 20 birds/pen) (internal data source)

Final considerations

A huge number of experimental and field trials have been carried out in poultry, albeit more in broilers than in turkeys, using a variety of alternatives to antibiotics. Whilst performance parameters such as growth or feed conversion are mostly used to evaluate effectiveness of feed additives this may be too simplistic and sometimes more advanced parameters (e.g. gut health indicators) are worthwhile to consider. The variety of feed additives used nowadays can behave as antibiotic alternatives or gut health stabilizers. Some may interact directly with microbiota stimulating the most favourable groups while inhibiting the harmful species, like stimbiotics. However, other additives may have the potential to interact indirectly with microbiota as revealed by improvements on the intestinal epithelial integrity, stimulation of the tolerance responses towards non-harmful bacteria, avoid excess of inflammation, stimulation of the host antibacterial responses (mucin and antimicrobial peptide production) and bring the host in a steady state of mutualism with its microbiota, like resin acids.

Identification of a consistent means of affecting the gastrointestinal health and function in a positive manner, to yield desirable end products capable of improving nutrient metabolism, barrier function and pathogen prevalence, would have significant implications for production and food safety for the poultry industry.

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ALERT: Introduction and Spread of Avian Metapneumovirus in the United States

Steven Clark, *DVM*

AMPV Alert: Navigating the Rapid Spread of Avian Metapneumovirus in North American Poultry Farms, USA

The introduction of avian metapneumovirus (aMPV) into the United States was suspected in late 2023, with subtype A detected in California and subtype B in North Carolina. By January 2024, the rapid dissemination of aMPV was confirmed in both states, and within four months, it had spread to most poultry-producing regions nationwide. Individual states and trade associations* continue to determine the exact number of poultry affected and the related economic impacts. By mid-summer of 2024, infections extended across all commercial poultry-producing states and into Canada.

In response to the rapid spread of avian metapneumovirus (aMPV), a collaborative effort swiftly emerged across all industry segments, including commercial poultry production, public and private research institutions, trade and allied associations, and government agencies. This prompt response culminated in the first confirmed diagnosis of aMPV Type B in the United States within one week of the initial call of the aMPV Working Group in December 2023.

By March 2024, this *ad hoc* aMPV Working Group expanded to over 200 participants, comprised of poultry veterinarians, researchers, regulatory officials, and animal health professionals, predominantly from the United States. These stakeholders represented a diverse array of sectors, including commercial production, trade associations, government bodies, and allied industries.

Collaboration among various laboratories and colleagues ensured that multiple facilities were equipped with the essential tools to effectively understand and combat the disease on a national scale. Within one month of the diagnosis, the first virus isolation of subgroup B was successfully achieved, followed by the isolation of subtype A. By April 2024, more than twenty laboratories were offering aMPV-related services, a significant increase from just four in December 2023, which had limited capabilities.

Throughout 2024, numerous meetings and several publications were conducted to enhance awareness of this emerging disease, underscoring the collective commitment of the industry to address the challenges posed by aMPV effectively. Within twelve months, United States Department of Agriculture (USDA) recognized the industry needs and approved the first-ever importation of a live vaccine, specifically a modified live avian metapneumovirus vaccine from Europe.

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The link between gut health and bone health in turkeys

Prof. D. R. Korver [1,2]
[1]*Alpine Poultry Nutrition, Edmonton, AB, Canada*
[2]*University of Alberta, Edmonton, AB, Canada*
dkorver@alpinepn.com

1. Introduction

Modern commercial turkeys have been highly selected over many generations for rapid, efficient growth and for a high proportion of breast meat yield. These characteristics place additional strain on the skeletal system due to the heavy body weights and associated physical stresses placed on the skeletal system. Turkey geneticists have done an excellent job of selecting for skeletal heath and reducing the incidence of leg deformities and breaks caused by slow bone mineralization. However, bacterial lesions in load-bearing joints can be a problem in turkeys (Clark and Froebel, 2020). In many cases, the bacteria present in articular cartilage arise from an ineffective intestinal barrier and bacterial translocation from the lumen of the gut into the bloodstream (Wideman and Prisby, 2012).

2. The gastrointestinal tract

Most countries around the world have either eliminated or are in the process of eliminating the use of in-feed, growth-promoting antibiotics in poultry diets. Antibiotic growth promotors (AGP) were important tools in maintaining gut health in turkeys, and a lack of approved efficacious drugs remains an important issue for the turkey industry (Clark and Froebel, 2020). The removal of AGP therefore requires the industry to have access to effective alternatives. A number of different alternatives have been tested with varying degrees of success. It is likely that multiple products with differing modes of action are required to reduce pathogen growth, maintain gut barrier function and reduce bacterially-induced bone problems in turkeys.

The digestive tract is the largest surface area of the body exposed to the outside environment, and in addition to its role in digestion and absorption, its barrier function is critical for bird health. The lumen of the digestive tract is outside of the body of the turkey, and intestinal microbes must be prevented from crossing the intestinal barrier and entering the blood and other tissues of the body. The maintenance of the intestinal barrier is nutritionally and energetically expensive, and intestinal microbial challenges lead to an increase in the cost of maintenance through increasing intestinal wall thickness, and also an increased cost of protective secretions and systemic inflammation. The reduction in growth rate due to a strong systemic inflammatory response in chicks is due mostly (~71%) due to reduced feed intake (Klasing, 2017). The remainder in performance loss is due to reduced efficiency of nutrient transport, increased energy expenditure in the form of a febrile response, mobilization of body nutrients to support immune mediators and protective molecules such as acute phase proteins, and the increased need to support immune defenses (Klasing, 2017). Although a similar study has not been performed in turkeys, it is likely the factors involved in reduced turkey performance due to inflammation are similar and proportional.

The intestinal barrier includes tight junction proteins, which prevent bacterial translocation across the intestine wall (Mehandru and Colombel, 2021). Various stressors including intestinal pathogens (Rafieian-Naeini et al., 2025; Sun et al., 2020), heat stress (Baxter et al., 2020) and high viscosity diets (Tellez et al., 2015; Tellez Jr et al., 2020) can reduce tight junction integrity, resulting in increased bacterial translocation, local and systemic inflammation, and bacterial movement to peripheral tissues.

3. The skeleton of turkeys

Genetic selection for increased growth rates, final body weights and breast muscle yield has put additional stress on the skeleton of the turkey, changing bird posture (Abourachid, 1993) and gait (Stover et al., 2018). Genetic selection for skeletal soundness has reduced incidence of skeletal abnormalities associated with poor mineralization and deformities.

However, the heavier body weights of modern turkeys increase shear stress on growth plates of immature birds, and result in microfractures in the cartilage. These microfractures can become a site of bacterial infections leading to degeneration of cartilage and bone tissue.

Independent of direct bone infection, systemic inflammation reduces bone mineralization and breaking strength. Inflammation induced through the injection of bacterial lipopolysaccharide reduced chick body weight, bone weight and fracture resistance in broiler chicks (Mireles et al., 2005). The use of a direct-fed microbial product to turkeys protected body weight gain and bone strength in turkeys fed a diet high in rye, intended to induce gut and systemic inflammation through increased intestinal permeability (Tellez et al., 2015; Tellez Jr et al., 2020).

4. Gut health and bone problems in turkeys

As mentioned previously, various stressors including pathogenic organisms and heat stress can reduce the effectiveness of the tight junction proteins, and cause "leaky gut" in turkeys (Ognik et al., 2020; Rafieian-Naeini et al., 2025). Once in the bloodstream, the bacteria can travel throughout the body. The clefts and microfractures in the cartilage of joints are an ideal location for the bacteria to colonize, and the relatively poor blood supply to these tissues make the bacteria somewhat hidden from the body's immune system (Wideman and Prisby, 2012). Once established, the bacteria can cause necrosis in the femoral and tibial heads (Blomvall et al., 2022), and at the free thoracic vertebra, a condition called bacterial chondronecrosis with osteomyelitis, or BCO (Alrubaye et al., 2020). Because the disease is often apparent in the femurs, this disease was commonly referred to as "femoral head necrosis" in the past. Over time, these lesions can lead to the separation of the cartilage cap from the underlying bone tissue, and in severe cases, destruction of the bone tissue. Birds with BCO show discomfort in walking, and as the disease increases in severity, animals can become unable to walk and reach food and water.

BCO is most commonly caused by staphylococci, but the lesions are not specific to these bacteria, and numerous other bacterial species have been isolated from BCO lesions; cultures from affected joints often show complex microbial communities (Wideman, 2016). The emergence of Enterococcus species as pathogens in turkeys can also result in joint lesions and lameness (Souillard et al., 2022).

5. Preventative measures

With the shift away from AGP in the poultry industry, effective alternative solutions must be identified, particularly given the current lack of effective drugs to treat turkey diseases (Clark and Froebel, 2020). Given that one of the primary growth promoting mechanisms of antibiotics is a reduction of inflammation, both directly and indirectly (Niewold, 2007), it is logical that effective AGP replacements will also involve the reduction of inflammation. Various categories of AGP alternative products are available. It is beyond the scope of this paper to give an exhaustive review of the various categories of products and their mechanisms of action, the reader is referred to several review articles (Gadde et al., 2017; Yadav and Jha, 2019). Categories include probiotics, prebiotics, synbiotics (probiotics + prebiotics), organic acids, essential oils, feed acidifiers, enzymes, phytogenics, bacteriophages and others. Through differing mechanisms, these products are intended to suppress the growth of pathogenic bacteria, or increase the growth of beneficial bacteria, this reducing the need for an active, systemic inflammatory response.

Another means of reducing bacterial translocation and therefore the effect of systemic inflammation on skeletal health, is the use of organic trace minerals. Copper, zinc and manganese are critical for cartilage formation and repair (Sirri et al., 2016). The use of organic trace minerals in turkey diets increased bone mineralization (Ghasemi et al., 2023) and reduced leg abnormalities (Ferket et al., 2009). Interestingly, organic trace minerals increased jejunum villus height and surface area in turkeys (Ghasemi et al., 2023), and nearly significantly increased occludin gene expression in broilers (Sun et al., 2020). Therefore, organic trace minerals may positively influence skeletal health directly at the level of the bone, and indirectly, at the level of the intestinal barrier.

Regardless of the mechanism, a reduction of inflammation caused by pathogens in the gut, and translocation of bacterial across the intestinal barrier will have a positive effect on skeletal health of turkeys. Many different AGP alternatives have shown effectiveness in laboratory and commercial farm settings, but in general, no single alternative product is as effective

as antibiotics. Therefore, it is likely that successful AGP-free production under commercial conditions will likely involve the use of multiple AGP replacement products. Adding to the challenge is the fact that different farm locations will be facing different microbial challenges, and the most effective combination of products will likely vary from farm to farm, and perhaps even over time on the same farm. Nevertheless, numerous examples of producers having successfully transitioned away from AGP use exist.

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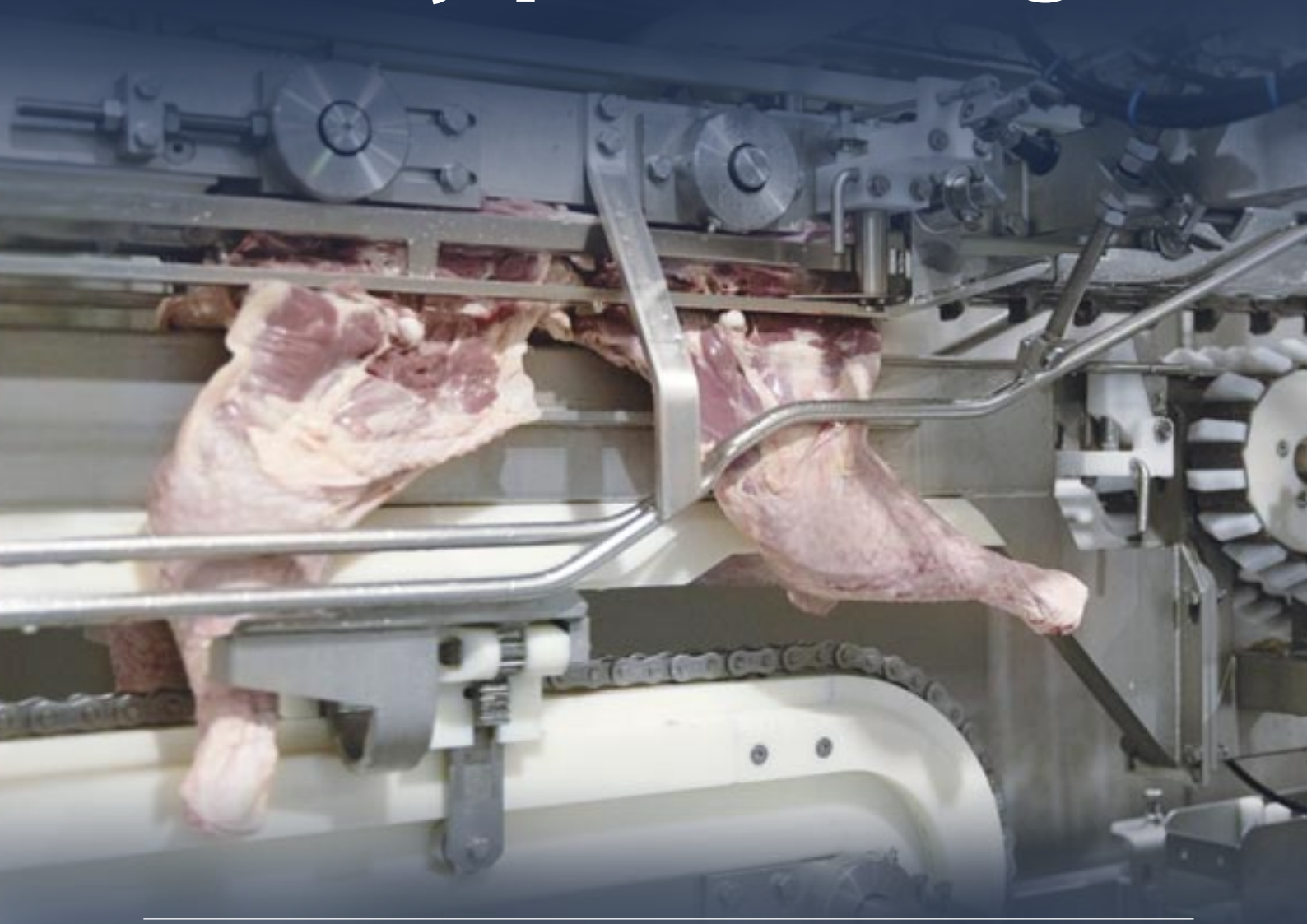

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Poultry House Air Filtration 101

Michael Czarick,
UGA Poultry Housing, USA

1) An individual virus is a approximately fraction of a micron (0.1 microns) in size and as a result would be extremely difficult if not impossible to keep out of a poultry house through filtration. The good news is that is that viruses tend to "travel" on "larger" particles (bioaerosols, generally one micron and larger) which are possible of being filtered out of the air...if you want to spend enough money.

2) Visible dust particles are 25 microns or larger. In general, less than 10% of the dust in the air is visible.

3) The hole size in a standard window screen is 1,000 microns.

4) Air filters are classified by their MERV rating (Minimum Efficiency Rating Value). The higher the MERV rating (1-20) the greater the level of filtration and the more resistive they are air flow. The U.S. swine industry considers MERV 14 filters adequate in low density production areas MERV 14 filters. In high density production areas MERV 15 or 16 filters are generally recommended. To eliminate virus from the incoming air requires a MERV 17 filter (HEPA).

MERV Rating	Will trap air particles size 0.3 to 1.0µm	Will trap air particles size 1.0 to 3.0µm	Will trap air particles size 3.0 to 10.0µm	Filter Type / common particles removed
MERV 1	<20%	<20%	<20%	Fiberglass & aluminum mesh / Pollen, Dust Mites, Spray Paint
MERV 2	<20%	<20%	<20%	
MERV 3	<20%	<20%	<20%	
MERV 4	<20%	<20%	20-34%	
MERV 5	<20%	<20%	35-49%	Cheap disposable filters / Mold spores, cooking dusts, hair spray, furniture polish.
MERV 6	<20%	<20%	50-69%	
MERV 7	<20%	<20%	70-85%	
MERV 8	<20%	<20%	>85%	
MERV 9	<20%	<50%	>85%	Better home box filters / Lead dust, flour, auto fumes, welding fumes.
MERV 10	<20%	50-64%	>85%	
MERV 11	<20%	65-79%	>90%	
MERV 12	<20%	80-90%	>90%	
MERV 13	<75%	>90%	>90%	Superior commercial filters / Bacteria, smoke, sneezes.
MERV 14	75-84%	>90%	>90%	
MERV 15	85-94%	>95%	>90%	
MERV 16	>95%	>95%	>90%	
MERV 17	99.97%	>99%	>99%	HEPA & ULPA / Viruses, carbon dust.
MERV 18	100.00%	>99%	>99%	
MERV 19	100.00%	>99%	>99%	
MERV 20	100.00%	>99%	>99%	

5) Prefilters, MERV 8, are typically used to increase the life of MERV14+ filters which increases the static pressure that fans are working against. Prefilters will typically need to be replaced multiple times a year, whereas secondary filters can generally last multiple years.

6) Filters become more effective over time, but also more resistive to air flow.

7) To remain effective air filters need to be kept as dry as possible.

8) In order to effectively eliminate viruses from enter a house ideally a positive-pressure ventilation system would be utilizedb. In a positive-pressure ventilation system all the air entering the house can be filtered. In a negative pressure ventilation a portion of the incoming air enter through cracks in the ceiling, side wall, end walls, fan shutters, etc will be unfiltered. In a positive-pressure system air flows out through any cracks in the house's envelope thus reducing the likelihood of a virus from entering the house.

8) In a filtered postive-pressure ventilation system (FAPP) air is often brought into a house by a one or two powerful, variable speed fans equipped with high efficiency filters. Duct systems are typically employed to distribute the fresh air which can lead to issues with house air quality and temperature uniformity.

9) FAPP ventilation systems are extremely expensive (roughly $2 per cmh) and historically only been used in research facilities and specific pathogen free (SPF) egg production for vaccines.

10) In the typical negative pressure ventilated poultry house during minimum ventilation over 50% of the fresh air is entering through cracks and not through air inlets which means if the inlets are equipped with filters over 50% of the air will not be filtered.

11) At a pressure difference of 25 PA air will enter through an air inlet at an air velocity of 6.25 m/sec. At the same pressure difference air will enter a house through a MERV14 filter air at a face velocity of only 1.5 m/sec. As a result, in a negative pressure system roughly four times the amount of air will enter through each square meter of leakage area that each square meter of air inlet area (equipped with a filter).

12) If filters are going to be added to a negative pressure ventilation house tightness is paramount. Houses should have a relative tightness rating of no more than 0.4 square meters of leakage per square meter of floor area (Poultry411-House Tightness Calculator) to minimize the amount of unfiltered outside air which will enter a house.

13) Poultry houses typically operate between a negative pressure of between 25 and 50 PA. The typical operating pressure for a HEPA filtration system is 250 PA or better.

14) The typical poultry house exhaust fan has a maximum operating pressure of approximately 80 PA. The amount of air moved by the typical poultry house fan will decrease by 50% or more as the pressure increases from 25 to 80 PA. As a result, filtration system should be designed so that the total pressure the exhaust fans are working against does not to exceed 50 - 60 PA at the maximum design air exchange rate.

15) It has been generally recommended when using typical poultry house axial fans that at least one square meter (face area) of MERV 14-15 filter, with MERV 8 prefilter, is provided for every 3,500 cmh of air inlet capacity to keep the to static pressure drop across the filter from exceeding 40 PA. This will vary significantly with filter type and design.

16) Negative pressure exhaust fans in houses with filtration should have an air flow ratio of 0.80 or greater. A fans air flow ratio is determined by dividing its air moving capacity at 50 PA by its air moving capacity at 13 PA. The higher the air flow ratio the less a fan's air moving capacity will decrease at as the pressure it is working against increases.

17) The typical modern poultry house exhaust fan has an energy efficiency rating of 20 cfm/watt at 25 PA of pressure. The typical fan in a HEPA filtration system has an energy efficiency rating of approximately 5 cfm/watt at 250 PA.

18) The approximate cost in the U.S. of a MERV14 filter is approximately $90-$130 per 1,000 cmh. The approximate cost of a MERV16 filter is $130-$200 per 1,000 cmh.

19) The typical poultry house requires a minimum hot weather ventilation system capacity of approximately 5 m3/hr per kg of maximum expected total bird weight. A filtration system should be sized to have a capacity of at least 50% of this value (2.5 m3/hr per kg) to handle ventilation needs in winter, early spring, and late fall.

20) If a person would like to filter the incoming air year round, a house's exhaust fan capacity will likely need to be increased 20 to 30 percent to compensate for the loss of air moving capacity caused by higher operating pressures.

21) In the U.S. swine industry, the installation of MERV 14-16 filtration systems has been shown to increase the cost of a house's ventilation system by more than five-fold (not including additional costs for construction/installation).

22) Filtration systems MUST be designed by a qualified engineer in order to ensure system effectiveness as well as to ensure that proper environmental conditions will be able to be maintained for the birds throughout the year.

23) Filtration does not guarantee that you will not have a disease break. Chances are a virus will "walk/crawl" its way into a house.

24) BID50 (Bird Infectious Dose - 50%) for high path H5N1is 3 to 4 logs lower with intranasal inoculation than through eating or drinking water.

25) Very difficult to isolate viruses past 100 meters from an infected house.

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Supplementary xylanase improves metabolisable energy of rape seed meals obtained by cold pressed hexane extraction when fed to turkey poults

V. Pirgozliev[1], E.S. Watts[1], S.C. Mansbridge[1], I.M. Whiting[1], S.P. Rose[1], M.R. Bedford[2]

[1]*National Institute of Poultry Husbandry, Harper Adams University, Newport, Shropshire, UK*
[2]*AB Vista Feed Ingredients, Marlborough, Wiltshire SN8 4AN, UK*

Correspondence: vpirgozliev@harper-adams.ac.uk

Introduction

Conventional, pre-press solvent extracted rapeseed meal (RSM), is the second worldwide feed protein ingredient and commonly used in poultry diets (Mielke 2018). Even though RSM is relatively high in dietary fibre, particularly non-starch polysaccharides, it is still a valuable source of protein for poultry (Watts et al., 2021). However, the process of oil recovery influences its nutritional value, e.g. reducing the exposure of the RSM to preliminary thermal treatments prior to solvent extraction may increase content of nitrogen corrected apparent metabolizable energy (AMEn) in RSM for broiler chickens (Olukosi et al., 2017; Watts et al., 2020). Olukosi et al., (2017) reported 1.3 MJ/kg greater AMEn in RSM produced via cold pressed hexane-extracted meals (CpHe) compared to the traditional pre-press solvent extraction method (TM). Watts et al. (2020) also reported that RSM produced via supercritical carbon dioxide extraction, a method involving less heat, had greater AMEn even compared to RSM produced via CpHe. However, the existing data on AMEn of RSM obtained via alternative methods is produced with broilers but not with turkeys. The aim of the study was to determine the AMEn of three RSM produced via CpHe method, when fed to turkey poults.

Animals and Experimental design

All RSM samples were produced under a CpHe method (Watts et al., 2020). In brief, the oil extraction of the seed from each of 3 cultivars was performed under standardized conditions at a pilot scale extraction plant (OLEAD, Pessac, France). Initially, seeds were dried in a batch dryer to a moisture content of approximately 70g/kg. The circulated air temperatures ranged from 60-70°C. Mechanical crushing was performed using an MBU 75 cold press (La Mechanique Moderne, Arras, France) at a rate of 250kg/h. Solvent extraction with hexane was performed in a six-stage continuous belt-diffuser (B-1930, Desmet-Balllestra, Zaventem, Belgium). Operational conditions were as follows: band velocity set at index 12 and solvent flow at 250 L/h. Hexane temperature was maintained at approximately 50°C. Residual hexane was flashed from the solvent laden marc in a six-tray desolventizing-toasting unit (Schumacher type, Desmet-Balllestra, Zaventem, Belgium) and was completed by injecting the meals with live steam. Residence time in the desolventising/toasting unit was 80 minutes with internal temperatures ranging from 104-108°C.

Energy and nutrient availability of the meals were examined in turkey poults experiment from 55 to 63 d age. Each of the three RSM samples were incorporated into a nutritionally complete basal feed (BF) in meal form at 200 g/kg (800 g of the BF + 200 g of each RSM sample) (Table 1). The nutrient specification of the BF met the breeder's recommendation (Aviagen Ltd.). The diets, including the BF, were then split in two parts and one of the parts was supplemented with 16 000 BXU/ kg xylanase (Econase XT 25P; AB Vista, UK) resulting in eight experimental diets in total. Diets contained 5 g/kg TiO2 as indigestible marker.

Ninety-six BUT Premium female turkeys (Faccenda Foods Ltd., Dalton, UK) were used in the study. All birds were reared in a single floor pen until the start of the study. Three weeks before the start of the study all birds were fed the BF. At 55 d age two birds were randomly allocated to one of 48 cages with 0.36 m2 floor area and each diet was fed to 6 cages

following randomisation. Each cage was equipped with a trough feeder and nipple drinker. Access to the feed and the water was ad libitum. The experimental house was equipped with a negative pressure ventilation system to meet commercial recommendations. A standard temperature and lighting programmes for turkeys were used (Aviagen, Turkeys Ltd.). At 60 d of age, after 5 d given to adjust to the diets, the droppings were collected for 4 d until the end of the study at 63 d age. Feed intake for the same period was recorded. Data were analysed by ANOVA following 2 x 3 factorial design.

Table 1. *Composition of experimental basal feed.*

Ingredients	Experimental diet (%)
Wheat	52.51
Prairie meal	2.50
Rye	2.0
Rape seed meal (RSM)	5.00
Soya ext hipro	29.50
L-Lysine HCl	0.35
DL-methionine	0.35
L-threonine	0.09
Soya oil	3.00
Limestone flour tru.270	1.00
Dicalcium phosphate flour	3.00
Salt	0.30
Turkey premix	0.40
Calculated provisions %	
Oil	4.56
CP	24.12
ME	12.16
Lysine av	1.39
M+C	1.08
Ca	1.28
P av	0.68
Determined values	
DM (g/kg)	885
Oil (g/kg)	358
CP (g/kg)	215

[1]Vitamin/mineral premix supplied per kilogram of diet: vitamin A, 16,000 IU; vitamin D3, 3000 IU; vitamin E, 25 IU; vitamin B1, 3 mg; vitamin B2, 10 mg; vitamin B6, 3 mg; vitamin B12, 15 mg; nicotinic acid, 60 mg; pantothenic acid, 14.7 mg; folic acid, 1.5 mg; biotin, 125 mg; choline chloride, 25 mg; Fe as iron sulfate, 20 mg; Cu as copper sulfate, 10 mg; Mn as manganese oxide, 100 mg; Co as cobalt oxide, 1.0 mg; Zn as zinc oxide, 82.222 mg; I as potassium iodide, 1 mg; Se as sodium selenite, 0.2 mg; and Mo as molybdenum oxide, 0.5 mg

Results and discussion

During the study period, the average daily feed intake was 166 g/kg bird and the body weight at 63 d age was 2.05 kg/bird, but no differences between treatments were detected (P > 0.05; data not in tables). As expected, turkeys fed on BF had a slight, primarily numerical, advantage on feed efficiency (Pirgozliev et al. 2022). Supplementary XYL tended to increase feed efficiency (P = 0.07; data not in tables) in agreement with previous research with turkeys (Zdunczyk et al., 2013; Kozlowski et al., 2018). The control diet had higher AMEn (P < 0.001) compared to the rest of the RSM diluted diets, lower NR (P < 0.001) and no differences (P > 0.05) in fat retention (FR) were detected (Table 2). This may be explained by the substitution of BF with RSM, which is high in fibre and N, i.e. reduced available energy, but increased N retention (Pirgozliev et al. 2022). There was no difference in NR and AMEn between the studied RSM samples (P > 0.05). The AMEn values of RSM were within the range reported by others (Olukosi et al., 2017; Watts et al., 2020) using the same cold pressed hexane extraction method when fed to chickens. Watts et al. (2020) also found no significant effect of RSM cultivars on AMEn or pre-caecal protein digestibility coefficients. Feeding xylanase improved (P = 0.022) AMEn by 2.64 MJ/kg and tended (P < 0.1) to improve nutrient retention coefficients, which agrees with previous research (Kozlowski et al., 2018). In a recent chicken broiler study, Olukosi et al. (2017) found that RSM produced with cold pressed hexane extraction has AMEn that is approximately 1.3 MJ greater than the AMEn of commercially produced RSM. We have not done a direct comparison but compared with the value of Premier Nutrition (2008) of commercially produced RSM for broilers, which is 6.99 MJ/kg DM, the AMEn of our RSM in our study is still about 0.7 MJ higher. In research with 4-week-old turkeys, Lessire et al. (2011) reported AMEn in RSM to be as low as 5.99 MJ/kg DM, which is 1.7 MJ lower than our value, thus further supporting the view that oil recovery methods that minimise the exposure of RSM to thermal treatments can increase the nutritional value of RSM for turkeys.

Table 2. *Nutrient retention coefficients and AMEn in diets and rapeseed meal.*

Variable	AMEn diet (MJ/kg) DM	NR	FR	AMEn RSM* (MJ/kg) DM
Diet				
Control	12.91[b]	0.635[a]	0.871	-
Caberne	12.15[a]	0.731[b]	0.889	9.13
Elgar	12.11[a]	0.714[b]	0.885	8.84
Quartz	12.09[a]	0.733[b]	0.896	8.79
SEM	0.149	0.0154	0.0065	0.644
Enzyme				
No	12.11	0.689	0.879	7.70
Yes	12.52	0.718	0.891	10.14
SEM	0.105	0.0109	0.0046	0.526
CV %	3.8	6.9	2.3	22.8
P values				
Diet	0.001	<0.001	0.073	0.923
Enzyme	0.010	0.067	0.076	0.004
Diet X Enzyme	0.725	0.893	0.061	0.774

*Data obtained via substitution method.

Conclusion

The reported results showed that there is no significant difference in AMEn obtained from different rapeseed cultivar samples and by adding a suitable NSP degrading enzyme there is scope to increase the nutritional value of RSM for turkeys and increase its utilisation in modern turkey production. Experiments involving comparisons of RSM obtained via different extraction techniques may provide further information on the development of feeding quality of RSM for turkeys. Experiments involving comparisons of more samples and obtained from various extraction plants may provide a wider and robust data set.

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The Impact of Bacterial Chondronecrosis with Osteomyelitis Lameness in Poultry and the Prophylactic role of Active Form of Vitamin D3 (1.25-Dihydroxycholecalciferol)

***Dr. Jan Dirk Van Der Klis*[1], *Kathrin Bühler*[1], *Fernando Lima*[1], *and Adnan Alrubaye*[2]**

[1] *Herbonis Animal Health, Innovad Group, Rheinstrasse 30, 4302 Augst, The Netherlands*

[2] *Department of Poultry Science, Center of Excellence for Poultry Science, University of Arkansas, USA*

Introduction

Lameness is one of the main issues facing the poultry industry worldwide. Knowles *et al* (2008) assessed the walking ability of 51,000 birds, representing 4.8 million birds in the UK within 176 flocks. They found that over 27% of birds showed poor locomotion, where 3.3% were almost unable to walk. A recent systematic study on causes of lameness in birds in Northern Ireland showed that Bacterial Chondronecrosis with Osteomyelitis (BCO), also known as Femur Head Necrosis, was the most common one, being present in 17.3% of lame birds (McNamee et al., 2000). The same authors indicated that in the UK, BCO was identified in 0.75% of all broiler placements. Their estimate was similar to others.

Lameness due to BCO in broilers and turkeys involves high bird growth rates, stress, immunosuppression and chronic respiratory and/or intestinal infections resulting in bacteraemia, where *Staphylococcus spp* is one of the main causative agents of bone infections (Szafraniec *et al*, 2022). These fast-growing poultry species are very susceptible to mechanical micro fracturing of the growth plate, followed by colonization by opportunistic bacteria via the bloodstream (Wideman, 2016). As BCO in young birds can only be detected by necropsy, such bone infection might spread within a flock before being noticed and lead to a fast deterioration of bird welfare. A field study in turkeys showed that Turkey Osteomyelitis Complex (TOC), which has a similar etiology as BCO, did not appear until week 9 of age but thereafter steadily increased up to the end of the study on week 15 (Huff *et al.*, 2000). This pattern resembles the development of BCO lameness in a broiler infection study, in which cumulative lameness started at day 38 and accumulated to 80% of the birds at to the end of the trial on day 56 (Asnayanti *et al*, 2024a), whereas others indicated BCO peak incidence around day 35.

Recently, it was observed that plasma from BCO-affected birds decreased cell viability of primary chondrocytes *in vitro* (Ramser *et al*, 2021), where elevated levels of TNFα and IL-1β due to BCO both can decrease chondrocyte proliferation and hypertrophy, which are both essential for bone growth and growth plate homeostasis. Moreover, these researchers demonstrated a relationship with increased Fibroblast Growth Factors (FGFs) and decreased FGF receptor expression in bones of BCO affected birds. Fibroblast Growth Factors also affect phosphate homeostasis and vitamin D metabolism (Quarles, 2012).

Two experiments were done at the University of Arkansas based on their aerosol transmission model in broilers to study the effect of *Solanum glaucophyllum*, a plant-based form of 1.25-dihydroxycholecalciferol as glycosides in broilers (Asnayanti *et al*, 2024a,b).

Experimental Set-up

The aerosol transmission model used is described in detail by Asnayanti *et al* (2024b). In brief, seeder birds were placed in two wire-floored pens, located upwind of the treatment groups to allow pathogen dissemination to the litter pens through forced-air circulation. Each pen had a separate water line and two feed bins. An empty buffer zone was created between the seeder birds and treatment groups to prevent direct contact. The dietary treatments in Exp. 1 were an unsupplemented basal diet (NC: negative control), or NC plus 1.0 µg/kg $1.25(OH)_2D_3$ as glycosides (other treatments were presented by Asnayanti *et al*, 2024b), and in Exp. 2 were an unsupplemented basal diet (NC: negative control), or NC plus 0.5 µg/kg (Trt. 2), 1.0 µg/kg (Trt. 3) or 2.0 µg/kg of $1.25(OH)_2D_3$ as glycosides (Trt. 4) fed during 56 days, or Diet 1 fed for 28 days, followed by Diet 3 for the remaining trial period (late supply of $1.25(OH)_2D_3$) (Trt. 5) or vice versa (early supply of $1.25(OH)_2D_3$) (Trt. 6). Clinically lame birds were assessed from d 22 to d 56 based on symptoms of goblet gait, kinky back, wing tip dipping to support the body, and complete paralysis, followed by necropsy and gross evaluation of tibial and femoral lesions based on the classification by Wideman (2016). Body weights of healthy birds were determined at the end of the trial to prevent stress during the trial.

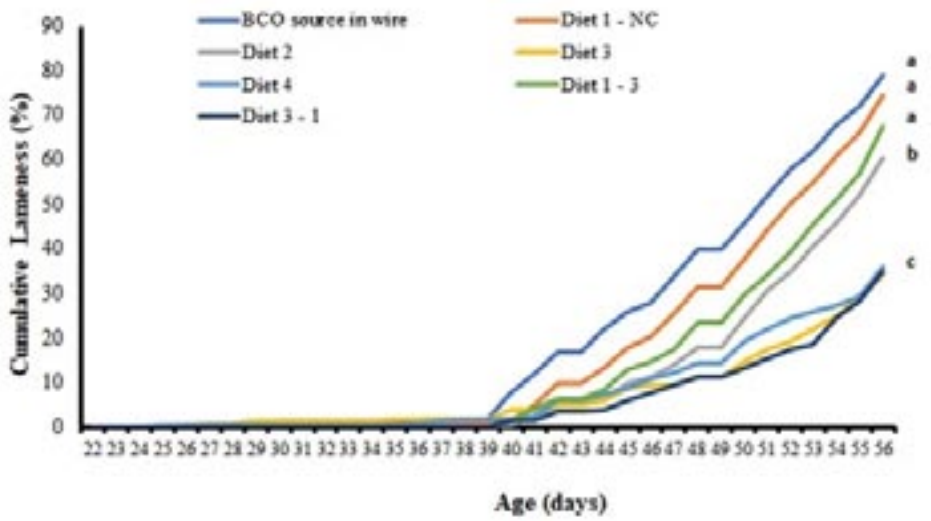
Results and Discussion

Cumulative lameness in both trials is reported in Table 1, whereas its development with age is shown in Figure 1.

Table 1. *Cumulative lameness in broilers at 56 days of age in seeder birds and when fed different dietary dose levels for the complete growth cycle or different duration of supply of $1.25(OH)_2D_3$ as glycosides from Solanum glaucophyllum.*

	Trial 1	Trial 2
Seeder birds	78.0 ± 3.0[a]	81.0 ± 3.0[a]
Negative control	73.5 ± 1.3[a]	74.5 ± 2.1[a]
0.5 µg/kg of $1.25(OH)_2D_3$	-	60.5 ± 1.5[b]
1.0 µg/kg of $1.25(OH)_2D_3$	32.5 ± 1.9[b]	34.5 ± 3.6[c]
2.0 µg/kg of $1.25(OH)_2D_3$	-	36.0 ± 3.9[c]
Late supply	-	68.0 ± 3.5[a]
Early supply	-	35.0 ± 4.0[c]

Figure 1. *Trajectory of the percent cumulative lameness during the 56-day long trial period per treatment group in Trial 2. Treatments are described in the text.*



Cumulative lameness in seeder birds and in negative control birds was similar in both trials, whereas 1.0 µg/kg of $1.25(OH)_2D_3$ significantly reduced lameness incidence. Half dose was less effective, where double dose didn't show extra effects. It was clearly demonstrated that early supply during the first 28 days was adequate, whereas late supply did not reduce the incidence of cumulative lameness compared to supply during the full cycle. Average body weight (kg) of clinically healthy birds at 56 days of age in Trial 2 was: Seeder birds: $4.28^a \pm 0.13$; Negative control: $4.53^a \pm 0.06$; Trt. 2: $4.59^a \pm 0.12$; Trt. 3: $4.88^b \pm 0.08$; Trt. 4: $4.69^a \pm 0.14$; Late supply, Trt. 5: $4.94^b \pm 0.15$; and Early supply, Trt. 6: $4.82^b \pm 0.12$. Therefore, it was demonstrated that the reduced lameness and thus BCO incidence was not associated with a reduced body weight gain. Finally, *Staphylococcus spp* was isolated from 65% of the femoral heads and proximal tibia of apparently healthy birds on D56 (summarized over all treatment groups) Asnayanti *et al* (2024a).

Although the exact mode of action is a topic of further research, most likely the effect of $1.25(OH)_2D_3$ on immunocompetence and/or the relationship with FGF metabolism in bones of BCO-affected birds (Ramser *et al.*, 2021) are part of it.

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Turkey Gut Health: A New Perspective on Synbiotics as an Efficient Solution

***Michaela Mohnl*[a], *Luis Valenzuela*[b*]**
[a]*dsm-firmenich/Research Center, Tulln, Austria*
[b]*dsm-firmenich/Technical Marketing, Getzersdorf, Austria*

The turkey industry continuously seeks innovative strategies to enhance the productivity and sustainability of commercial turkey production. This series of studies evaluates the effects of a poultry-specific synbiotic supplementation on growth performance and intestinal health in turkey poults challenged with a mixed *Eimeria* inoculation. The synbiotic feed additive consisted of probiotic bacterial strains (*Enterococcus faecium*, *Bifidobacterium animalis*, *and Lactobacillus salivarius*) combined with a prebiotic derived from inulin fructo-oligosaccharide (FOS). In one group (T2), turkeys were fed a diet supplemented with the synbiotic, while a control group (T1) received a standard unsupplemented diet. This experimental setup was repeated across two 14-week trials. Key performance indicators, such as body weight gain, feed conversion ratio (FCR), and mortality, were assessed. Across both trials, a significant improvement ($P<0.05$) in FCR was observed at 12 weeks (T1 = 2.07 vs. T2 = 2.00) and 14 weeks (T1 = 2.33 vs. T2 = 2.27). Additionally, by the end of the trials, turkeys in the supplemented group exhibited greater body weight gain (T1 = 8.24 kg vs. T2 = 8.30 kg).

In a third trial, poults were divided into two groups: a control group (T1) on a standard diet and a second group (T2) on the same diet supplemented with the synbiotic. On day 16, each group was further subdivided into *Eimeria*-challenged (T1I, T2I) and unchallenged subgroups, creating a 2 × 2 factorial arrangement. Weekly body weight measurements were taken post-challenge, and fecal samples were collected between days 21 and 33 to quantify oocysts per gram of feces. Between days 21 and 28, the *Eimeria*-challenged, synbiotic-supplemented group (T2I) showed a 23% improvement ($P<0.001$) in percent body weight gain (%ΔBWG) compared to the challenged control (T1I). Moreover, the overall weight gain in T2I was comparable to that of the unchallenged control (T1).

The findings demonstrate that incorporating a poultry-specific synbiotic into the diet of poults up to 42 days of age mitigates performance losses and intestinal health issues caused by mixed *Eimeria* infections. Overall, synbiotic supplementation effectively improved the performance of turkeys raised for fattening while supporting gut health in *Eimeria*-challenged birds.

Key words: *Synbiotic supplementation, gut health, Eimeria spp., challenge, growth performance, feed conversion ratio* (FCR).

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The Nutrigenomic Potential of a Lysolecithin Based Product

Veerle Van Hoeck*, *David Gonzalez Sanchez*, *Frederika Somers*, *Jody Shaw*, *Bart Forier
Kemin Europa, Herentals, Belgium
**veerle.vanhoeck@kemin.com; 0032(0)486410950*

Optimal feed efficiency is key for successful livestock and poultry production. But in times of rising feed costs, it can be a balancing act to maximize efficiency or reduce input costs while still meeting the nutritional requirements for optimal animal performance. Lysolecithins contain functional lysophospholipids (LPL) and are commonly known for their surface chemistry properties to enhance the emulsification, digestion and absorption of dietary lipids in monogastric feed formulations. More recent scientific findings have now revealed another important pathway in the mode of action of LPL, namely that they can also serve as signalling compounds triggering intestinal cell gene expression and nutrient absorption through so-called nutrigenomic action. Hence, the objective of this study was to determine the full nutrigenomic potential of the currently used lysolecithin source in LYSOFORTE®. An in vitro cell culture trans-well assay with intestinal CaCo-2 cells was designed.

The apical side mimicked the intestinal lumen, whereas the basolateral compartment represented the blood. Then, 0.5% lysolecithin, which corresponds with 500 g/t feed, was applied on top of the intestinal cells for 6 hours. In vitro cell culture data with intestinal CaCo-2 cells found that the lysolecithins can significantly improve intestinal cell viability ($P<0.05$). Furthermore, a trans-well culture experiment showed these lysolecithins can significantly trigger gene expression. When comparing the control versus lysolecithin, 274 genes were differently expressed, in which 163 were up-regulated and 111 were down-regulated ($padj<0.05$). The most significantly affected genes could be correlated to G-coupled protein signalling which might explain the increased cell viability. Enrichment analyses shows that amino acid transport and lipid metabolism pathways are significantly stimulated.

Finally, a polarized cell culture revealed that the lysolecithins, when applied apically, can affect the abundance of metabolites/nutrients in the basolateral compartment. In total 220 metabolites were differently abundant (with $P<0.05$), in which 155 were up-regulated and 65 were down-regulated. Interestingly, the nutrients that were increased by the lysolecithin group showed a much higher abundance (reflected in bigger fold change) numerically than the down-regulated ones. In conclusion, these findings on intestinal cell viability, gene expression and metabolite abundance, clearly show that the specific lysolecithins used in this study exhibit a broader mechanism of action beyond just surface chemistry, and that that the nutrigenomic effects contribute substantially to the potential of the lysolecithin as a nutrient absorption enhancer for pigs and poultry. Both surface chemistry and nutrigenomic activities do directly result in financial gains as feed efficiency is increased for several nutrients. The latter is an incentive for a more cost effective (re)formulation of the diets.

Keywords: *Lysolecithin, CaCo2-cells, Nutrigenomics, Lysophospholipids, Nutrient Absorption*

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Microbial supplementation: three decades of practical applications and learnings in turkey production

***P. Lebreton*[1], *A. Sacy*[1], *V. Demey*[1]**
[1] *Lallemand S.A.S., France*

Abstract
The poultry production industry has relied heavily on in-feed antimicrobials to enhance health and productivity over the past fifty years. However, the EU ban on growth-promoting antibiotics (AGPs) due to resistance and residue concerns, coupled with the physiological and metabolic challenges of modern turkey rearing, has necessitated the exploration of alternative solutions. Probiotics, defined as live microorganisms that confer health benefits to the host, have emerged as a promising solution. This study evaluates the efficacy of probiotics in turkey production, focusing on the strains *Pediococcus acidilactici* CNCM I-4622 (previously MA 18/5M) **(PA)** and *Saccharomyces cerevisiae boulardii* CNCM I-1079 **(SB)**. A multi-analysis of 14 trials on fattening turkeys revealed significant improvements in body weight (BW) and feed conversion ratio (FCR) in turkeys receiving probiotics compared to a negative control. Specifically, PA showed the highest BW increase (+0.071 kg) with a significant FCR reduction (-0.04 units), while SB demonstrated the best FCR improvement (-0.08 units), with a BW increase of 0.044 kg. Additionally, a PA supplementation field trial with turkey breeders resulted in a 0.4% increase in laying rate, reduced feed consumption by 1.7%, and improved egg quality. These findings suggest that probiotics can enhance turkey health and productivity, offering a sustainable and effective alternative to antibiotics.

Introduction
Over the last half-century, the poultry production industry became more dependent on continuous and uncontrolled in-feed use of antibiotics, which led to the EU ban of growth-promoting antibiotics (AGPs) due to resistance and residue concerns. In addition, intensive farm management procedures of modern turkey rearing systems also result in physiological, biochemical and metabolic changes that are difficult to address. Consequently, turkey growers were faced with the challenge of maintaining productivity and preventing disease on their farms, which facilitated the development and evaluation of novel antibiotic alternatives. The use of novel feed additives such as probiotics may improve the overall health status of turkeys and production efficiency in a sustainable, economic, environmental and animal welfare-friendly way whilst enabling safe food for consumers (Bozkurt *et al.*, 2023). Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (FAO, 2002). Based on this definition, live bacteria and live yeasts, that provide benefits to the host, are considered probiotics.

Benefits of Probiotics in Turkey production
Whilst the microbiota of chickens and turkeys share some similarities and can inform some general principles, they also exhibit notable differences, which is why more turkey specific trials must be conducted. Genetics and other factors such as diet, digesta passage rate, and rearing environment may account for the differences in intestinal microbiome composition between chickens and turkeys (Pan *et al.*, 2014).

Turkeys are highly sensitive to dysbiosis, and inflammation can easily be trigged. They are also more sensitive to dietary changes than other poultry, and their microbiota can be significantly affected as reported by high prevalence of intestinal disease (Figure 1) (Shehata *et al.*, 2021).

Turkey issue	2017	2016	2015
Lack of efficient antibiotics	4.8	4.6	4.4
Colibacillosis	3.8	3.9	3.2
Necrotic dermatitis (clostridium)	3.5	3.4	3.3
Lameness – footpad issue	3.2	3.1	2.4
Salmonella	2.9	3.0	3.0
Poult enteritidis	2.8	2.5	2.3

Figure 1. *Main turkey disease ranking (USA studies from 2015 to 2017)*
(1: no issue – 5: serious issue)

Utilizing probiotics in turkey farming presents several challenges, despite their potential benefits (Shehata *et al.*, 2021). Achieving robust and consistent results requires the careful consideration of various criteria. First, selecting the right probiotic strain at the appropriate dosage is crucial. Consistency and quality of the product must also be ensured, demonstrating good stability under varying conditions such as temperature, humidity, and pressure. Compatibility with antimicrobial molecules is needed to guarantee the survival and effective delivery of the microorganisms. Furthermore, the benefits of probiotic use must be clearly demonstrated to justify the return on investment. Finally, obtaining regulatory approval and conducting thorough safety analyses are essential steps in the process.

Multi-analysis of LAN probiotics in turkeys reared for meat

Over the past three decades, literature has extensively documented the use of the probiotic *Pediococcus acidilactici* CNCM I-4622 (Bactocell) with more than 100 publications. This lactic acid bacteria has been specifically selected for monogastrics due to its ability to produce high levels of L-lactic acid exclusively. L-lactic acid is a direct source of energy for the birds, either via its absorption at gut epithelial level or indirectly through a cross-feeding pathway (as a substrate for bacteria producing short-chain fatty acids, like butyrate producers). The probiotic has been authorized in the European Union for its use in broilers since 2003 (EC, 2003), in laying hens since 2010 (EFSA, 2010) and finally for all avian species since 2020 (Reg EU 2020/151).

Saccharomyces cerevisiae boulardii CNCM I-1079 (Levucell SB) is currently the only live yeast registered for poultry in Europe with a unique claim on the reduction of Salmonella on carcass contamination (EFSA, 2017 and 2019) and has a specific patent in the US for the ability of this strain to diminish Campylobacter and Salmonella in poultry (Line *et al.*, 2000).

A multi-analysis was performed to evaluate the effects of these 2 probiotics (*Saccharomyces boulardii* CNCM I-1079 (SB) and *Pediococcus acidilactici* CNCM I-4622 (PA)) compared to a negative control (NC) on the growth performance, feed conversion ratio (FCR), mortality rates, and gut histology of turkeys, providing an overview of their efficacy in turkey production. Data were compiled from 16 trials (covering different breeds) (Table 1), with 66% of the trials being performed at research institutes and 44% being field evaluations. Of these 16 trials, 14 analysed body weight (BW), feed conversion ratio (FCR), and mortality rates. Statistical analyses were performed using Generalized Linear Mixed Models and non-parametric Mann-Whitney tests (SPSS Statistics 29.0, IBM).

Country	Year	Probiotic	Duration
USA	1994	SB	3 weeks
France	2005	PA	Full cycle
Lithuania	2008	PA	12 weeks
Lithuania	2008	PA	12 weeks
France	2008	PA	4 weeks
France	2016	PA	Full cycle
France	2016	PA	Full cycle
France	2016	PA	Full cycle
France	2016	PA	Full cycle
France	2016	PA	Full cycle
Iran	2016	PA	12 weeks
Turkey	2021	PA	3 weeks
Turkey	2021	PA	6 weeks
France	2022	SB	Full cycle
France	2022	SB	Full cycle
Italy	2024	SB	12 weeks

Table 1. *Overview of trials included in multi-analysis*

Results indicated that probiotics significantly improved BW and FCR overall. Turkeys receiving probiotics showed an average BW increase of 0.052 kg and a reduction in FCR by 0.039 (P-values: 0.015 and 0.004, respectively) (Table 1). When comparing different probiotic types (PA, SB), PA showed the highest BW increase (+0.071 kg; $P<0.05$) with an FCR reduction of -0.04 units ($P<0.05$) compared to NC. SB on the other hand showed best improvement in FCR (-0.08, $P<0.05$) combined with an improved BW of 0.044kg ($P<0.05$) compared to NC (Figure 2).

	BW (kg)	FCR
Control	4.585	1.92
Probiotic	4.637	1.88
SEM	1.385	0.10
P-value	0.015	0.004

Table 2. *Effect of probiotic supplementation on meat turkey production performance*

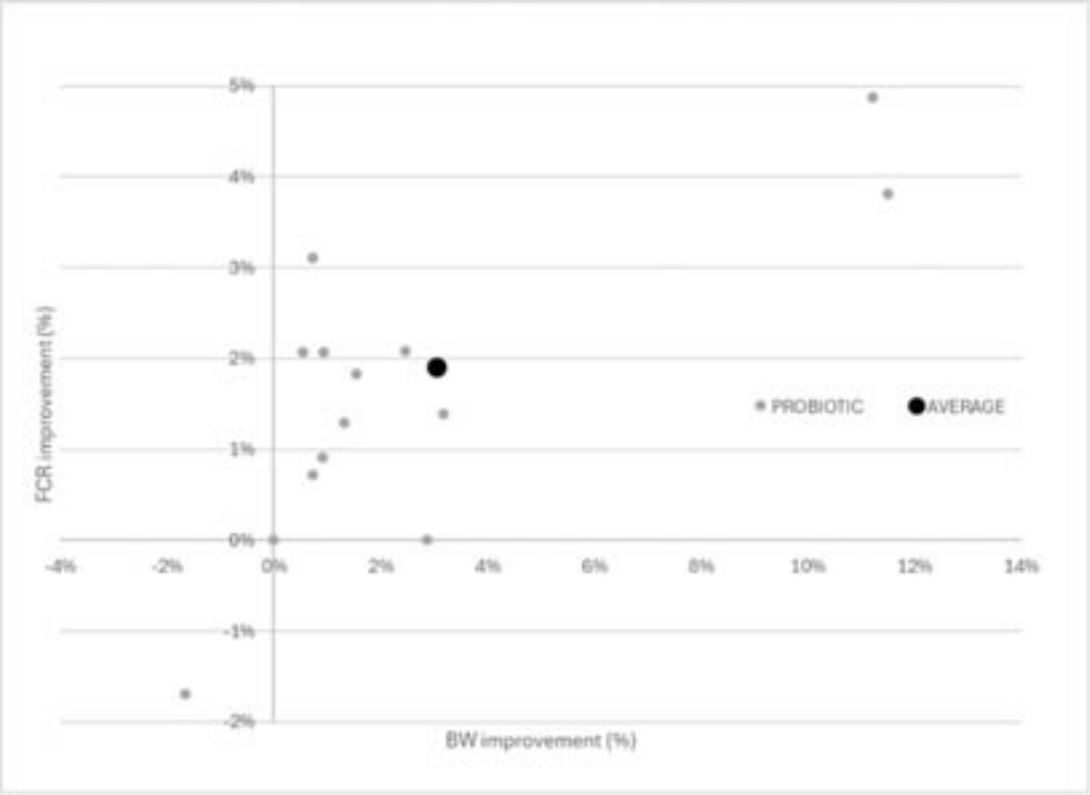

Figure 2. *Effect of probiotic supplementation on meat turkey production performance (1994-2024)*

The mechanisms of action of probiotics are multifactorial and not fully characterized, but potential modes of action are improved nutrition absorption combined with improved gut histology. A numerical improvement in gut histology (increase in villi height) is illustrated in several studies included in our multi-analysis (Figure 3). The beneficial effect of PA supplementation on gut architecture is already documented in unchallenged broilers (Mozafari *et al.*, 2016; Temin *et al.*, 2009) as well as in *Salmonella* challenged broilers (Jazi *et al.*, 2018).

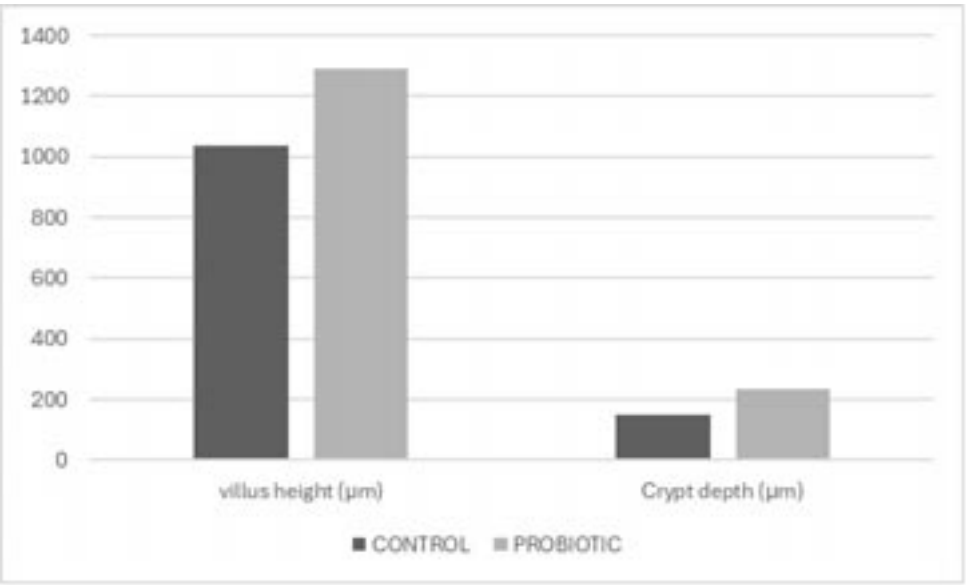

Figure 3. *Effect of probiotic supplementation on gut histology (Chamani et al., 2016; Bradley et al., 2014) (average value for duodenum, ileum and jejunum in µm)*

Some authors highlight the benefits of probiotics to regulate lipid metabolism, reduce body fat deposition and cholesterol concentration, but it is not always consistent between studies with different probiotic strains. With this in mind, different trials have been collected in broilers and layers to look at the impact of lipid metabolism on cholesterol content. The documented strain *Pediococcus acidilactici* CNCM I-4622 shows a consistent effect on the reduction of cholesterol in blood and egg yolk (Demey *et al.*, 2018). Interestingly, the same benefits on lipid metabolism are reported with this probiotic strain in turkeys (Figure 4). A clear relationship has been reported between cholesterol, intestinal inflammation, gut microbiome dysbiosis and bile acid disorders (Kriaa *et al.*, 2019).

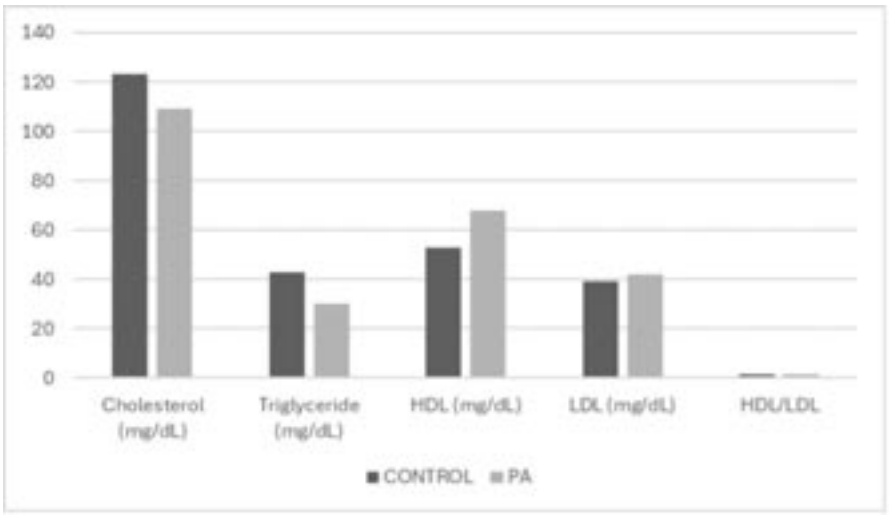

Figure 4. *Effect of probiotic on blood parameters of turkey poults at 84 days of age (Chamani et al., 2016)*

Effect of PA in turkey breeder laying performance

A study was conducted to evaluate the performance and egg parameters of Nicholas turkeys (Aviagen Turkeys) under two different dietary treatments. The trial was carried out at a commercial breeder farm in Brittany, France in 2008, with 4,540 turkeys aged from 28 to 57 weeks. The turkeys were housed in two contemporaneous commercial barns with straw bedding. The study compared a control group fed a basal diet with a treatment group (PA) receiving the basal diet supplemented with probiotic added post heat treatment process at 10e10 CFU/kg feeds. Performance metrics, including laying rate, feed intake, and mortality, were measured weekly. Egg parameters were also assessed. Performance data were analyzed via paired t-tests and egg classification and mortality analyzed using binary logistics (SPSS Statistics 29.0, IBM).

The supplementation of PA demonstrated several significant improvements in turkey breeder performance. Laying rate increased by 0.4% ($P<0.05$) for PA birds which resulted in a significant rise in the total number of eggs produced for PA compared to CTR ($P<0.01$). This was combined with a 1.7% reduction in feed consumption. The positive effect of probiotic PA supplementation on production performance is in line with the results obtained for laying hens, as previously reported in the meta-analysis performed studying the average effect of the same probiotic (Demey *et al.*, 2023).

With regards to the classification of eggs, a significant increase in category A and a significant decrease in category B eggs was documented for PA ($P<0.01$). A trend towards fewer broken eggs for PA (1.25%) compared to CTR (1.30%) in overall egg production ($P<0.1$) resulted in a significant reduction in the percentage of broken eggs among declassified eggs ($P<0.05$). Again, this is similar to observations in laying hens where the incidence of broken or downgraded eggs was substantially reduced for PA supplemented hens compared to a negative control (Demey *et al.*, 2023).

Mortality rate tended to be lower (4.22% vs 5.29%; $P<0.10$) for PA supplemented birds (Figure 5). Additionally, PA supplementation improved litter quality, with 5.2% less bedding used in the PA barn, while feathering quality was also enhanced for supplemented birds.

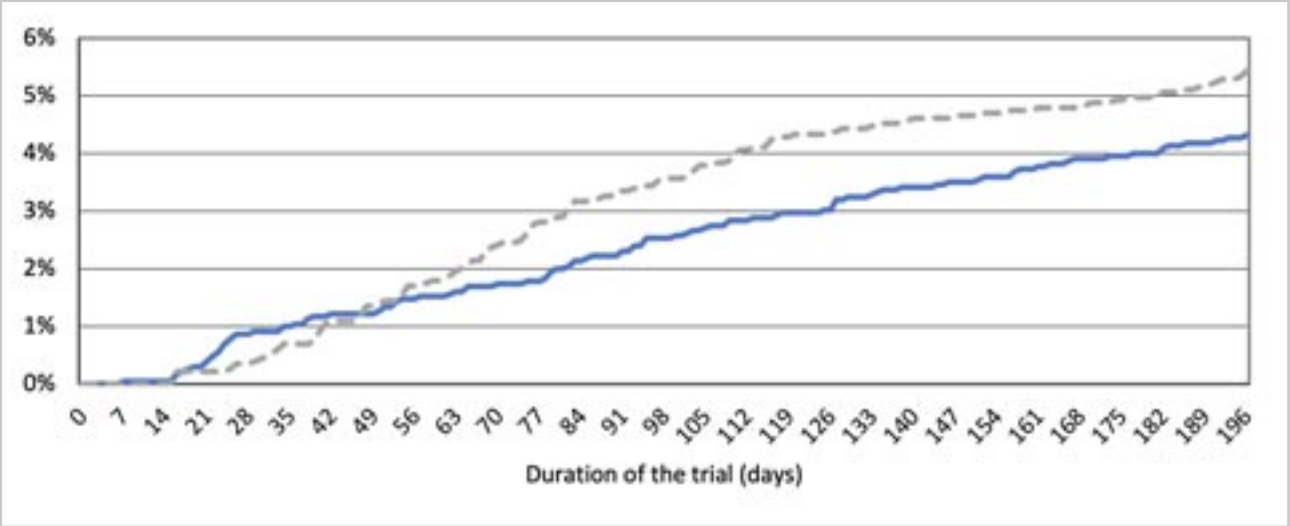

Figure 5. *Evolution of mortality (%) during trial for both Control (– – –) and PA (—)*

Taking into account the increased laying rate, lower feed intake, and reduced mortality, the return on investment (ROI) for PA supplementation was calculated to be 1:8.

Conclusion

The use of probiotics in turkey farming presents a promising approach to enhancing animal health and productivity. By selecting the appropriate microbial strains (registered, documented, compatible with turkey production management) and ensuring proper administration, farmers can achieve significant benefits, including improved gut health, enhanced immune function, and reduced reliance on antibiotics.

Perspectives

Beyond the use of single-strain probiotics, competitive exclusion products have shown promise in protecting the microbiota of turkeys from opportunistic pathogens such as *Salmonella* and *E. coli*. This beneficial effect has been documented by Cameron *et al.* (1997). Further investigation into the application of these products could provide valuable insights into enhancing the health and productivity of turkey flocks.

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