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Poster Abstracts

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Future perspectives for improved threat reduction to turkey industry from HPAI

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Background

High Pathogenicity Avian Influenza (HPAI) has been a major challenge for the Turkey industry in particular in the last five years. There have been dramatic global changes in the disease, its ability to evolve and spread and find its way into kept birds. It is well established with HPAI that turkeys carry a higher susceptibility than other kept bird species. This means that turkeys are less resistant to infection. A lower exposure dose is required to establish infection in individual birds and flocks and the virus has a high ability to spread amongst birds. Infection in non-vaccinated birds results in 100% mortality with rapid onset of clinical signs and death. The dramatic changes in risk to the UK turkey industry have occurred as a result of significant changes in the properties of the virus itself. These properties have conferred even more infectivity and infectiousness to birds, greater survival in the environment including in close proximity to turkey holdings. Increased carriage of virus by wild birds is a key factor. The number of cases in the last three years in domestic poultry in the UK have exceeded 390 cases. Turkey outbreak numbers will be presented.

Risk to Turkeys and underlying factors

As a result of these changes in disease risk the UK has been a continuous high status with risk to turkey populations with good biosecurity only recently being moved into the low risk category. In the preceding three years this risk was a medium or high. The global situation remains dynamic and evolving including of greater importance the UK turkey industry, the risk currently occurring on the European continent. Cases in Europe at present are slightly higher than in the UK but at a much reduced level. One critical feature of the disease in recent years has been the ability to maintain and persist in wild bird populations throughout the 12 months of the year. Historically risk periods have increased in the autumn with incoming migratory waterfowl and reduced in the spring as those birds depart. The virus itself has acquired an ability to spread to a greater proportion of different wild bird populations thereby increasing the risk year-round. It still however remains predominantly and autumn winter virus since the populations of birds carrying the virus during the summer months are those that have much less connectivity with the turkey sector.

Current perspective and future scenario

Since 1st October 2023 there has only been a single break of HPAI in turkeys in Great Britain (amongst the total of 5 outbreaks in kept birds overall). This is obviously a very positive situation compared to recent years and its probably a result of a number of factors.

I. Increased awareness in industry of risk

II. Improved biosecurity and hygiene practices in the turkey sector to take preventative actions

III. Greater communications of risk

IV. A reduction in the amount of virus being carried by UK wild bird populations.

The key question scientists are addressing is why has this reduced? Simply it's the lower amount of infection in the wild bird population which reduces environmental contamination and thereby less risk in the immediate environment of kept birds.

Preliminary indications are this reduction in risk wild birds is due to several factors. Firstly, after many years of cycling of the same or similar H5N1 strain in wild birds the population has started to build immunity to the virus since not all wild birds will naturally succumb to infection and die. This therefore disadvantages the virus and it becomes less able to infect as many birds in a population which then translates into less virus shed into the environment creating therefore less risk to turkey farms.

However as has been shown in recent years the influenza virus is capable of evolving at quite a fast rate and there may come a point where this virus mutates and acquires the ability to escape those wild bird flock level immune responses and persist more strongly as it has done in recent years. We enter a period of extreme uncertainty so whilst cautiously optimistic it is important that the industry continues to improve and maintain the advances made in biosecurity standards and vigilance of course is always required for this high impact disease.

Biosecurity challenges

The Field Epidemiology Team in the Animal and Plant Health Agency (APHA) have carried out detailed epidemiological investigation of several hundred infected premises and report cases since 2020/2021. Since the 2021/2022 HPAI season there has been no change in our understanding of the biosecurity risk factors likely to drive any future HPAI outbreaks.

This paper through presentation seeks to offer practical insight and advice on where to prioritise efforts to make businesses as biosecure as practical, taking into account that premises are commercial businesses and there is a limit as to how biosecure it is possible to make typical turkey houses, many of which are temporary, open-fronted structures or multi-purpose buildings being used seasonally. How many of these risk factors to address is a commercial decision. To put the risk into perspective, there is a reason that research institutes handle HPAIV in highly biosecure laboratories - one faecal dropping from an infected bird can contain sufficient virus to infect 100,000 birds and can survive in the environment for several months under UK conditions.

The paper will cover key observations from the recent outbreaks, a summary of the most important factors that increase the risk of becoming infected, and the most important factors that reduce the likelihood of becoming infected. It is important to consider the factors that impact the resilience of the business (i.e. reducing the impact of becoming infected with HPAI), as well as the biosecurity (reducing the likelihood of becoming infected).

Future preparedness: vaccination?

A great deal of planning and consideration has been given to better interventions to protect against this disease which we anticipate may return at some point in the future undefined. Accordingly, there has been a great deal of interest across Europe in routinely vaccinating birds to protect against this infection. Vaccination can protect birds against disease, it can increase the amount of virus required to infect a bird and it can stop transmission between birds. However even when using good vaccines vaccinated birds may still become infected, even if silently since the vaccine protects against the disease. Currently there is a GB cross sector working group including representatives of the turkey industry that are reviewing the UK's preparedness and future options for vaccination. Any use of vaccination will have to have cross sector commitment with obviously strong support from the competent authority and under their control. Key tenants would be appropriate vaccines to match the current wild type viruses nationally circulating and ability to deliver those vaccines effectively at large scale with low cost. In addition, it will be vital to monitor and track populations that are vaccinated to ensure there is no exposure and infection with wild type virus. Finally, to underpin and assure other sectors and trading partners that the GB even if vaccinating truly remains free of the disease. The challenges around vaccination and the progress made to date will be discussed in the presentation. It is clear from preliminary work done in Europe that vaccinating turkeys will be more challenging than other sectors given the birds particularly high susceptibility. Some options under consideration are the use of a prime boost strategy using two different vaccines. Work remains ongoing to develop the evidence base. The GB vaccination group is drawing together its conclusions and there has been strong interest in the turkey sector to routinely vaccinate. The challenge is that this comes at a high cost to industry and needs to be balanced against the real and likely risk of disease incursion whilst also considering the next epidemic strain (it might be different). Given the uncertainty ahead and the current reduction in infection risk it is still useful to map out potential future options and scenarios.

In summary HPAI continues to pose a significant threat to turkey production. However current risk levels are much reduced and it is likely in the future changes in the virus may be required in order to circumvent the expected building of immunity in wild bird populations. This directly correlates with environmental contamination and therefore risks incursion into turkey farms where biosecurity is a vital line of defence. It is however prudent given the global threat and situation with the disease that alternative strategies are considered and planned even if not applied and as such vaccination is being considered as a contingency for future action if required at GB level.

Air filtration in naturally ventilated turkey houses as a transitional solution in months at risk of AI

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Introduction

In recent years, highly pathogenic avian influenza (HPAI) has led to outbreaks with high economic losses in poultry production. Different species such as chickens, turkeys, geese and ducks can be affected. The virus is spread by wild birds and environmental contamination on affected farms. Also farm-to-farm disease transmission seems to occur. Biosecurity is practiced in order to protect poultry from HPAI entry into farms. Separating poultry from wild birds, keeping visitors out, hand hygiene, changing clothes and boots, water and feed hygiene are typical biosecurity measures to prevent the entry of the infectious agent. The airborne transmission by contaminated dust, feathers, faecal particles or droplets seems to play a minor role. However, this route is difficult to control or even not controllable at all. Furthermore, there are hints that the risk of farm-to-farm spread increases in areas with high poultry densities. Potentially infectious viruses can form agglomerates with dust particles. These are known as bioaerosols, each consisting of a living part (microorganisms) and an inanimate part (dust). This composition can provide the living part with better protection against environmental influences and increases its resistance against irradiation, for instance. As a result, the so-called tenacity increases and the viruses have the potential to survive longer in the air and thus remain infectious over longer distances and periods of time. In secondary outbreaks of HPAI, the most common type of affected barn is the naturally ventilated barn. Compared to forced ventilated barns, the incoming air cannot be filtered because the counter pressure would impair the necessary air exchange rate. Otherwise, naturally ventilated turkey barns, which have to ensure a high level of air exchange, were particularly affected by the most recent outbreaks in Germany. Turkeys are highly susceptible to HPAI. To prevent the entry of the disease via the previously uncontrollable airborne route, a technical solution was developed and implemented at a naturally ventilated turkey barn in this project. The idea of the system is that a naturally ventilated barn can be converted to a forced ventilated barn during high-risk situations. The air is filtered at the air inlets to protect flocks from virus-loaded particles. This flexible ventilated barn can be operated mainly cost-effectively by natural ventilation, but still offers the possibility to protect from airborne entry of diseases in high-risk situations. Furthermore, the modules with fine dust filters and fans were also equipped with cooling pads. This additionally protects the birds from heat stress during the summer with high enthalpy of incoming air. Such a system cannot only contribute to biosecurity, but also to the welfare of birds. In the present study, the system was investigated concerning the particle and bioaerosol retention and the effects on climate parameters within the barn.

Figure 1: Two filter boxes for air supply installed at one side of a naturally ventilated turkey barn. The curtains remained closed during forced ventilation.

Methods

After the successful adaptation of the filter modules (see Figure 1 showing the filter modules with cooling pads, pre-filter, F-Filter and ventilator within the box), the barn was operated continuously in the simulation mode of an acute hazard situation. Air samples of the outdoor air, the filtered air and the barn air were taken at fortnightly intervals. Particle counters and air samplers were used to determine particle and bioaerosol concentrations. Total bacteria, fungi and yeasts were analysed in the laboratory. The readings were carried out over a period of one year and included three fattening periods. From the start of the second fattening period, the measurement technology was extended. A stationary sampling point was added to continuously separate particles on a Teflon filter in front of and behind the filter unit. These separated particles, and those collected with the air samplers were analysed via PCR for influenza A, the avian metapneumovirus responsible for Turkey Rhinotracheitis (TRT) and *Ornithobacterium rhinotracheale* (ORT).

Results and discussion

Average retention rates for the particulate matter (PM) 10.0, 2.5 and 1.0 ranged between 94% and 98% (Table 1). The observed decreasing retention rate for smaller particles could have been expected. Considering the literature, it can be assumed that virus loaded particles can be efficiently removed from incoming air with F-filters. However, HEPA filters would be probably more efficient in retaining particles. Nonetheless, these are costly, need more pressure and clog more quickly in dusty environments. A clear reduction in the total bacterial count, fungi and yeasts was achieved in the supply air through the filter unit. These indicator microorganisms can be consistently retained by the system (Figure 2).

Additionally, it was demonstrated that the filter unit efficiently retained the indicator ORT. With the stationary collection point, ORT DNA was found in all of the seven examinations (CT 26-34), while DNA was only found once behind the filters with a significantly higher CT value (CT 38). Influenza viruses were not detected during the production period. To date, no outbreak of HPAI has been reported within a 10 km radius of the test barn. Measurements are ongoing until spring 2024.

In Germany, there are specific limits for climate parameters in turkey barns. For harmful gases, the concentration of CO_2 should not exceed 3,000 ppm and that of ammonia 20 ppm. The specified minimum value for light intensity is 20 lux. Despite the conversion and modification into a forced ventilation barn, these climate parameters were not exceeded in our study. The highest CO_2 level measured was 2,300 ppm. The maximum value for ammonia was 17 ppm and the average value was 7.4 ppm. Sufficient daylight continued to enter through the closed curtains. The prescribed value of 20 lux was never exceeded on any of the days readings were taken.

Table 1: Percentage of particulate matter (PM) retention of filter modules at three fattening periods (A, B, C).

Period	PM10.0 [%]	PM2.5 [%]	PM1.0[%]
A	96.60	94.37	92.55
B	98.85	97.60	96.37
C	99.51	98.49	96.92
average	97.89	96.27	94.27

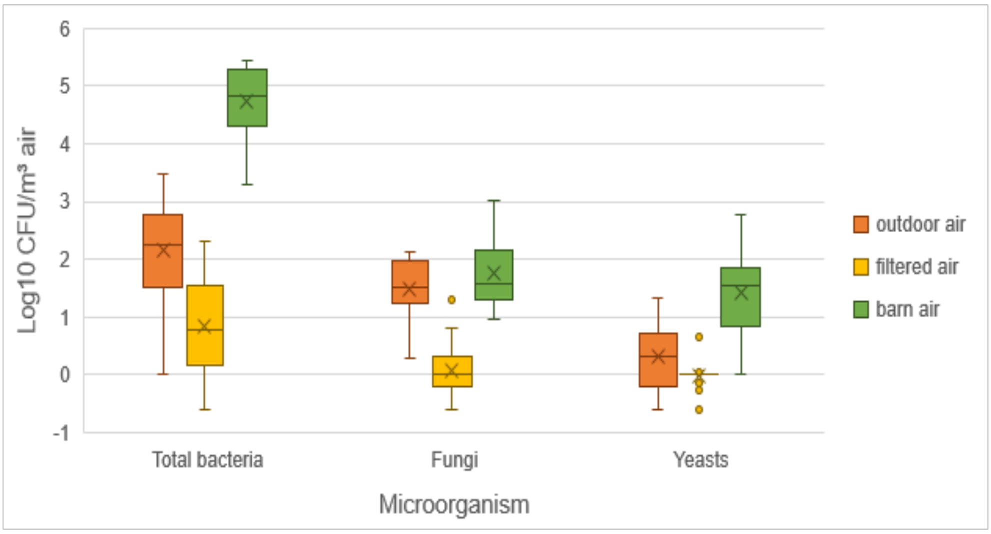

Figure 2: Average values for total bacterial count, fungi and yeasts in the outside air, in the filtered air and in the barn. The results are logarithmised and shown for one cubic metre of air.

Conclusions

This flexible system for converting a naturally ventilated turkey barn into a forced ventilation barn can efficiently filtering particles and bioaerosols from the outside air. The system can be used all year round and can prevent airborne entry of pathogens. Furthermore, no adverse effects on climate parameters were observed. Therefore, the possibility of cooling down incoming filtered air may contribute to the welfare of poultry in naturally ventilated barns.

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Optimizing Genetic Potential Through Management in Turkey Production

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Optimizing Genetic Potential Through Management in turkey production encompasses key considerations throughout the production cycle. From set-up placement to monitoring, nutritional management, and addressing environmental influences, a comprehensive approach is vital for unlocking the full genetic potential of turkey flocks. Strategic decisions during set-up placement lay the foundation for the birds' growth trajectory. Continuous monitoring ensures timely adjustments, guaranteeing optimal conditions for development. Nutritional management, tailored to the specific genetic lines, is pivotal in enhancing performance and efficiency. Concurrently, addressing environmental influences, such as housing conditions and stressors, is integral for sustaining genetic potential. This holistic strategy underscores the intricate balance required in managing various aspects of turkey production to achieve the highest genetic potential and overall success in the industry.

Poult quality, transport, and delivery

The poult start is an extension of the hatching process. Once set, the egg must be monitored through the entire incubation and hatching process. At hatch, the poult must be kept in conditions to ensure a correct body temperature, through the selection, sexing and holding period. During transportation, it is mandatory to continuously monitor the environmental conditions in which the poults are transported. If the birds arrive too hot or too cold, and to overcome challenges during placement, there should be a plan in place to allow the birds to recover without adding additional stress.

Set-up and placement

The correct access to feed and water upon arrival at the farm, and the control of a proper barn environment, will support the hatching and delivery process already done. All the equipment must be properly functioning and should allow adequate access to feed and water.

In 2016, Dr. Vern Christensen demonstrated the correlation between delivery temperature and 7-day body weight, as well as 7-day mortality in toms' placement. The findings revealed that maintaining poult body temperatures (Figure 1) within the range of 39.4-40.0˚C (103-104˚F) resulted in the most consistent body weights and consistently low 7-day mortality rates

Figure 1. Optimal vent temperature must be between 39.4 to 40.0°C (103-104°F)

Environmental control: Temperature and gases

It is also important to ensure the correct heat and ventilation exchange on the farm. If the poults are too cool at delivery, room temperature can be raised a couple of degrees as needed but avoid big jumps in temperature. Even in warm conditions, preheating the litter is essential. Newer brooders with electronic heating systems can be synchronized with the barn's computer timer. The average floor temperature across the feed and water sections should not exceed 35-35.6˚C (95-96˚F) (Figure 2). If it becomes too warm, there is no place for the poults to find their comfort zone, thus producing added stress on the birds. The heat should be reduced 8-10 hours after placement back to a normal 33.3-34.4˚C (92-94˚F) floor temperature. Following temperature guidelines, maintaining an environmental temperature of 31.1˚C (88˚F) at day 5 and 30.5˚C (87˚F) at day 7. This promotes early feeding behavior for optimal growth. To optimize poult performance in extreme temperature conditions, it is crucial to manage the heater and ventilation systems effectively. Regular monitoring of poult behavior and body temperatures is necessary, adjusting room and floor temperatures accordingly. Attached to the temperature control, the air moisture must be monitored and controlled. After the first week, the humidity must be between 55-58%.

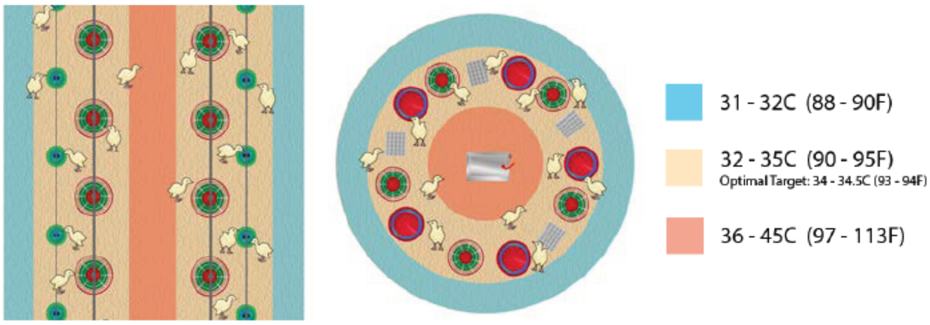

Figure 2: Floor temperature in a whole room brooding system

Monitoring gas levels is crucial, with CO2 less than 2500 ppm and CO less than 20 ppm. Adequate light intensity, measured at 8-10 ft candles, 80-100 lux for the first 5-7 days, ensures active behavior and encourages early eating, positively impacting overall performance.

Stress avoidance, behavioral development, and welfare

Poult activity must be monitored once placed at the farm. They should be alert and active while also seeking out food and water. It is beneficial to assess poult temperature upon arrival to the farm and 6-12 hours after placement to ensure they are comfortable in their environment. Alterations in behavior serve as a reliable indicator of poult comfort. The monitoring must be precise to be able to identify the difference between all the signs, huddling poults not always means cold and can be also caused by high concentration of CO2.

Implementing the crop test after 24 hours is a reliable method to assess the conditions. Check the crop by palping after 24 hours of placement, if 90% of the poults have content, all the setup and conditions are adequate. If the crop test shows less than 90%, there is something that can be improved.

Biosecurity

Having an adequate biosecurity culture and measures are an important part of the key checkpoints to success. These include a delimited perimeter, clear entrance procedures, Danish houses per barn, proper exchange of boots and coveralls, a clear bird disposal system, and correct litter management.

Conclusion

The culmination of incubation, hatching, delivery, and barn preparation all play a role in the overall success or failure of a poult start. In the realm of turkey production, optimizing genetic potential through comprehensive management practices is paramount for ensuring flock health, welfare, and productivity. The process involves strategic decisions during set-up placement, continuous monitoring, tailored nutritional management, and addressing environmental influences. The first week of a turkey's life is crucial, setting the trajectory for subsequent performance. Attention to early nutrition, water intake, and environmental factors is pivotal. Effective control of temperature, gases, and light intensity contributes to optimal growth. Stress avoidance, behavioral development monitoring, and biosecurity measures further enhance success. In essence, a holistic approach to turkey production management is essential for unlocking the industry's highest genetic potential.

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Warm Weather Ventilation Strategies

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Introduction

The face of the turkey industry in North America has changed significantly since 2010 when I did my last discussion on warm weather ventilation at the Turkey Science and Production Conference. In North America, tom turkey weights have dramatically increased and pressure from consumers has also driven demand for antibiotic free and organic turkey production, which have placed a strain on the ability to raise turkeys efficiently and support sound animal welfare. Tunnel ventilation has long been associated with hot weather ventilation control in older turkeys in North America. However, as genetic, and dietary improvements allow for increased weight and lower feed conversions, this will also increase heat production in turkeys which already have difficulties coping with extreme environmental conditions, consequently impacting their productivity (Mendez et al., 2015). Higher energy diets fed for this heavier, faster growing turkey increases heat production (Uemura, D., 2021). Heat stress is a concern for the turkey industry and has negative impacts on welfare and performance (Midwest Plan Service, 1987). Methods to maintain bird comfort within the thermal neutral zone are no longer confined to hot weather. This can require more detailed control throughout the growing cycle all year.

Genetic improvements have been significant for turkey production. Weight gain and feed conversion have significantly improved as seen in Table 1. This information is a sample from North America looking at 2003, 2010, and 2023, in both hens and toms. From this example, both weight gain and feed conversion have improved for hens, which are primarily used for whole body turkey sales. Tom production, which is widely used for breast meat, parts, and further processed sales, weights have significantly increased while feed conversion utilization has been lower. Livability, however, has suffered due to reduced effective antibiotic use and increased stress on the birds including increased heat production noted earlier.

This coupled with the potential increase in environmental temperatures over time, will require the industry to improve temperature control in turkey production facilities. "Recent increase in extreme heat wave events and enhanced sensitivity of modern poultry genotypes to heat stress has become a major concern leading to significant economic losses to the poultry industry" (Pawar et al., 2016). In North America, the Pennsylvania Climate Impacts Assessment 2021 report documented that by mid-century "the average annual temperature statewide will continue to rise and is expected to increase by 5.9°F

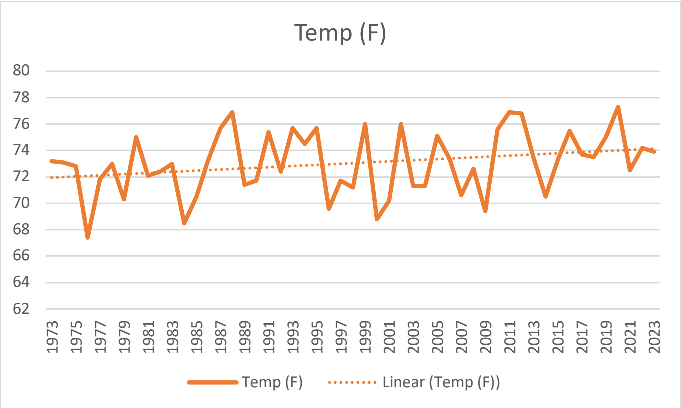
Table 1. Performance Examples

Hens	2003	2010	2023
Livability %	93.30	91.20	92.50
Age (Days)	94	94	90
Average Weight (kg)	6.68	7.52	7.56
ADG	0.0711	0.0800	0.0840
FCR	2.22	2.27	2.10
Toms	**2003**	**2010**	**2023**
Livability %	87.80	87.12	85.50
Age (Days)	131	137	137
Average Weight (kg)	14.45	18.63	21.17
ADG	0.1103	0.1360	0.1545
FCR	2.57	2.57	2.29

(3.3°C) compared to the 1971-2000 baseline". Additionally, the report states that there will be more frequent and intense extreme heat events – temperatures are expected to reach 90°F (32.2°C) during 37 days compared to 5 days for the baseline period.

Climate change is not the point of my paper, however, based on the information in Figure 1., temperatures have been increasing in North America as documented in Pennsylvania from 1973-2023 (Pittsburgh Historical Temperature Averages, https://www.weather.gov).

Figure 1. Pittsburgh, PA Historical Temperature Averages



The bottom line is that we must be ready to use and manage the necessary tools to keep turkeys comfortable to reach the genetic potential of the breeds available and deal with the environmental conditions that we cannot control.

Hot weather can have a negative impact on turkey performance. Understanding when this occurs by measuring temperature and humidity as well as some old-fashioned turkey husbandry experience is necessary. When turkeys are exposed to high temperatures and/or humidity combination, they begin to pant. This is easily recognized by gaping mouths as seen in the picture below where 10-week-old toms are exposed to temperatures above their thermal neutral zone. Dr. Sally Noll (University of Minnesota, 2022) defined the thermal neutral zone as "the range of environmental temperatures that an organism can maintain their body temperature. For most poultry, this zone is between 60°F (15.5°C) and 75°F (23.9°C)".

When turkeys are exposed to temperatures above 23.9°C, especially for prolonged periods, there is potential for large losses due to heat prostration. However, prior to that, daily weight gain and feed conversion will suffer. Information in Table 2 from the North Carolina State University (Anderson and Carter, 1993) identifies the temperatures at which turkeys are comfortable and when they are heat stressed. When turkeys begin to pant, the process of energy loss has already started. Therefore, any management techniques that can be implemented to reduce this stress and maintain bird comfort before hot weather will benefit the turkeys and the bottom line. These same techniques can also be a vital component in an animal welfare program which addresses bird comfort. Tunnel ventilation can be used as a management tool to control the environment all year long, but especially during hot weather.

Table 2. Heat Stress and Ambient Temperature

13° to 24°C (55° to 75° F) - Thermal neutral zone – bird does not need to alter its basic metabolic rate to maintain its body temperature
24° to 29°C (75° to 85° F) - Slight reduction in feed consumption, feed conversion compromised - panting
29° to 32°C (85° to 90° F) - Feed consumption falls further, weight gains lower, feed conversion further compromised – excessive panting
32° to 35°C (90° to 95° F) - Feed consumption continues to drop, birds in survival mode
35°C (95°F) and up – mortality

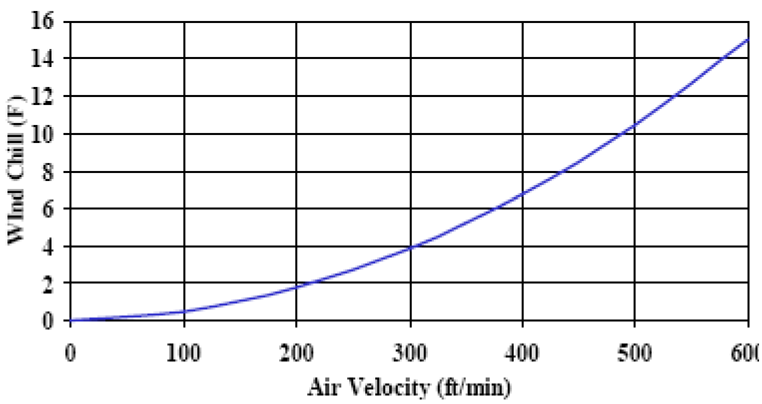
The information presented in Table 2 is from 1993. Turkey performance – weights, gains, and feed conversions are certainly different than that period. When observing turkeys - depending on age, air temperature, and relative humidity – panting may begin prior to the 24° C temperature listed. Additional work on the effect of temperature, relative humidity, and ventilation rate on turkey wattle temperatures has shown a positive relationship (Mendez et al., 2016). It has also been demonstrated that air velocity from 1.5 to 2.5 m/s and temperature at 30°C is an optimal combination for young turkeys (Yahav S., et al., 2007).

Tunnel ventilation, as defined in my 2010 paper, is a type of ventilation where air enters at one end of a poultry barn and is exhausted by ventilation fans at the opposite end

(www.epa.gov/oecaagct/ag101/porkglossary.html). This creates airspeed allowing the environment to be controlled. The advantages of tunnel ventilation have been demonstrated with improved weight gain and feed conversion. Conventional open curtain sided or ‘naturally’ ventilated structures, which are dependent on natural cross winds and low airspeeds from circulation fans, do not afford the ability to maintain consistent wind speed and therefore effective temperatures. These stir fans normally reach airspeeds of approximately 0.51 m/s (100 fpm) for a short distance directly in front of the fan, allowing for little cooling effect on birds. Additionally, in areas of North America, uncontrolled high winds can lead to increased chilling of younger birds if not regulated. Tunnel ventilation, if designed properly, can provide constant desired airspeeds typically ranging from 0.45 m/s to as high as 3.5 m/s. The advantage of this constant wind speed is that it creates a windchill over the turkeys, so they feel a lower ‘effective temperature’ than the actual ambient air temperature (Donald, 1995). Figure 1 shows that as the airspeed increases, wind chill also increases thus lowering the effective temperature that the turkey feels.

Figure 2.

Wind Chill Effect at 29°C (85°F)



Adapted from 2008 Univ. of Georgia

Ventilation Workshop, Czarick

Table 3 is an example of annual performance from November 2022 through November 2023 from North America using tunnel ventilation to maintain 15.5 to 23.9°C and farms not using tunnel ventilation to maintain those temperatures, fed the same diets. The economic improvement in performance of three measurable factors in effectively managed tunnel barns is clear. Livability, weight gain, and feed conversion are all improved.

Table 3. Tunnel maintained 15.5°C to 23.9°C range vs. not

Toms	Tunnel	Not tunnel
Livability %	87.68	85.25
Age (Days)	135	136
Average Weight (kg)	20.69	19.70
ADG	0.1533	0.1449
FCR	2.29	2.35

Transitional ventilation – the ability to maintain environment and temperature during both warm and cool weather is a key to good performance. In North America, this happens primarily in Fall and Spring, which can be the most challenging times of the year to ventilate poultry houses due to large swings in temperature. In some parts of North America, temperatures can be below freezing as the sun rises and get into the mid 20°C range in the afternoon. Today's poultry housing and ventilation equipment allows us to control the environment inside at any time of the year to meet the comfort zone demands of the bird, as we understand it. Our goal in managing the ventilation in the barn is to maintain temperature for bird comfort and performance while at the same time controlling ammonia and moisture levels. It can be done with proper equipment and controller settings. The days of "the weather is tough and making it hard to ventilate" should be a thing of the past if we are setting our systems up properly. For barns with inadequate tightness, without tunnel ventilation, and/or inadequate fan power and inletting, the weather will be a problem.

Table 4 is an example of a basic controller setup that incorporates minimum, transitional, and gradual tunnel applications for turkeys that are around 16 weeks of age. Notice that the controller will be able to manage temperatures that are cold with timer fans running in minimum ventilation, negative pressure. The controller then transitions to increase negative pressure ventilation when the outside air warms up and inside temperatures move through the 16°C range. When the inside temperature reaches 20°C, the house converts to tunnel ventilation at a low speed and gradually increases as the house warms. The Effective Temperature column shows the approximate effective temperature that the bird 'feels' due to the wind speed as it increases. The birds remain in their comfort zone, even though the actual temperature in the house is as high as 26.6°C. Effective temperatures are based on Figure 2 which shows the relationship between airspeed and effective real feel temperatures for poultry at an ambient temperature of 29.4°C. Just remember, this is only a guide. Reading the birds' reactions to airspeed is critical. The two biggest mistakes in tunnel ventilation are 1. waiting too long (temperatures too high) to go into tunnel and 2. going into tunnel too early (young age and low temperature) and sitting birds tight to the floor chilling them. Figure 3 is an example of younger birds that have been exposed to higher than desirable airspeed for their age. They are sitting tight to the floor with ruffled feathers.

Figure 3. Too much airspeed at a younger age

Table 4. Typical environmental controller setup

Controller set up								
19.22m x 213.5m	Turkey finisher							
				Estimated				
				Wind chill	Effective	Effective		
Target Temperature	**16.7**			at 29.4˚C	Temp On	Temp Off		
	Fans	On	Off					
Ventilation rate	1250 m³/min	Timer					Negative pressure	
	1250 m³/min	17.81	17.25				Negative pressure	
	1250 m³/min	17.81	16.70				Negative pressure	
	2295 m³/min	20.04	18.92	0.56	19.48	18.36	Tunnel	about 0.65 m/s
	3060 m³/min	21.15	20.04	0.97	20.18	19.07	Tunnel	about 0.87 m/s
	3825 m³/min	22.26	21.15	1.11	21.15	20.04	Tunnel	about 1.09 m/s
	4590 m³/min	23.37	22.26	1.39	21.98	20.87	Tunnel	about 1.31 m/s
	6120 m³/min	24.48	23.37	2.50	21.98	20.87	Tunnel	about 1.74 m/s
	7650 m³/min	25.60	24.48	3.75	21.85	20.73	Tunnel	about 2.18 m/s
	9180 m³/min	26.71	25.60	6.00	20.71	19.60	Tunnel	about 2.61 m/s
	10710 m³/min	27.82	26.71	8.33	19.49	18.38	Tunnel	about 3.05 m/s
	Foggers 1	31.71	30.60	Foggers operate on temperature, not timer. There should be a 2.8°C separation between the last tunnel fan coming on and foggers cutting off. Foggers should not come on before 30.5°C.				
	Foggers 2	34.49	33.38					
	- It's important to keep in mind that wind speeds (m/s) are estimates and should be checked with a wind speed meter							
	- Wind chill and therefore effective temperatures are theoretical values.							

It is well understood that this wind tunnel is effective at removing radiant heat that birds produce during hot weather. Reducing the effective air temperature with wind speed created in a tunnel ventilated barn enables producers to maintain temperatures within the bird's comfort zone. Table 5 is an illustration I have used many times to show that removing heat from the birds in hot weather with airspeed is the most efficient way to remove heat from the building, based on the heat calculation inside the barn. Evaporative cooling techniques employed in conjunction with wind speed can further improve the temperature reduction ability in a turkey barn.

Table 5.

2,787 m² (30,000 ft²) Turkey Finisher – heat calculation
- 8,500 toms, 18 kg (40 lbs)

- Ceiling – 70,000 Btu/hr
- Side walls - 10,000 Btu/hr
- End walls - 3,500 Btu/hr
- **Birds - 1,700,000 Btu/hr**
- Lights - 8,100 Btu/hr
- **TOTAL 1,791,600 Btu/hr**

Clearly, it is more important to remove the heat from the turkeys than to try to reduce heat entering the building structure from the outside. Sizing the ventilation system properly, including fans and inlets, is necessary to remove the heat production and to provide the airspeed necessary to create reduced effective temperatures.

Evaporative cooling can be an effective method to reduce the temperature of incoming air. This method is most effective when relative humidity levels are below 80%. In most cases, depending on temperature and humidity levels, incoming air temperatures can be less than 32°C. This coupled with the wind chill effect will keep the real feel temperature for the birds within their thermal neutral zone. Evaporative cooling can be accomplished with cool cell pads or high-pressure fogging.

Cool cell pads, typically 15.25 cm (6 in) in thickness, are mounted at the tunnel inlet end and water is slowly sprayed or trickled over the wet pads to cool the incoming air. While this system may be the most effective method to lower temperatures, it is also the most expensive and usually the most difficult to maintain. Also, in many North American commercial turkey production systems, cool cell pads are only used for a short period during the hottest time of the year, making them even less cost-effective.

High-pressure fogging is also used to evaporate water into the air, lowering the temperature. High-pressure pumps running at 200 psi are used to push water through nozzles rated at 3.8 L (1 gal) per minute to create a fine mist. Nozzle lines are normally placed over each tunnel inlet as well within a line spaced strategically across the house, stopping approximately 21 m (70 ft) away from the tunnel fans.

Evaporative cooling is effective when the temperatures are over 27°C (80°F) and humidity levels are below 80% (Poultry Housing Tips, The University of Georgia, Vol.12, No.9). Evaporative cooling is detrimental when humidity and temperature levels are both over this mark, as turkeys can no longer lose heat through respiration. Overuse of evaporative cooling can cause high humidity levels in turkey barns causing wet litter and dangerous humidity levels if not monitored properly.

Figure 4.

Temperature and humidity relationship

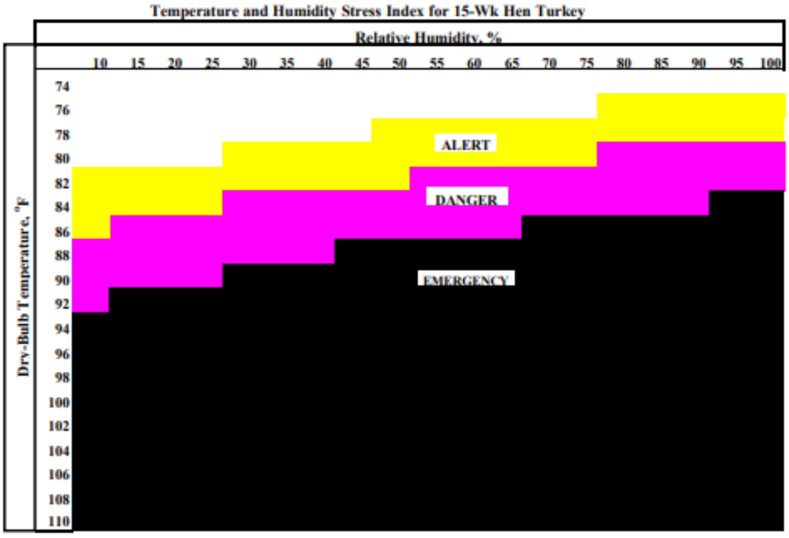


Figure 4 is an illustration from Xin and Harmon, Iowa State University, March 1998, showing the relationship between temperature and humidity and the potential dangers when they are both above 80°F (26.6°C) and 80% humidity. Czarick and Lacy, University of Georgia, also refer to this as the 80-80 rule. It is important to measure both temperature and humidity inside the turkey barn. High humidity decreases poultry heat loss from the lungs and increases heat stress (Noll, 2022).

Summary

Managing the growing space environment has changed significantly in the 35 years since I started in the turkey business. Genetics have allowed us to realize performance potential to reach heavier weights earlier and at lower feed conversions. This comes at a cost as we need to improve ventilation strategies with tunnel ventilation and evaporative cooling to maintain the thermal neutral zone for bird comfort to realize these gains and improve animal welfare. Tunnel ventilation is a tool that must be designed properly to create the wind speeds necessary to provide wind chill and evaporative cooling necessary to achieve the goals stated. Barns must be tight, fans and inlet openings must be sized properly, and evaporative cooling designed to match the airspeed and temperature drop desired. Finally, once the system is in place, it must be maintained at peak performance.

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Can distraction prevent pecking events? Results from beak trimmed and beak intact turkey hen flocks on four commercial farms in Germany

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Background

In Germany the renunciation of non-curative interventions on animals, such as beak trimming in turkeys, is a declared goal regarding animal welfare (Kulke et al., 2016). According to the German Animal Welfare Act (TierSchG), exceptions to the existing ban on amputation may only be granted if “the intervention is essential for protection of the animals with regard to the intended use” (§6 (3)).

However, as the causes of injurious pecking in turkey farming have not yet been sufficiently clarified, the multifactorial behavioural disorder often leads to serious welfare problems in affected flocks (Dalton et al., 2013; Duggan et al., 2014). The consequences of pecking outbreaks are diverse and may range from the affected animals’ suffering to secondary infections of the injuries to death. Even healed injuries can still lead to parts of or whole carcasses being discarded at the slaughterhouse ultimately, which stands in contrast to responsible and resource-conserving utilisation. On top of that, beak intact turkeys can inflict more severe injuries onto each other in less time. All those risk factors combined make many farmers throughout Germany still rely on beak trimming in order to reduce damage in live flocks as well as in the resulting meat products today.

Further research regarding the origin and containment of injurious pecking as well as practical experience with beak intact turkey flocks is needed in order to refrain from beak trimming in conventional turkey farming. At the same time, a much discussed element in relation to injurious pecking, for both turkeys with trimmed and intact beaks, is the optimisation of husbandry. Local management guides (such as the one from the Lower Saxony Ministry of Food, Agriculture and Consumer Protection, Germany, 2019) suggest the use of additional occupational and structural elements in the barn in order to adapt the housing environment to the animals’ needs. Further, aspects such as stocking densities, lighting program, health management as well as adapted feeding are considered influential on ethopathies, such as injurious pecking (Dalton et al., 2013; Marchewka et al., 2013). Nevertheless, a strategy of using different approaches simultaneously in form of “optimisation packages” is not yet widespread. This might be due to a lack of information, possible applications as well as the economical aspects in terms of additional workload.

Animals, Material & Methods

The project, in which all underlying data was collected, is called #Pute@Praxis and was conducted from May 2020 to December 2023. #Pute@Praxis aimed at transferring existing scientific knowledge and procedures for improving animal welfare in turkey farming into practice in order to test their practicability and to process and demonstrate the experiences through new forms and media for a broad specialist audience. The project was based on six practical turkey farms throughout Germany, four of which operated conventionally. The conventional farms kept beak trimmed turkey hens prior to participating in the project, even though in some cases there were single previous experiences with beak intact turkeys. The remaining two farms used alternative farming strategies and already worked with beak intact turkeys routinely for several years (labels “Neuland” and “Naturland”) before participating, thus differed greatly methodically from the conventional farms as well as from each other, and are therefore excluded from this publication.

Together, the project team and farmers established different optimisation measures, which were implemented on the farms’ participating turkey hen herds. This included reducing stocking density to at least 48 kg/m², two 1 h light breaks daily, supplementation of either whole oats or oat bran (2-3 % of the complete ration), one structural element per 1,000 turkeys in the barn and one occupational element per 500 turkeys in the barn. Additionally, each farmer had an “emergency kit” at hand, which included several more occupational elements that were only in use while behavioural deviations in the herds were observed. Each farm conducted three fattening runs with one to two flocks of turkey hens within the project. On the conventional farms, the first fattening run was conducted with beak trimmed turkeys to let the farmers get used to the project measures in form of "optimization packages" first. After that first fattening run, the measures were evaluated by the project team and each farmer, customised indivdually if required, and then implemented in the second and third fattening run with beak intact turkey hens. A detailed description of the individual environmental enrichments on the different farms is listed in table 1 below. An impression of the different elements is given in the following figures 1, 2, and 3.

Tab. 1: Individual set ups of environmental enrichments on the participating conventional farms along with the contents of the emergency kit. Beyond that, the farms all agreed to work with a stocking density of 48 kg/m², 1h light breaks twice daily, and oat supplementation (2-3 % of the ration in form of whole oats or oat bran).

Conven-tional Farms	**Structural elements** (1/1,000 turkeys)	**Occupational elements** (1/500 turkeys)	**Emergency kit** (as many as seem fit to distract a herd behaving abnormally)
A	- Elevated perforated platforms	- Metal mobiles	- Green corn chips
			- Lifting systems connected to activity dispensers and hay baskets filled with hay bales - Straw bales
B	- Straw square bales	- Nibbling tubes filled with miscanthus briquettes - Pecking blocks	- Green corn chips - Sawdust - Green plastic buckets - Metal mobiles - Lucerne bales
C	- Elevated platforms - Straw square bales	- Activity dispensers filled with oats or grit	- Green corn chips - Milk powder filling for activity dispensers - Lifting systems connected to hay baskets filled with hay or hay bale on platforms
D	- Dust baths filled with rock flour or spelt husk pellets - "Escape" boxes (animals inside and on top)	- Dust baths filled with rock flour or spelt husk pellets - Activity dispensers filled with calf cereal - Pecking blocks	- Green corn chips - Hay baskets filled with hay bales

Fig. 1: Structural elements: Escape box (1), dust bath (2), straw bales (3), elevated, perforated platforms (4) (© #Pute@Praxis)

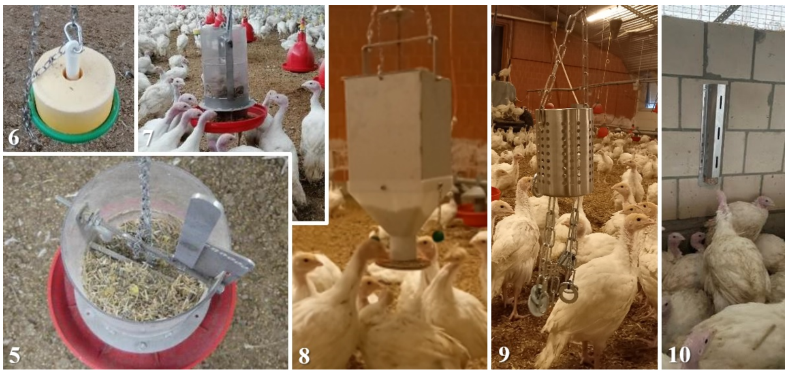


Fig. 2: Occupational elements: Calve cereal in activity dispenser (5 & 7), pecking block (6), activity dispenser with oats/grit (8), metal mobile (9), nibbling tubes filled with miscanthus briquettes (9) (© #Pute@Praxis)

Fig. 3: Contents of the "emergency kit": Lifting system (11) with connected hay basket (11 & 15), sawdust (12), green maize chips (13), activity dispenser filled with milk powder (14), hay bale offered on elevated, perforated platforms (16), lucerne hay offered in basket screwed to the wall (17) (© #Pute@Praxis)

In order to carry out the daylight breaks to synchronize flock behaviour, dimming curtains were installed on each farm. The turkey barns on the farms A-C were typical free ventilated barns with openable side walls. Depending on the position of the sun, bright patches of light fall through the gaps of the side walls of this type of barn, which can induce restlessness or even pecking in turkey flocks. In order to avoid this, additional elements were installed at the ends of the side walls. This created an even incidence of daylight and avoided light dots while opening them. At the same time an additional, controlled ventilation system was installed to ensure the desired air flow. The turkey barn on farm D was a hybrid type that was equipped with both a controlled and a free ventilation system. The side walls were transparent, letting in diffused daylight. Here, roller shudders were installed in order to realise daylight breaks.

All Farmers and staff were sensitised to immediately separate animals from the herd once they detected minor injuries and to only reintegrate them after the animals' wounds had fully recovered. To implement this, most farmers used fences to build separate compartments in the main barn, which were supplied by the main feed and water pipes. Most farms were already equipped with well-structured separation compartments, the sizes of which they could enlarge and reduce depending on the number of animals brought there.

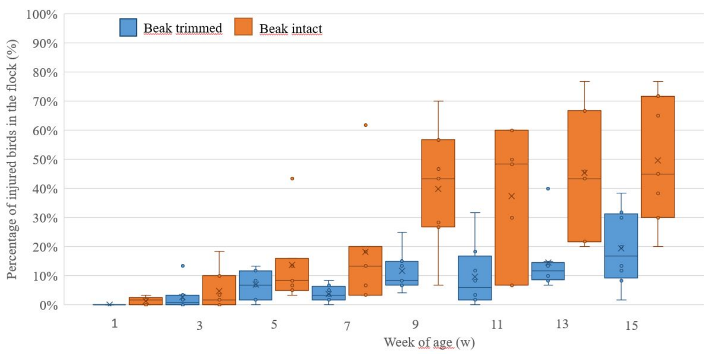
The four conventional farms housed between 2,000 and 7,500 B.U.T. 6 turkey hens (Aviagen Ltd., Tattenhall, UK). As part of the project, animal numbers, causes of loss and separation details were recorded continually by the farmers while the numbers regarding injuries in the flocks were collected every second week by the project team with a random sample of 60 birds on each observation day. At the end of each fattening run, the slaughterhouse reported discarded whole carcasses or single carcass parts. Across all conventional farms, data of eight beak trimmed (BT) and seven beak intact (BI) turkey hen flocks were collected in eight time intervals each (total numbers: BT= 39,560 hens; BI= 30,010 hens). The data was then merged by the project team, visualised and analysed descriptively using MS Excel (Microsoft Corporation, Redmont, WA, USA).

Results

Even though both types of turkey hen flocks used the offered structural and occupational elements steadily, there was still a big difference between beak intact (BI) and beak trimmed (BT) turkeys in terms of injuries, separation effort, mortality, and discard at the slaughterhouse.

On average, throughout all fattening runs across all conventional farms, 26.2 % birds of the BI flocks showed injuries somewhere in the head and/or body region. This was three times as much as in the BT flocks (8.7 %). For both flock types, injuries occurred more often towards the end of the fattening phase (see Fig. 4). In week 15 the average rate of injured birds in the flock was 49.5 % for BI and 19.4 % for BT.

Fig. 4: Proportion of injured birds found in the flocks at different weeks of age, separated by beak trimmed (blue, n=3,260) and beak intact (red, n=4,020)



The body area most affected by injuries was the snood for both flock types (ø BT=4.28 %; ø BI=14.66 %), followed by the head to neck area (ø BT=2.63 %; ø BI=5.72 %) and the tail area (ø BT=0.55 %; ø BI=6.41 %). The higher number of injured birds in the BI flocks also led to an extensive separation management. With an average number of 14.7 % of animals being separated from the flocks in order to be treated as well as shielded from opponents, the BI flocks were almost three times as labour intensive to take care of than the BT flocks (5.5 %). Despite the effort of separating birds, even with the smallest of injuries, injurious pecking alone led to mortalities almost three times as high in the BI flocks (2.6 %) compared to the BT flocks (0.9 %) as you can see in figure 5. In figure 5, the span width regarding the BI flocks represents not only different outcomes between flocks on the same farm but also different outcomes between the different farms.

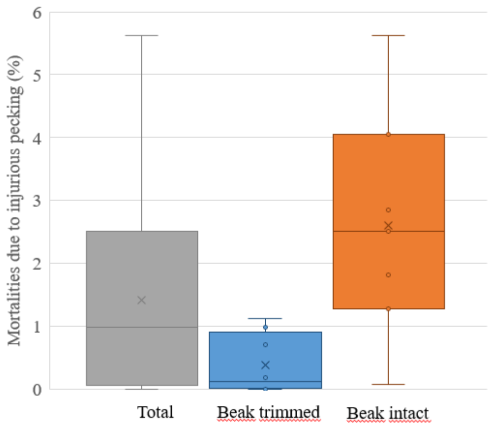


Fig. 5: Mortality rates attributable to injurious pecking only, separated by total (grey, n=69,570), beak trimmed (blue, n=39,560) and beak intact (red, n=30,010)

BI flocks showed only a slightly higher average percentage of discarded carcasses at the slaughterhouses with 0.5 % compared to 0.4 % of the BT flocks (see Fig. 6). But there was a wider range of outcomes for the BI flocks, starting at the minimum of 0.2 % and ending at the maximum of 1.15 % discarded carcasses, while the range was narrower for the BT flocks (0.2 – 0.6 %). All values were still below the “alarm values” recommended in Germany (here, the discard rate shall not exceed 2.0 %).

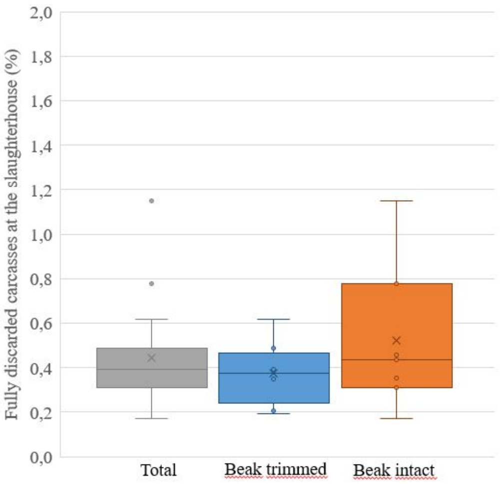


Fig. 6: Percentage of fully discarded carcasses at the slaughterhouses, separated by total (grey, n=64,890), beak trimmed (blue, n=37,340) and beak intact (red, n=27,550)

In terms of carcass parts, the slaughterhouse had to discard 1.1 % of parts on average in both the BI flocks and the BT flocks. This time the ranges of values were similar (see figure 7), even though the discard of parts of the carcass tended to be in the higher range for the BI flocks, starting at a minimum of 0.4 %, whereas the minimum for the BT flocks started at 0.1 %. The maximum was 1.78 % for both types of flocks. All values were still below the “alarm values” recommended in Germany (here, the discard rate should not exceed 3.0 %).

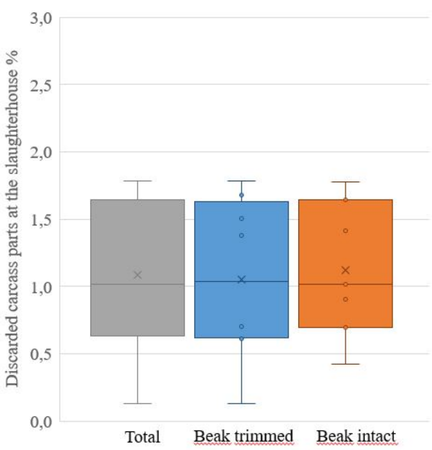


Fig. 7: Percentage of discarded parts of the carcasses at the slaughterhouses, separated by total (grey, n=64,610), beak trimmed (blue, n=37,200) and beak intact (red, n=27,410)

Conclusion

The increased incidence of injuries and mortality in the beak intact flocks shows that the environmental enrichment through "optimisation packages" could not prevent pecking outbreaks. Instead, there was a strong flock specific variation, regarding the occurrence and extent of injurious pecking, as the wide ranges of results regarding the beak intact flocks show. A very high level of separation management of injured birds in combination with elements of the emergency kit, including light reduction, was needed in order to maintain healthy flocks. This demanded quick, situation-dependent action from the farmers and was accompanied by high costs in terms of labour and materials. Not to forget the emotional burden the farmers had to endure when seeing injuries of this extent potentially every day.

Acknowledgements

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Adaption of Infrared Beak Treatment to Genetic Progress of Commercial Turkeys

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Infrared beak treatment (IRBT) is a commonly used practice to reduce injury, pain, and stress associated with aggression and feather pecking in commercial turkeys. IRBT is a welfare-friendly, precise, and fully automated treatment method, which uses a high energy infrared light to treat the beak tissue without creating an open wound thus reducing the risk of infection.

Due to genetic improvements of commercial turkeys and ongoing animal welfare discussions, it is desirable to adapt the method of IRBT. The study aimed to achieve a natural beak profile while minimizing the risk of feather pecking and cannibalism.

Several approaches were evaluated to achieve the desired beak treatment results. All tests were done with Nova-Tech Engineering's Poultry Service Processor (PSP) using BUT 6 commercial turkeys. The most successful way to minimize beak treatment is to reduce the size of the opening for the beak so that a smaller part of the beak tip is exposed during infrared light treatment. Therefore, a new interface plate for infrared beak treatment on the PSP was developed (Figure 1, Right) to replace the original interface plate (Figure 1, Left).

Figure 1

36 hours after treatment, it becomes evident that the treatment lines of the test birds are more closely located to the beak tip in comparison to the standard group. The comparison in treatment can be seen when comparing treatment lines (Figure 2).

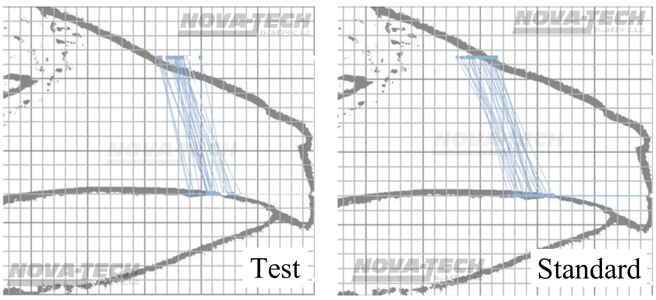

Figure 2

At 146 days, the upper and the lower beaks of the test birds are nearly equal in length, whereas birds of the standard group tend to show an offset between the upper and lower beaks (Figure 3). Although the beak treatment was less intense compared to the standard group, there was no significant difference between the test and standard group regarding weight development and mortality caused by injuries pecking.

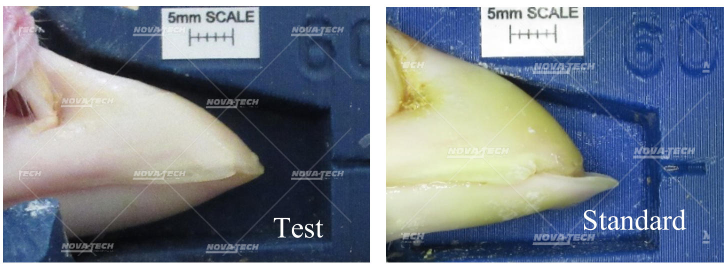

Figure 3

Figure 3

In conclusion, by implementing the test interface plate, with appropriate lamp power, we identified a beak treatment process for today's European turkey genetics that displays a natural beak profile.

Achievements in breeding for turkey welfare and future challenges

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Welfare landscape

Welfare aspects surrounding the production of turkeys is under increasing scrutiny. Societal concerns around the welfare of animal production are relayed through retail channels and regulatory authorities, resulting in increasing expectations of the standards of welfare. Examples of this are the current undertaking of European Food Safety Authority to generate a scientific opinion on the welfare of turkeys whilst in the UK, the Animal Welfare Committee is reviewing the welfare of livestock breeding and breeding technologies. Facts based and practical reviews such as these help understand the welfare status of the turkey industry and can highlight possible opportunities to further improve turkey welfare.

Management of bird health, nutrition, genetics and husbandry play a key role in the welfare of turkeys. This paper will focus on the work by the primary breeder to improve turkey welfare and highlights the selection technologies and measurable achievements that have been attained and the future challenges we need to address.

Breeding for improved welfare

Good animal welfare takes account of the bird's physical wellbeing. Incorporation of welfare related traits into the breeding goal can assist in development of breeds which are suited to the farming environment and have good health, vigour, resistance to disease and reduced likelihood of injury, pain or suffering.

By taking a balanced approach to selective breeding, it is possible to improve many different traits at the same time. Selection of some traits may impact positively or negatively on the development of other traits. Unfavourable relationships are regularly observed between production traits and health and welfare traits. Such antagonisms are handled by simultaneously considering multiple traits in the breeding goal and selecting birds which have better than average breeding values than the population average for those traits.

Whilst consumer preferences are evolving to an increasing awareness of welfare in animal production, long term breeding targets for improving economic efficiency are closely aligned to these goals. For many years, turkey breeding has focussed on minimising inputs of feed and antibiotics and maximising meat produced through a balance of welfare, weight, yield, and liveability.

Evidence for genetic improvement in welfare traits

Evidence for improvements in welfare traits can come from a number of sources.

Firstly, genetic trends can be used to track the improvement in genetic merit over generations using the vast quantities of data collected within the breeding programme. As the selected breeders are at the start of the multiplication chain, this can be used to predict the expected improvements for commercial producers.

Breeding programme information can also be used to generate phenotypic (observed) performance trends from within the breeding operation. These trends are a reflection of both the genetic improvement and environmental influences which, although controlled, vary between facilities and across time.

Finally, field performance of the final commercial cross is powerful evidence of improvement in turkey welfare. Collating data can be challenging as commercially sensitive information needs to be shared by producers, trials need to be conducted, or information retrieved from government reporting systems. This data is significantly affected by non-genetic factors which change over time or with differences between production systems so it requires some careful interpretation and is more valuable when available over a long-term basis.

Welfare traits

<u>Leg Health</u>

Leg Health has been a key feature of the turkey breeding programme since the 1970's. This began with walking assessment and selection against defects (Figure 1). Each bird is also screened for footpad dermatitis (FPD), hock lesions and toe defects. Any birds displaying any type of leg defects are not considered for selection (to contribute to the next generation). This policy has been a driving factor in reducing the genes associated with leg defects within our populations as demonstrated by Kapell *et al.*, 2017.

Figure 1. Leg health assessment in turkeys. Healthy (left), valgus (middle) and varus (right) deformities.

The scope of leg health assessment has been expanded to include the use of a hand-held x-ray device (Lixiscope) for the detection of clinical and sub-clinical tibial dyschondroplasia (TD) (Figure 2).

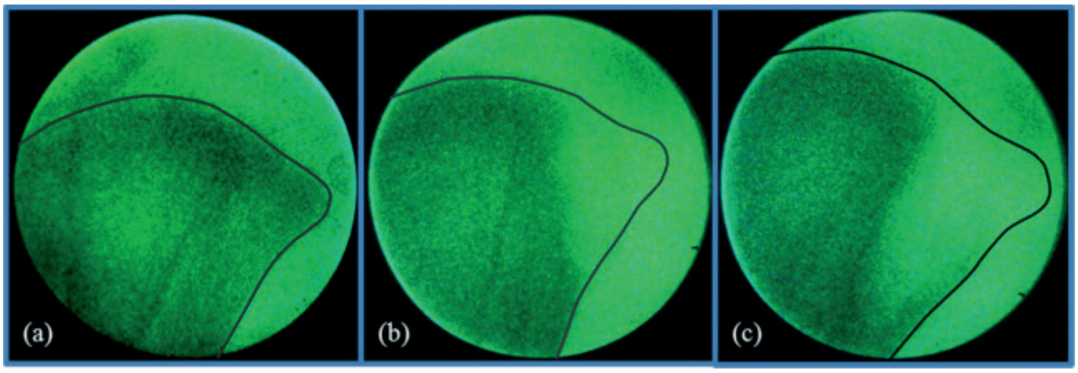

Figure 2 Lixiscope x-ray images showing tibial dyschondroplasia assessment in turkeys: (a) no lesions, (b) moderate lesions, and (c) severe lesions (Kapell *et al.*, 2017).

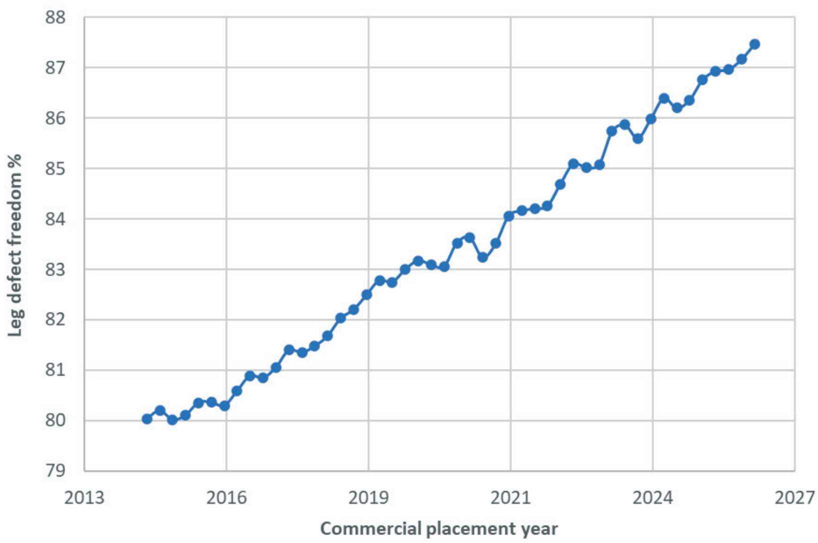

Figure 3. BUT6 genetic trend for selected leg health traits. The trend depicts the improvement in % defect free legs using information from clinical and subclinical leg health assessment and gait evaluations (Neeteson *et al.,* 2023).

The favourable effect of inclusion of a range of leg health traits in the breeding goal is clearly seen in the genetic trend (Figure 3) and improved leg health in the final commercial breed (Figure 4). Improvement in leg health has been achieved alongside liveweight gains. This is an example of the application of balanced breeding where adversely correlated traits can be improved simultaneously.

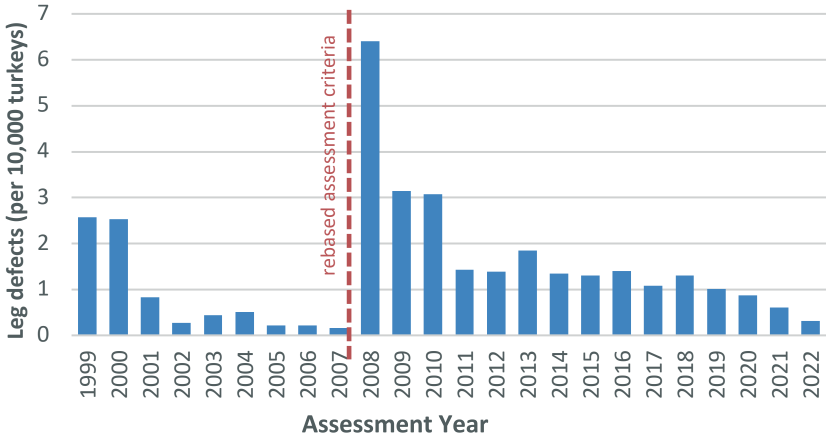

Figure 4. Leg health condemnation rates in turkeys per 10,000 turkeys from 1999-2022. Assessment prior to 2007 was for valgus/varus leg issues, after 2007 wider leg health criteria were used. Source: Agriculture and Agri-Food Canada (AAFC), 2023).

Footpad dermatitis (FPD) is a common welfare indicator. Genetic selection is performed by scoring footpads on every pedigree individual and selecting birds showing a low genetic predisposition to develop FPD. FPD scoring takes place in the pedigree environment and on siblings in the more challenging environment, to ensure robustness under field conditions.

Wet litter is a key contributor to the incidence of FPD (Mayne *et al.,* 2007). Individual water intake measurement is used to identify birds with excessive water consumption which has been shown to contribute significantly to litter moisture. The combination of targeted exclusion of individuals creating wet litter as well as those with a lower tendency to develop FPD is an effective genetic means of improving foot pad health of the population (Figure 5), (Neeteson *et al.,* 2023).

To maximise progress, the challenge for the breeding programme is to develop more effective methods of assessing traits. The measurement of FPD was improved by adding footpad shape which is very highly correlated to FPD but with around twice the heritability allowing greater levels of progress to be achieved.

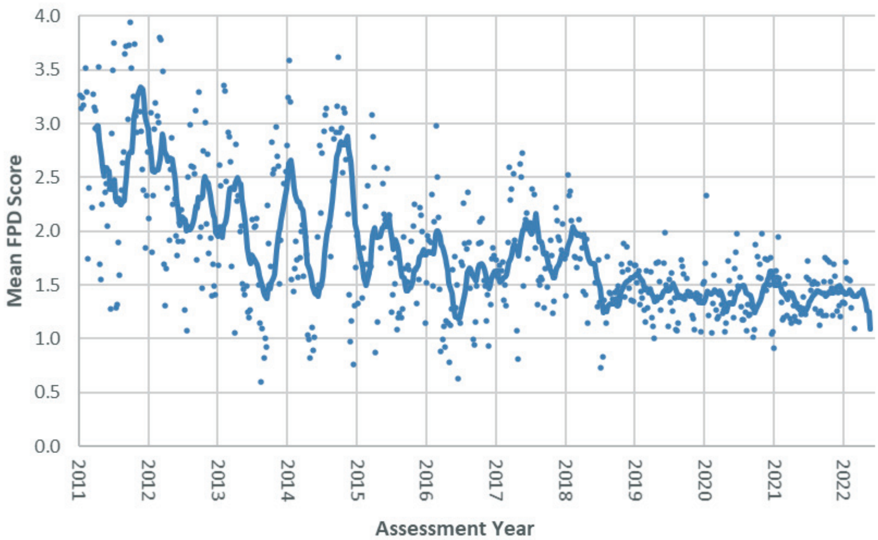

Figure 5. Trend graph showing mean FPD scores of BUT6 pedigree birds in the pedigree environment. Scoring: 0=clear, no FPD, 1 = less than 25% of the pad; 2= less than 50% of the pad; 3 = greater than 50% of the pad; 4 = pad and toepads affected. FPD = footpad dermatitis.

Future opportunities to further improve rates of progress in leg health may come from developments in medical imaging technologies. For example, computed tomography (CT) is already used for accurate measurement of meat yield in turkey breeding and algorithms have been developed to automatically detect sub-clinical incidence of TD. CT imaging also allows recording of a variety of novel skeletal and morphological features that could make it possible to determine the ideal architecture for good gait and balance.

Robustness

A key component of good welfare is the ability of birds to thrive in a variety of production environments. Pedigree facilities replicate typical farming environment, management and nutrition conditions and these have been continually refined over the years to keep them relevant to commercial practice. As an example, in 2013, a higher density brooding regime was introduced to reflect industry practice, which altered the expression of traits such as leg defects.

As pedigree birds contribute to future generations, the pedigree facilities are maintained to the highest biosecurity standards. This means that pedigree birds do not meet the spectrum of natural health challenges found in commercial poultry production. To measure the potential of birds when grown under natural health challenges, a parallel, dedicated commercial turkey farming system has been established where siblings of pedigree birds are grown and assessed in lower hygiene conditions, this is also known as the sib-test. Pedigree selections are then based on performance measurements from both locations (multi-environment selection), ensuring that only the families that perform well in both types of environments pass their genes on to the next generation.

Over time, this process of 'multi-environment selection' has had a dramatic effect on the robustness to various management and immune challenges, ensuring a higher level of welfare is experienced by the birds. This multi-environment strategy has made current generations of birds better able to adapt to the wider range of management circumstances they may encounter in the field. This testing of siblings has led to more robust animal populations with higher liveability and better uniformity.

Liveability

The turkey breeding program targets improvements in liveability through a number of traits. Mortality is recorded at all stages of the production cycle as well as in pedigree and sib-test environments. Liveability is also indirectly improved through selection for traits like leg health, carcass defects and feather coverage (to protect against scratching damage).

Incidence of mortality is recorded and linked through the pedigree to identify families that may be predisposed to higher mortality. By including this as a trait within our balanced selections, the commercial cross continues to see improved liveability each year (Figure 6).

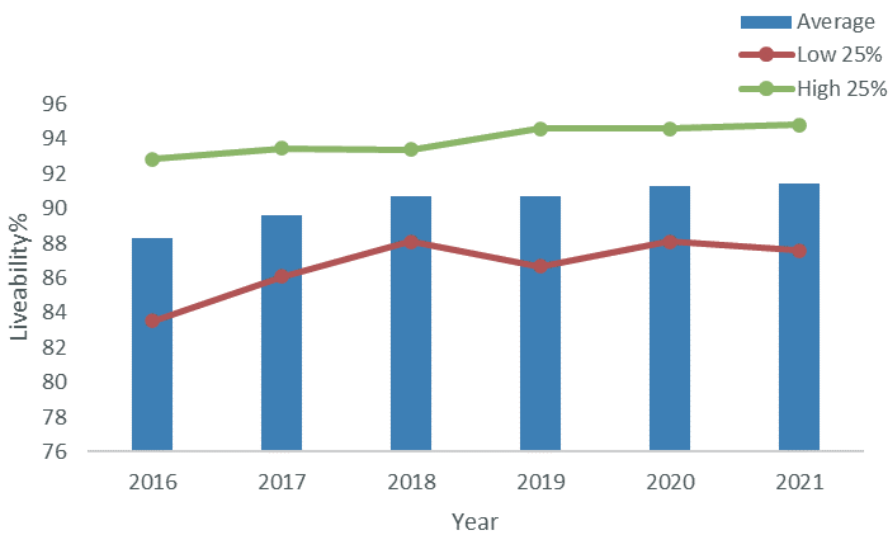

Figure 6. Field liveability of BUT6 commercial males in Europe. Chart shows average liveability and the average of the highest and lowest 25% of flocks for each year (Neeteson *et al.,* 2023).

Much of the improvements in liveability coming through improved leg health and robustness are observed in the second half of the bird's life. Selection for early liveability is also included in the breeding goal and our recent breeding research has been looking at novel traits to enhance rates of progress.

Turkey poults require relatively high management inputs in the first few weeks of life. With increasing automation, widespread movement to whole house brooding systems and reduced labour inputs, good start-ability of the turkey poult with an eagerness to find food and water is more essential than ever. The addition to the breeding programme of crop-fill scoring as an assessment of feed and water intake shortly after placement has a good heritability and a strong favourable correlation with early liveability.

Antisocial behaviour

Pecking and cannibalistic behaviours are part of the natural behaviour repertoire of turkeys. A range of on-farm interventions are used to counter the negative welfare effects of pecking e.g., infra-red beak treatment of newly hatched poults, environmental enrichment, nutrition, lighting levels.

Selection against pecking behaviour has been demonstrated in research populations of layer chickens (Kjaer & Sørensenb, G., 2001). It is also clear that there are differences in the level of pecking activity and damage between turkey breeds, indicating that genetic factors may also play a role.

Potential breeding strategies to reduce pecking related damage focus on (i) reducing the likelihood of birds being damaged (victims) (ii) reducing the ability to damage others (beak shape), and (iii) reduce the inclination to peck (pecking behaviour).

Most traits measured in the breeding programme are assessed by measuring birds and recording the bird identity at a handling event. Selection against behavioural activities such as pecking presents a significant measurement difficulty for breeders as the birds need to be allowed to express their normal behaviour in an undisturbed environment yet at the same time, we need to record the behaviour and identification of the individual involved. This challenge requires the development of novel approaches to gather the data required and our research in this area has resulted in practical selection techniques to reduce pecking behaviour.

Conclusion

Animal welfare is at the core of the selection processes in turkey breeding. Through balanced selection for better welfare outcomes alongside productivity, breeding is helping develop highly efficient breeds which perform well in a variety of environments and maintaining excellent standards of health and welfare.

The turkey breeding operation is continually looking to enhance rates of improvements in existing welfare traits and developing novel traits to address industry and wider stakeholder needs. Leg health, liveability and robustness are the current core welfare areas and there is good evidence for improvements being achieved. Future challenges surround novel and complex welfare traits, particularly those involving behavioural aspects. Innovation in measurement and recording technologies will have a role to play.

For welfare traits, the variation due to genetics is a small proportion of the overall observed variation. Management factors often have a large bearing on the welfare and production outcomes and here too the breeder has a role to play in developing and providing producers with management, nutrition and veterinary care advice that will further enhance welfare and optimise performance through the expression of genetic potential.

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Environmental enrichment: providing behavioral opportunities to enhance turkey health and welfare

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Introduction

Advancements in genetic selection and breeding practices, nutrition, management, and biosecurity have greatly increased turkey meat yield and productive efficiency. However, multifactorial issues such as injurious pecking (e.g., aggression, feather pecking, cannibalism) and leg health problems are still common across production systems. Among turkey toms in particular, lameness, and leg and skeletal health issues remain top health and welfare concerns (e.g. van Staaveren et al., 2020). One potential approach to reduce damaging pecking behavior and improve turkey leg health and walking ability is to provide turkeys with environmental enrichment. The use of environmental enrichment is commonplace in situations where non-domesticated animals are maintained in captivity, such as in zoos and aquariums, and the importance of environmental enrichment for animal health and welfare are broadly recognized in these contexts. However, the use of environmental enrichment in commercial turkey production is comparatively limited, yet environmental enrichment is becoming increasingly important as public interest in animal welfare continues to increase, alongside a growing number of animal welfare certification organizations that recommend or require the use of environmental enrichment. While research into effective environmental enrichment for modern, commercial turkeys is still limited, evidence from recent studies indicates that certain types of environmental enrichment can benefit turkey health and welfare by reducing injurious pecking and potentially delaying the onset of gait issues (e.g. Dong et al., 2023).

Environmental enrichment for turkeys

Environmental enrichment refers to changes in animals' environments that lead to improved biological functioning (including enhanced health status) of the animals (Newberry, 1995). Therefore, environmental enrichment is more than simply a change to an animal's environment. Environmental enrichment is generally categorized as social, occupational, sensory, nutritional, and physical (ADSA ASAS PSA, 2020; Table 1). However, some categories of environmental enrichment may be more feasible for use on commercial turkey farms than others. As outlined by van de Weerd and Day (2009), for enrichment to be successful, the enrichment used should meet four criteria including increasing species-specific behavior, enhancing animal health, improving production economics, and being practical and cost-effective to implement and use.

Table 1. Categories of environmental enrichment with examples (adapted from information in ADSA ASAS PSA, 2020)

Enrichment category	Description	Example
Social	Contact with animals of same species and/or humans	Housing turkeys together in flocks (standard industry practice)
Occupational	Objects or items that promote exercise and that provide turkeys with the opportunity to control some aspects of their environment or provide turkeys with some type of challenge	Providing turkeys with substrate to forage in, such as wood shavings
Sensory	Enrichments that rely on animals to use their senses (sight, sound, touch, taste, smell)	Music, video stimulation, or odors could be used but limited research is available regarding their use as enrichment for turkeys
Nutritional	Enrichment related to how or what animals are fed	Hanging feeders with supplemental feed, or scattering grain can be used but have not been assessed for effectiveness as enrichment for turkeys
Physical	Adding features or changing aspects of the animals' environment	Providing turkeys with platforms, straw bales, or visual barriers

Reducing injurious behavior and improving feather condition

Injurious pecking includes feather pecking, cannibalism, and aggression, and can lead to injuries, as well as economic and production losses due to reduced feed conversion efficiency, mortality and culling of injured birds (reviewed in Erasmus, 2018). Preventing injurious pecking and reducing pecking-related injuries are important goals within turkey production, and environmental enrichment can help reduce damage resulting from injurious pecking, but the benefits and effectiveness of enrichment depend on the type of enrichment provided. Results from studies conducted more than 20 years ago in experimental settings indicated that providing turkeys with straw, various objects that could be pecked at, visual barriers, and in some cases, perches, resulted in fewer injuries due to pecking compared to turkeys housed without these types of items (Crowe and Forbes, 1999; Sherwin et al., 1999; Moinard et al., 2001; Martrenchar et al., 2001). However, another study examined the use of plastic balls for reducing injurious pecking among turkey toms in commercial barns and reported no improvement in feather condition (Duggan et al., 2014). More recently, Lindenwald et al. (2021) reported that the use of a structure that provided elevated platforms ("turkey tree") for female turkeys resulted in lower levels of feather pecking and aggression, and improved feather and skin condition compared to turkeys that were not provided with the elevated platforms. Similarly, Dong et al. (2023) reported improved wing and tail feather condition when male turkeys were provided with elevated platforms, straw bales, or both a platform and a straw bale compared to turkeys that were not provided with enrichment.

Improving walking ability and skeletal health

Leg problems, including lameness and reduced walking ability, affect a large percentage of turkeys and result in important economic losses due to mortality, culling, and carcass downgrading. Leg problems have been reported as one of the main reasons that turkeys are culled (van Staaveren et al., 2020); consequently, any environmental changes or genetic selection strategies that can reduce leg problems can have profound benefits for turkey health and welfare. To date, research examining the benefits of environmental enrichment for turkeys is limited, but two studies have yielded promising results. Sherwin et al. (1999) reported that turkeys provided with occupational, physical and sensory enrichment (i.e., a wooden board with rope and screws attached, reflective sheeting sandwiched between clear acrylic sheets, cabbages, and supplemental spot lights that turned on and off) took longer to sit back down after being forced to stand, suggesting that these turkeys had improved musculoskeletal function compared to turkeys that were not provided with these enrichments. In a recent study, Dong et al. (2023) assessed the walking ability of turkey toms provided with various types of occupational and physical enrichment, including straw bales, wooden platforms, pecking blocks and structures that turkeys could use to hide from conspecifics (tunnels). Walking ability was assessed at 8, 12, 16, and 19 weeks of age. Results indicated that walking ability started to decline at 16 weeks for turkeys provided with the tunnel or no enrichment, whereas walking ability started to decline at 19 weeks for turkeys provided with a straw bale, platform, platform and straw bale, or pecking block. Studies with broiler chickens, for which lameness and walking ability are also major welfare concerns, have provided evidence that environmental enrichment can improve walking ability and reduce lameness (reviewed in Riber et al., 2018). For example, Bailie et al. (2013) reported that lameness was worse in broiler chickens that did not have access to straw bales and Kaukonen et al. (2016) reported that walking ability and leg health were better for broiler chickens that were provided with raised platforms compared to broilers that were not provided with platforms. Taken together, there is evidence that environmental enrichment may benefit turkey musculoskeletal health and walking ability, but further research will provide important insights to the types of enrichment that are most beneficial.

Considerations for the use of environmental enrichment in practice

Effective use of environmental enrichment for improving turkey health and welfare requires a programmatic approach that includes setting goals (e.g., reducing injurious pecking, improving leg health) and considering factors such as hygiene and biosecurity; some enrichments may increase the risk and spread of disease; and labor and cost; enrichments impose additional time, financial and labor constraints that may limit the feasibility of using certain types of environmental enrichment. Examples of environmental enrichments that are being used on commercial turkey farms are also listed within the audit tools of these organizations and include, among others, straw or hay bales (American Humane Association, 2020; Global Animal Partnership, 2022), elevated structures or ramps (Global Animal Partnership, 2022), vertical panels to divide the space, perches or vegetation (Humane Farm Animal Care, 2014; American Humane Association, 2020).

Conclusions

In general, the benefits of environmental enrichment for animal welfare include reduced performance of injurious behavior, such as feather pecking, and providing opportunities for behavior, such as foraging, and exercise. Environmental enrichment can also potentially improve leg health and delay the onset of gait problems, but further research is needed. Environmental enrichment is one tool that turkey producers have at their disposal to improve turkey health and welfare, and a programmatic approach to the use of enrichment will ensure that environmental enrichment is successful in improving turkey health and welfare.

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Role of probiotics in managing turkey health

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Summary

The 13th ESVAC report published at the end of last year shows that the industry is moving in a positive direction by reducing the use of antibiotics in animals. From the 2018 (118.3 mg/PCU) sales in the 27 EU Member States figures have decreased by 28.3% in 2022 (84.8 mg/PCU), reaching more than halfway the target to decrease aggregated sales to 59.2 mg/PCU by 2030 *(Thirteenth ESVAC report).*

However, growing consumer demands and new trends increasingly emphasised by poultry integration do not allow the cessation of efforts reducing the use of antimicrobials. And today's consumer demands meat of the highest quality, from farms with high animal welfare and, above all, from farms where antimicrobial agents are rationally limited or ideally completely eliminated during the production cycle.

The use of alternative solutions, such as probiotics (*Bacillus* sp. PB6) in turkey production, can significantly minimize the need to use antibiotics. Combination strategy of two alternative solutions, such as an immunomodulator (β(1,3)-glucan from *Euglena gracilis* algae and a probiotic (*Bacillus* sp. PB6), can result not only in strengthening the immune response after vaccination but also in improving the health status of the flock, which will result in lower bird mortality and better economic profitability.

Introduction

Turkeys are susceptible to a range of diseases. They can be particularly sensitive to respiratory diseases, gastrointestinal diseases, and viral infections. Some common diseases that affect turkeys include: *Avian Influenza, Newcastle Disease, Enteritis, Coccidiosis, Ornithosis, Mycoplasma Infections, Leg problems, Colibacillosis.*

To minimize the impact of diseases on turkey flocks, it is important to implement proper biosecurity measures, vaccination programs, regular health monitoring and good management practices, such as proper nutrition and hygiene.

There are several alternative preventive strategies that can be used to reduce the need for antibiotics in turkey production and maintain better health, such as: probiotics (beneficial bacteria that can improve gut health and enhance the immune system of turkeys), prebiotics (non-digestible dietary fibres that promote the growth and activity of beneficial bacteria in the gut), herbal supplements (various herbs and plant extracts have antimicrobial and immune-boosting properties), essential oils (derived from plants have been shown to have antimicrobial properties and can be used as natural alternatives to antibiotics), acidifiers (substances that lower the pH in the digestive system, creating an unfavourable environment for pathogenic bacteria. Organic acids, such as formic acid, propionic acid, and citric acid, can be added to the turkey's diet to improve gut health and reduce the need for antibiotics), immunostimulants (immunostimulants are substances that enhance the immune response of the turkeys, making them more resistant to diseases and responsive to vaccinations).

Many of the alternatives can be used in combination to reduce the need to treat with antibiotics, moving the focus away from treatment towards resilience and prevention. The holistic approach to successfully reduce antibiotic use is based on fundamental management practices. These in turn are based on biosecurity, disease prevention by vaccination, optimal farm management, good husbandry practices, as well as good feed and water management to optimise nutrient use and control microbial proliferation. Added via the feed or drinking water supplements are an integral part of this approach. *Bacillus* sp. PB6 and β-(1,3)-glucan from *Euglena gracilis* algae have proven to be efficient tools to support that.

Example from commercial production with ***Bacillus*** sp. PB6

Materials and Methods

In cooperation with a commercial turkey integrator, *Bacillus* sp. PB6 (CLOSTAT®) was applied across all flocks via water ($3x10^8$ CFU/L) in instances where indications of beginning intestinal health problems were observed. Data were collected for the first 27 calendar weeks of the year and historic data of the three previous years were the negative control. Data collected included diseases, antibiotic treatments and weather information. In the trial period, *Bacillus* sp. PB6 treatments were added to the recorded parameters.

Results and discussion Effects on antibiotic usage

Figure 1 shows the number of colistin treatments, per week. The usage of colistin was significantly (p-value = 0.004) decreased by 44 % (Figure 1). This did not lead to a shift towards other antibiotics, as their use equally reduced by 51 % over the reference period. The effect of *Bacillus* sp. PB6 application on the usage of beta-lactams was not statistically significant (p< 0.05), there was a numerical decrease of 13 % when the usage of beta-lactams in the trial year was compared to the usage in prior year (data not shown).

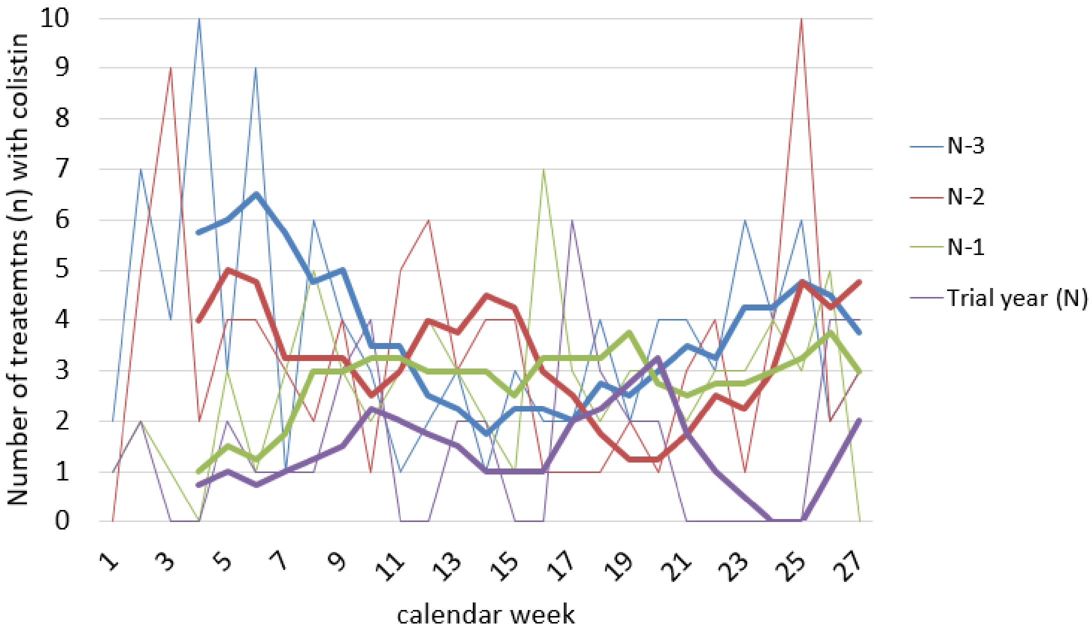

Figure 1 Number of colistin treatments by weeks.

Disease incidence requiring treatment was recorded by week. The incidence of enteritis reduced by 38 %, colibacillosis by 34 % and ORT by 38 %.

This trial generated evidence that a specific strain of *Bacillus* sp. (PB6) may reduce therapeutic antibiotic use in commercial farming.

The reduction in antibiotic treatments did not lead to a deterioration in animal health, but an improvement. The effect on the "other" antibiotics recorded showed significant reduction (p- value 0.002). Applications of tylosin, doxycycline, fluoroquinolones, TMPs reduced by 51 % over the reference period. Colistin is typically used for intestinal disorders. Despite the fact that a greater reduction could be observed in colistin usage, compared to the effect on beta- lactams. Despite this decrease in colistin use, colibacillosis incidence decreased by 34 %.

Overall, the trial presented here clearly indicated that it is possible to *Bacillus* sp. PB6 to reduce therapeutic antibiotic use in turkeys.

Example from commercial production with Bacillus sp. PB6 and β-(1,3)-glucan from

***Euglena gracilis* algae as a combination of alternative solutions**

Materials and Methods

The farm run fully antibiotic free production, and they were interested in any tool to improve the productivity of the farm, as its production cycle is susceptible to many threats. *Bacillus* sp PB6 was already used in feed at 4 x 108 CFU/kg and drinking water dosed depending on litter quality, but it was clear that β-(1,3)-glucan from *Euglena* *gracilis* algae was a potential candidate for an additional tool to improve the performance of the farm. This farm run a specific production cycle: until 23 days of age, all 25 000 birds are housed in one building, called P3. From 23 days of age, the birds are divided over 3 houses, 2 of those houses were included in this trial (P3 and P1). All birds received β-(1,3)-glucan from *Euglena gracilis* algae (Aleta™ D) at 1 kg per ton of feed until 23 days. At 23 days of age, the P3 building with 10300 animals on a surface of 1200m2 (8,6 animals/ m^2) continued to be supplemented with β-(1,3)-glucan from *Euglena gracilis* algae (1 kg/ton of feed) and the other building P1 containing 4700 animals on a surface of 700m^2 (6,7 animals/m2) was not. Body weight per square meter and mortality were monitored until slaughter age (110 days). Additionally, the response to vaccination (Dindoral SPF, a live hemorrhagic enteritis virus vaccine) was measured.

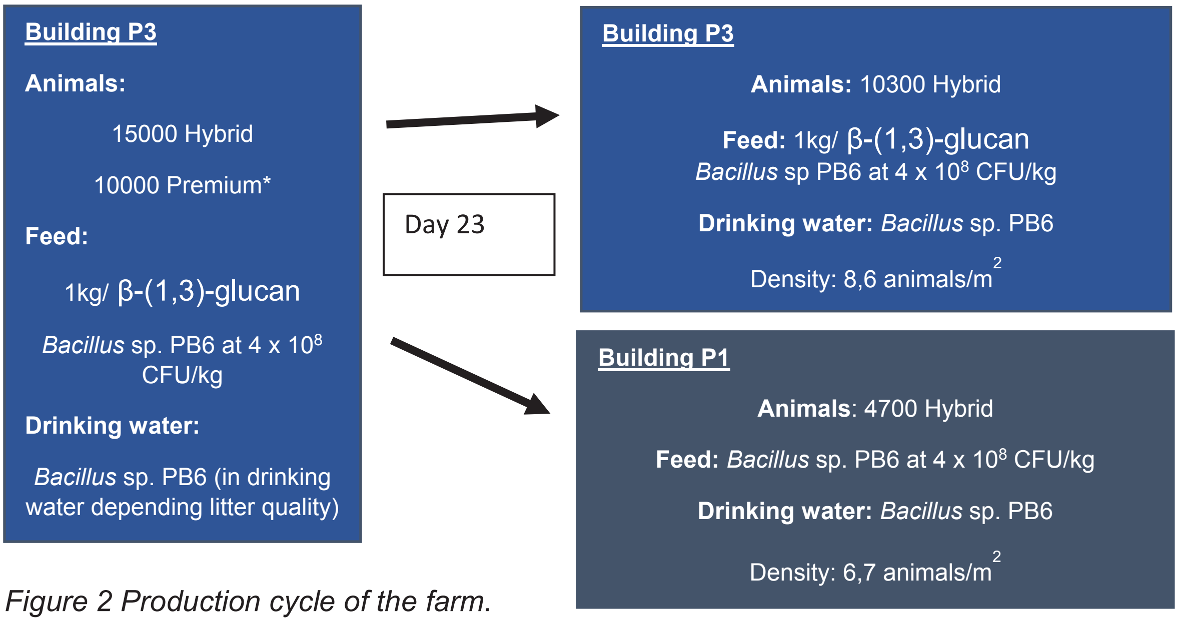

Figure 2 Production cycle of the farm.

Results and discussion

During this trial we could observe a 14% higher body weight per square meter and a 32% lower cumulative mortality rate in the β-(1,3)-glucan supplemented building compared to the control building. At 25 and 72 days after vaccination the antibody titers (IgG) in response to vaccination were respectively 36% and 32% higher in the β-(1,3)-glucan supplemented group compared to the non-treated group. Due to the improved immune status and disease resistance, mortality was reduced and birds were performing better.

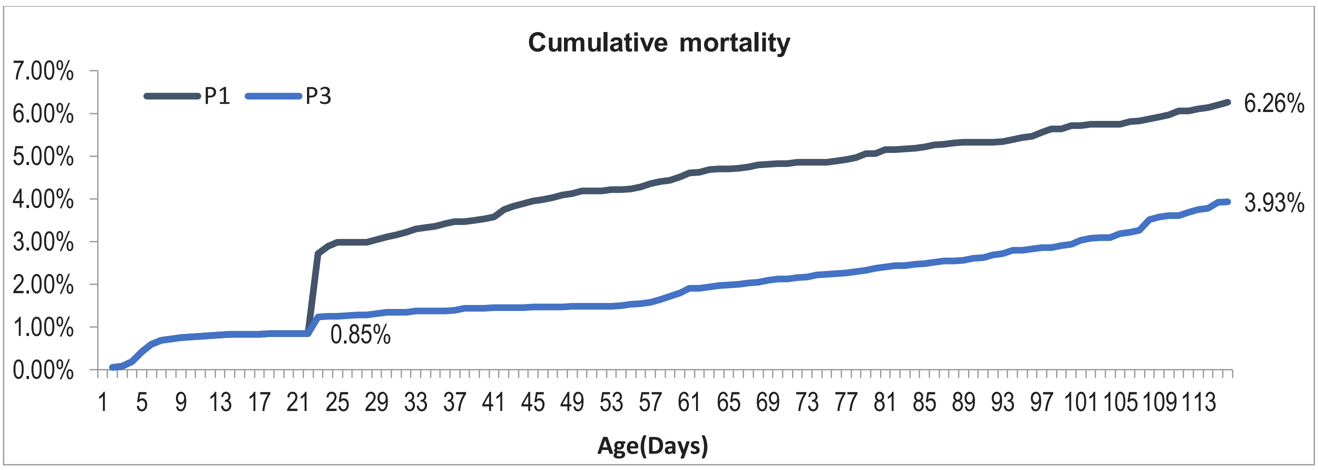

Figure 3 Cumulative mortality.

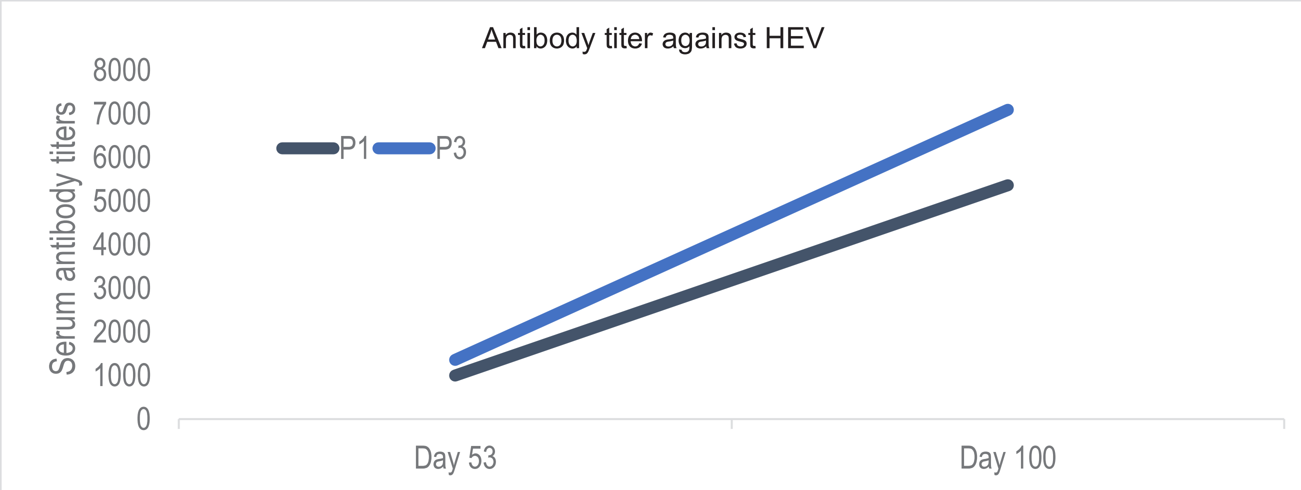

Figure 4 Antibody titer against hemorrhagic enteritis virus.

Conclusion

In conclusion, both the antibiotic reduction by application of *Bacillus* sp. PB6, and the effect being more pronounced with antibiotics aimed at intestinal disorders could be proven in the commercial field trial. To achieve further reductions in the need to treat with antibiotics, a new idea was therefore to look for a synergy between promoting good immunity and a favourable gut microbiome via probiotic application. Accordingly, a new product synergism was identified, β-(1,3)-glucans and *Bacillus* sp., providing a more complete solution to antibiotic free turkey producers.

References on request

Innovative selection methods to drive product performance

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Introduction

As the first link in the turkey value chain, our goal is to continuously improve performance. Selections are made to create sustainable progress for a variety of traits including egg production, livability, and behavior, but particular focus is placed on plant economic traits, including breast meat yield, feed efficiency, and live weight. Innovative tools and technology can speed up genetic progress when selecting for these traits. New technology can also have a positive impact on operational efficiency by automating processes, making farm laborers' tasks less labor intensive. Since Hybrid is a part of Hendrix Genetics, we can leverage our multi-species knowledge. Here we want to give a few interesting examples of technology cross-pollination in Hendrix Genetics. We will look at farrowing in sows, rooster mating behavior for traditional poultry, smart trap-nests, and walk-over-weighing for turkeys.

Technological challenges for breeding companies

There are many animal management systems available that have sophisticated metrics at the flock level. Some examples are feed and water consumption, barn temperature and humidity, lighting control. However, for our breeding programs, we need these metrics not at the flock level, but for every individual animal in the barn. Often, there are no commercially available solutions that meet our needs. This is where the Digital Phenotyping team at Hendrix Genetics comes in. Our goal is to help our breeding programs in layers, swine, traditional poultry, shrimp, trout, salmon, and turkeys to digitize the way in which individual performance characteristics, or traits, are measured.

The technologies we use to record behavior can be grouped into two camps: wearables, cameras. Wearables are devices that are worn by the individual animal, much like a smartwatch. Cameras on the other hand, are observing the animals from a distance. Both technologies have their pros and cons. Sometimes we use both at the same time.

From data collection to decision making

All the data coming in is digital. In the case of wearables, it's high frequency measurements of movement in all directions. In the case of cameras, it's high-resolution video. This "raw" data does not provide any insights. We design and build data pipelines for data driven decision making. These are software processes that ingest raw digital data, aggregate the information within and present the data to the end-user in a human-readable format. These steps require knowledge of software and data engineering for efficiency, as well as data science for prediction models. Another important aspect of the data pipeline is the prediction model. Nowadays we mostly train artificial intelligence models for this. To learn what parts of the raw data are important, these models are trained with examples, so it's important to have access to a large quantity and good quality examples, such as phenotypes. Fortunately, as a breeding company, we have plenty of those.

Below, we will demonstrate a few of our recent digital phenotyping projects, to give an idea of what is possible. We start with automated farrowing detection, then we look at natural mating behavior in traditional poultry and we finish with our new turkey smartnest.

Farrowing performance of sows

For our swine breeding program, we wanted to get a better understanding of the farrowing process in our pure lines. What started out as a simple and quick video of a sow in a farrowing crate with a mobile phone, turned into a fully scaled-up automated system that records and collects insights on 1000s of farrowing events.

Every farrowing crate has a camera module mounted above the farrowing crate (Figure 2). This module is set to record an image every minute. Once the farrowing starts, in the camera image, most of the time the piglets are visible. We have trained a computer vision model to recognize the piglets in the images. Once we were able to accurately recognize piglets, we were also able to count them over time. A farrowing process takes between 2 to 5 hours (200 data points per sow), so a few inaccuracies here and there do not matter much. With the piglet count increasing over time, we can then look at the total farrowing time (the time it took the sow to birth all her piglets) and the variation of the time between piglets consecutive piglets. These new phenotypes are used by our geneticists breed for more sustainable pigs. Our Hypor sows farrow piglets at a more constant rate, leading to better piglet survival.

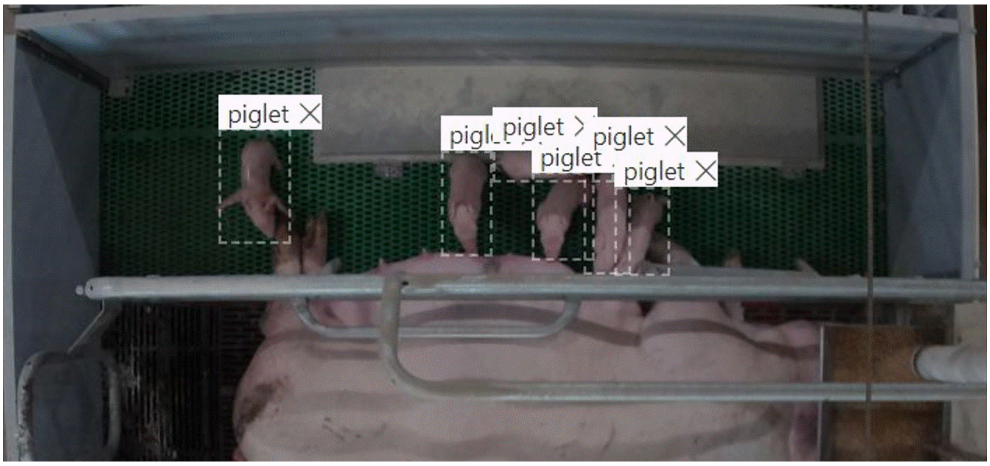

Figure 1: An image from a farrowing camera.

Natural mating in traditional poultry

About five years ago, we invested in some expensive wireless trackers for turkey breeding. Originally these trackers are designed for Hollywood productions, to track human motion very accurately and to transform that into movement of animated characters like the Avengers. The idea was that we could log the raw data from these trackers and then, with enough examples from human experts, train a machine learning model to automatically predict individual turkey walking scores.

More recently, we got a request from our traditional poultry geneticists, to investigate natural mating behavior. We want to select for roosters that create plenty of offspring. Without automation, natural mating of individual roosters is hard and costly to quantify without a lot of hard work by human experts. Because we could leverage our experience with the turkey walking score trackers, we were able to quickly do a proof of concept for our traditional poultry business. In this proof of concept, we were able to demonstrate that it was technologically feasible to accurately quantify rooster natural mating. Now, we have scaled up the technology and designed and built bespoke trackers for natural mating. We were able to do this efficiently, because of our expertise with the turkey trackers.

New turkey smartnest

We have automatic nests for all our poultry species, these nests automatically collect egg production data per individual hen for our pure line animals in group housing. For turkeys we already have an extensive trap-nest infrastructure globally, so for this project we opted to retrofit existing trapnests with smart electronics.

We have developed a modular unit for every nest, complete with egg sensors, RFID antennas for hen detection and door sensors to detect nest occupancy and lay duration. We were able to develop this system, consisting of more than 100 custom components, completely in-house, through our rapid prototyping workshop. We were able to do this quickly, because of our experience with programming development boards (small computers) from earlier aquaculture and laying hen projects. The new turkey smartnests are now collecting a wealth of data on egg production and behavior traits such as nest visit duration and nest preference (Figure 2).

The automated collection of individual egg production has great potential for downstream application to gather information on the effects of genetics combined with different environments and then to feed this information back into the genetic engine. In doing so the industry will see enhanced progress in breeder hen performance.

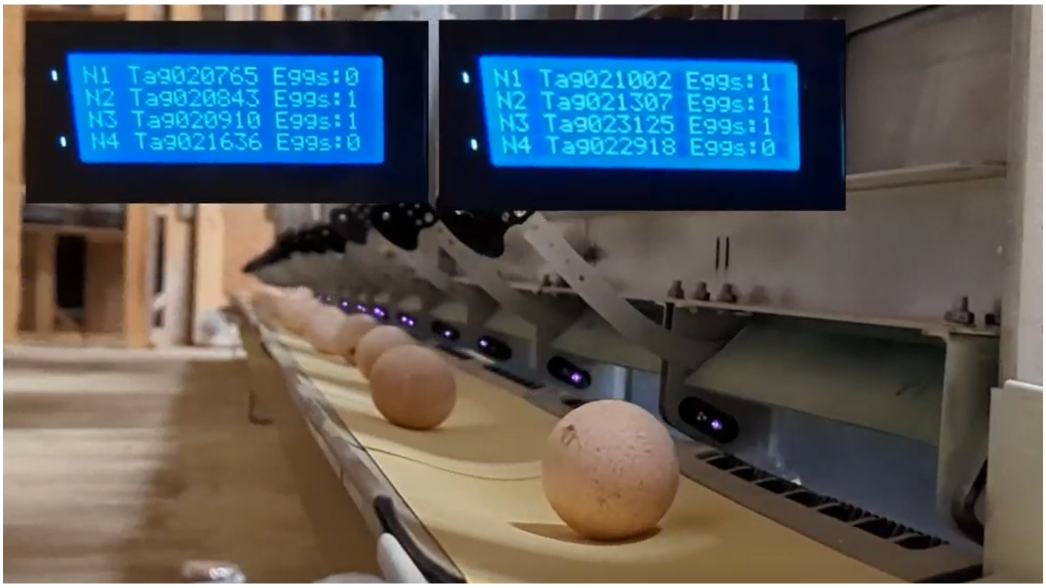

Figure 2: Eggs counted in our new turkey smartnest.

Benefits of a multi-species approach

Innovative selection methods are an important part of fueling the genetic engine. Technology continues to evolve and improve at a breakneck pace, and there is huge untapped potential of how these tools can be applied in the field of breeding and genetics. At Hendrix Genetics we strive to maximize the use of these innovative technologies through our multi-species portfolio of technology and data solutions. We continue to explore the use of new developments to deliver excellent performance at all levels of the turkey value chain.

Turkey embryo comfort during incubation and hatch

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One significant problem facing the turkey industry is hatchery losses.

Incubating and successfully hatching turkey eggs, with as minimum losses as possible, has always been a more challenging task.

Data going back to 1995 showed that, in all the commercial hatcheries in US, a total of 412 million turkey eggs were set and only 317 million poults were placed. About 95 million eggs (23%) either failed to hatch or produced weak poults. The average hatchability was below 80%. The situation was no different in Europe.

But over the past almost 30 years, with the help of new incubation technology, the added value of considerably new information regarding turkey embryology and the tremendous work of genetics companies, hatcheries all over the world have been reporting rewarding results.

The early development of the chicken embryo has been long categorized into 14 stages. A similar staging sequence for the turkey was not proposed until 1993, when it was described the early development of the turkey embryo, which was divided into 11 stages. Obviously, chicken embryo staging didn't apply to turkey embryo staging.

Of significance is the observation that at oviposition the turkey embryo is in Stage VII. In contrast, the chicken embryo at oviposition is in Stage X. Similarly, the hypoblast, which is already apparent in the Stage X chicken embryo, does not appear in the turkey embryo until the egg is incubated.

It is apparent that the turkey embryo is less mature than the chicken embryo at oviposition. This could be related to differences between the hatchability of turkey and chicken eggs and the concept that turkey eggs need more "comforting", from oviposition until hatching the final product.

Even though the variability amongst hybrids is high, if we refer to % of hatchability, we at Petersime believe that, if we best comfort the developing embryos as it transitions through its fundamentally different stages of incubation, we can achieve the top rate of hatching the best quality poults.

Assuming that the hatchery receives good quality turkey eggs, how do we best comfort the preincubation & incubation embryo stages?

WE DO RESTORE (SPIDES): considering that the turkey embryo at oviposition is only in stage VII of preincubation development, ReStore is a very effective method of advancing the germinal disks to a more mature strong stage and aligning more embryos to a very similar start, thus producing a shorter hatch window.

WE PROPERLY PREHEAT the turkey eggs: a good preheating is to be done in the setter. It will properly thermally balance the air content inside the setter and all around the eggs. Let's keep in mind that some turkey hybrids have thicker shell and less pores compared to others and that the germinal disk, located in the center of the egg, is the one that needs to "feel" the comforting prewarming temperatures. If your setters do not reach air temperature set point in max 5 hours since the start of the incubation program, most likely the duration of the preheating was not enough (referring to standard Petersime preheating program).

A short preheating will produce a wider span in the start of embryo development, so in the end a wider hatch window.

THE ENDOTHERMIC PHASE promotes the developing of a strong heart and blood system. Why are these needed? Because the genetic companies have built the hybrids to have phases of rapid growth. These phases of fast development of the skeleton, muscles and organs challenge the blood system of the bird to deliver to the fast-growing tissues a correspondent amount of nutrients and oxygen. If the heart and the circulatory system cannot cope with the high demands of the rapid growing tissues, consequently the rate of growth will be affected, and the birds will not achieve the targeted production specifications.

More than that, some birds can even suffer from heart attacks and bleedings in the breast muscles, which is the most valuable piece of the carcass of a commercial turkey. The bleedings in the breast muscles, that could root back to a weak blood system of the developing embryo, are an unpleasant surprise discovered in the slaughterhouses and automatically will increase the number of losses of the slaughterhouse.

During this "construction phase" of the turkey embryos, keeping the setters closed until day 11 of incubation, with hardly any ventilation open – regulated by CO2 set points, will help build up good levels of CO2 that will trigger the production of a good number of red cells in the blood. The red cells are the ones carrying oxygen to the growing tissues, thus feeding the metabolism.

THE EXOTHERMIC PHASE is the period we have at our disposal, as incubation experts, to drive the eggs towards **a proper weight loss by the time of the transfer.** We cannot influence much the growth of the air cell during the first 11 days of incubation, due to the high increase of humidity in the "closed" setter. But, starting day 12-13, until day 24:12, we must ensure the size of the air cell decreases towards 1/3 of the longitudinal dynameter of the egg. That translates into a minimum of 11-12% moisture loss of the eggs at 24d:12 hrs approximately.

No batch of turkey eggs should be incubated without knowing the initial average weight of the eggs at set, so that, throughout the incubation period, regular checks of the weight loss are performed and ventilation adjusted accordingly.

Why is weight loss so important for comforting the embryos?

Improper weight loss is only bound to cause losses because:

- The size of air cell will be above the 1/3 of the longitudinal diameter of the eggs and the embryos will not be able to find it, when the time comes to pip inside the air cell and transition from CAM respiration to lung respiration. As a result, some will drown in the amniotic fluid, others will desperately extend the head so high looking for the air cell (the instinct of IP is so strong that it will overcome anything else) that will push the head above the right wing. If by doing this they find the air cell, they are only half saved, "lucky "only to be alive for a little longer, but that "head above the wing" position will not ensure the next necessary action of pipping the shell and finish the hatch.
- The size of the yolk sack will be too big, the embryo, like an "athlete" getting ready to run the marathon, will have a heavy load to carry which is totally not comfortable. The consequences of carrying a big yolk sack are easy to notice when doing breakouts at hatch:

-the embryos get stuck into abnormal positions because the uncomfortable big yolk sack prevents a smooth positioning of the embryos in the correct position. For me, after 10 years of hatching turkey eggs, any abnormal position of unhatched embryos, automatically means improper wight loss of that certain egg.

-Sometimes, as they push with their feet against the pointed end of the eggshell, looking for the upper positioned air cell (due to insufficient weight loss), they break the big yolk sack with the claws and yolk content will automatically cause a septic shock, killing them.

- the uncomfortable weight of a big yolk sack, even if it gets retracted in time and the navel closes properly, will force the hatching embryo to consume more energy and get tired more easily during the hatch. Let us not forget about the complex processing of the poults the day of the collection in the hatchery. That also consumes a lot of their energy reserves. Why don't we make sure we "push" the eggs to a proper weight loss, thus creating "fit" athletes that can comfortably run "the marathon" and save their energy reserves for the tiring day of the processing and the first days in the rearing farm?

I had the best results when I did not take for granted the evolution of the weight loss of any flock, no matter how many times I incubated the eggs of a same flock. Every time I had to incubate turkey eggs of the same flock, I made control trays for each of the flocks processed. It is the best way to comfort the embryos and guide the moisture loss of the eggs towards a good size of the air cell.

Should we fail to achieve 11-12% weight loss at the end of the exothermic phase, the "battle" for comforting the embryos in the setter is not entirely lost, we can continue the actions of further comforting the embryos in the hatchers, until about 26d:18 hrs when we should start triggering them for IP. I will come back to this once we address the comforting during the hatch.

THE EGG SHELL TEMPERATURE is one very important detail we need to account for, as it is the one that actually regulates the metabolic rate of the embryos.

Just like us or any living being, the embryos also need a good balance between anabolism(growth, maintaining and renewing of tissues) and catabolism (obtaining of energy). The perfect balance between these two processes is regulated, for birds' embryos, by the temperature applied during incubation.

As we all know, commercial turkey embryos were under tremendous genetic improvement in the past decade and nowadays have a considerably bigger body mass, as the size of the eggs has gradually increased. Thus, the modern varieties of the commercial turkey embryos cannot find comfort in the previous incubation programs, regarding eggshell temperature. 100.0 F turkey eggshell temperature at transfer might have been comforting a decade ago but not anymore. Having a bigger body mass automatically increased the production of metabolic heat. The overall higher heat production of the eggs of a very fertile flock requires lower air temperatures as the embryos progress throughout the incubation days, to prevent the embryos of going into excessive catabolic activity due to overheating.

High eggshell temperatures will push the metabolic rate into a faster rhythm. Carbohydrates and fats, which are the main source of producing energy but take longer to break down, will no longer be sufficient for the "thirsty" metabolism driven by high environmental air temperatures. The metabolism will look for an alternative "fuel" that is easier to break down and provide the necessary energy faster. That alternative fuel are the proteins. Using this "fuel" will suffice the high demands of a fast metabolic rate. But that is not "fair" as the proteins are the main "fuel" of the immune system that will be weakened.

Weak, tired, uncomfortable embryos will no longer be able to perform properly during the hatch, thus delivering poorer hatch results. Some, as they had finished the energy reserves, will just stop during the last hours of their embryonic life. Others, hatched, will no longer be able to fight the normal bacterial load that they meet the very few hours after hatch and will become sure victims of E Coli or Preudomonas.

These are losses that are also an expression of how comfortable the embryos were during incubation. They could be prevented if the embryos were allowed to have a "comfortable" metabolic balance between anabolism and catabolism, by gradually reducing the eggshell temperature as they progress throughout incubation days.

AFTER TRANSFER

Between day 24:12 and 25:16, when the main retraction of the yolk sack should take place, good ventilation rates promote that because the retraction of the yolk sack is a pure metabolic act: oxygen is needed for the rhythmic contractions of the umbilicus muscle to draw the yolk sack into the body cavity leaving a direct connection to the small intestine via the yolk stalk.

Making sure that plenty of air is available to all the hatching eggs, while the stimuli like CO2 and temperature are on lower set points, we comfort the embryos by resting them, allowing them to rebuild the glycogen reserves in the muscles and prepare for the stimulation steps. The lower air temperatures in the resting steps of the hatch program also keep the embryos away from the instinct of internal pipping, while the retraction of the yolk sack advances or even finishes. Should we stimulate them with higher air temperatures in these resting steps or hatch them in a program that has no resting steps, we only expose them to discomfort because:

- We promote a fast metabolic rate that will drain their energy reserves.
- We expose them to the instinct of an early internal pipping, while the yolk sack hasn't been retracted yet. They will be "multitasking" retracting the yolk sack while also pushing for internal pipping. This is a huge consumption of energy and drainage of the glycogen reserves, a sure path to tired embryos and weak poults.

Respecting these resting periods only comforts the embryos, as we expect them to perform like "good athletes" during the hatch. Not accounting for these needs will promote hatching of weaker poults, more second quality poults, pipped not hatched or late dead in shell.

Let's pay a closer attention to their comfort during each step of their evolution from early embryo stages of development until they leave the hatchery and the results will be rewarding.

The Latin American Turkey Industry

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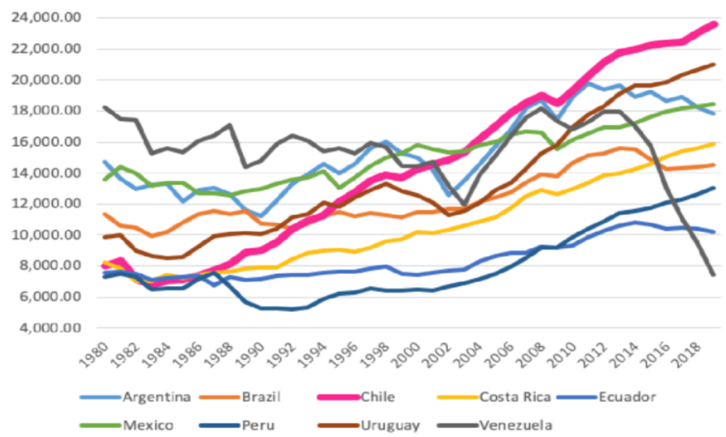
General Overview

Latin America is a diverse continent, in cultural, political, income and many other terms. Climate across the area, is diverse too, ranging from tropical rainforest areas, to arid desert and tundra places. This influences the economies and population behavior, inside each country and across the region.

Gross domestic product (GDP)

GDP is a good indicator to express the difference in income by countries and plays a role on the turkey meat consumption, as the price is higher than other sources of animal protein.

Chart 1: GDP of Latin American countries



Per capita GDP, selected Latin American countries (1980-2019)

Population in Latin America.

Including Central, Caribbean and South America, the population is 667MM. This represents 8.3% of the world population with Brazil (216 MM), Mexico (129 MM), Colombia (52 MM) and Argentina (45 MM) being the most populated countries in the region (Source: www.Worldometers.info).

The net migration for the region is -170,000 per year, indicating people leaving the region to live and work. The median age is 31.0 years.

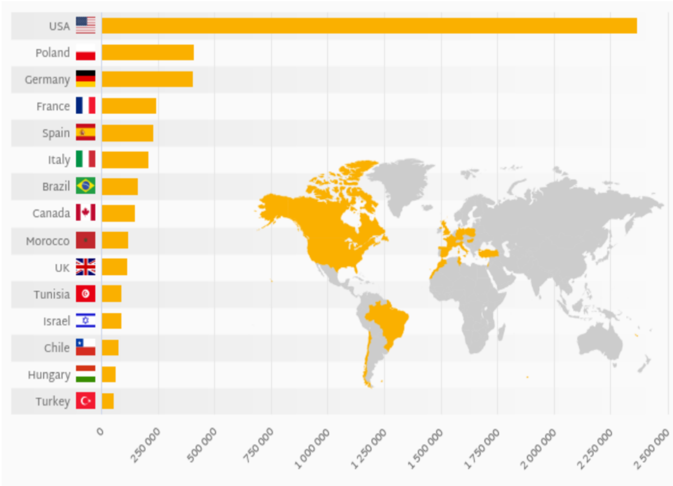
According to the IMF, in 2023, 84% of the population is urban, which creates a challenge for agriculture, making it difficult to find people willing to work in rural locations where poultry activity is being developed.

Labor to work in agriculture used to be a competitive advantage for the region but currently it is difficult to hire good people willing to do a good job in a continuous process like the poultry sector.

Turkey Production in Central and South America.

Countries with industrial turkey production in the region of Latin America, are Brazil, Chile, Peru, Bolivia, Ecuador, Colombia, Mexico. Looking at the largest turkey meat producing countries in the world, (*chart 2)* 2 out of top 15, are based in South America. These are Brazil and Chile and both of them are important exporters.

Chart 2: Turkey meat production (tonnes) by country, 2022. (Source Faostats)

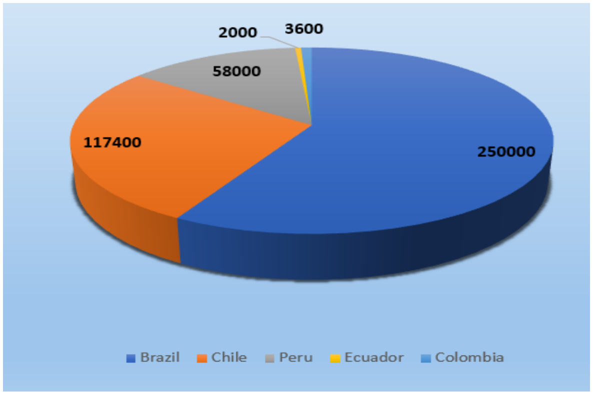


Unfortunately, during the last 10 years, both Brazil and Chile, have reduced their turkey production due to company decisions and the challenges that European markets have imposed on the exporters.

Some countries and companies have year-round production, as these companies have breeders or an annual placement program of day old poults. Other countries/companies, are seasonal, focused on end of the year consumption.

With, 250,000 breeders hens lit per year, Brazil is the largest in turkey production, followed by Chile with 117,400; Peru, 58,000; Colombia with 3,600 and Ecuador placing 2,000 in production (*Chart 3)*

Chart 3: Breeders hens lit, by country in South America



With this number of breeders, companies based in South America are able to produce 28.5 million birds and Brazil is the largest with 18 million. Mexico, Ecuador, Bolivia and Venezuela import day old poults, both for their seasonal demand or throughout the year.

Particularities of the Latin American turkey market.

With the exception of Brazil, most of the turkey producing countries do not produce raw materials. Soybean is the Brazilian main crop, producing 318 million tons. Corn production in Brazil is about 120 million tons (Conav.gob.br).

Turkey feed is approximately 8% more expensive than broiler diets and the yield per square meter is about 15% less. Companies located in countries far from the main sources of raw materials must be extremely efficient, looking for a great technical performance, maximizing the genetic potential and the cost of production and processing.

South America is still an immature turkey meat market as the demand is very seasonal. In some countries up to 70% of the production is focused on the end of the year. Some companies are in a process of developing cut-up and added value products to flatten the demand.

In this sense, Chile is the country with a broader product portfolio, allocating about 30% of the production for the domestic added value market.

A fact that complicates the development of the turkey industry is that many companies in the region are multi species agricultural groups. Inside these groups, they have a turkey business unit, which is usually the smallest in terms of sales, revenue and volume.

According to the income of the countries, domestic markets in many cases, aren't willing to pay this extra cost and consumers preference is for the cheaper animal proteins.

Some companies, but not countries, have invested significant financial resources on marketing to promote the domestic consumption of turkey meat as well as product development processes to add value to the meat. This, in addition to market research to properly position products have allowed some development of the turkey business units.

This explains why companies in Brazil and Chile, have put their focus on exports to the Northern hemisphere markets of North America and Europe, looking for consistency in prices. This of course, is an additional challenge, as the livestock practices and management at processing plant must meet the requirements of the importers.

As turkey business units have to compete internally for the allocation of economic resources, companies in the region are extremely focused on maximizing performance, getting in general, really good results in breeders, commercials, poultry health and processing.

BRAZIL.

Brazil is the largest country in Latin America, both in surface and population. The companies are Brazil Foods and Seara. The consumption is 0.5 Kg per person, per year. Until 2018, Brazil was the 2nd producer, with about 400,000 tons per year. However, internal difficulties, regulations and challenges had a negative impact on the production. Current production: 157,000 tons per year (Chart 4).

Processing weights: 24% of the production goes at 4.2 – 5 Kg hens. Same percentage for heavy hens (6.5 – 10Kg). Males at 22 – 23 Kg.

Chart 4: Turkey meat production in Brazil (X1000)

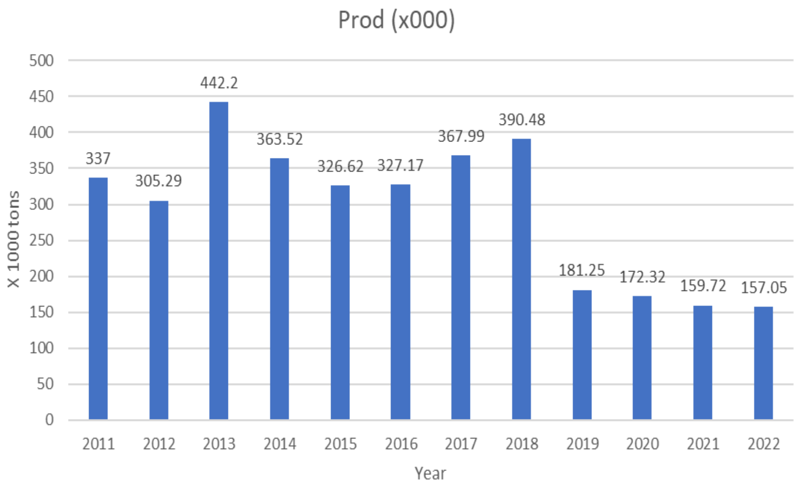
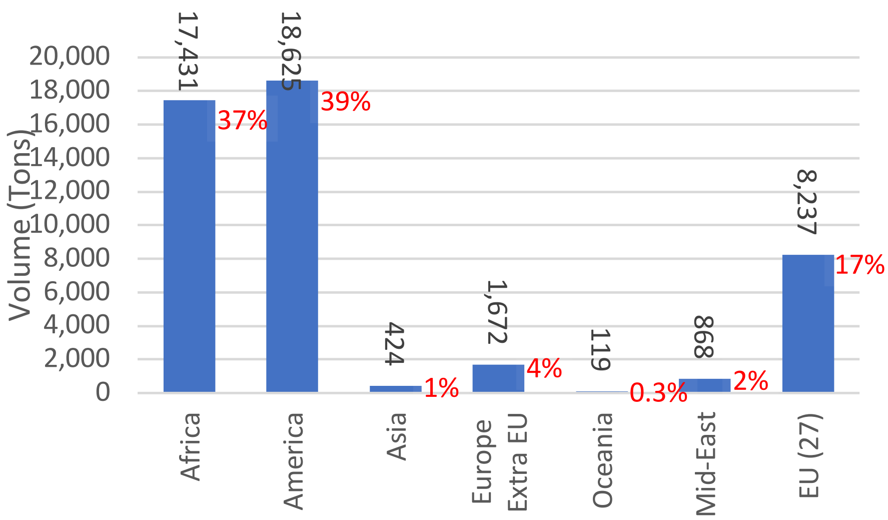


Chart 5: Importing regions of Brazilian Turkey Meat (2022-2023)



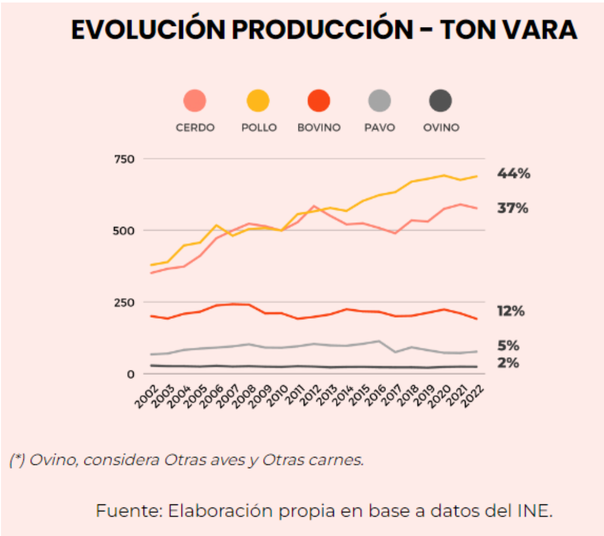
More than 87% is exported cut and 11.5% further processed. Currently, almost 40% of the exports, are sent to America. Similar percentage to Africa and more than 17% to Europe (Chart 5)

CHILE.

Population is 19 million. Suitable locations for poultry competes against other agriculture, making turkey production tighter in terms of resources allocation. Chilean corn production is less than 2% of the needs. Soy bean is less than 1%.

Despite this, Chile used to be ranked in the top 10 turkey producing countries in the world, with more than 130,000 tons per year. Domestic market for turkey meat is strong, both in prices and per capita consumption. Early 2,000's, it was 4.7 Kg. Current consumption is 3.5 Kg per year, which represents 2% of the meat produced in the country.

Chart 6: Evolution of meat production in Chile.



Changes in business ownership and the continuous fight against the other species, have positioned Chile with about 77,000 tons of carcass. One of the main reasons of this reduction, is that the largest turkey company, looking for higher efficiency in the use of assets and feed, decided to produce only males. This production represents about 4% of the meat produced in Chile.

Chile takes some advantages of the free trade agreements that has signed with Canada, United States and Europe, to export mainly chicken and pork meat. Turkey exports are about 31,000 tons per year, which represents 4.6% of the meat exports.

Also, Chile imports about 9,000 tons of frozen breasts for end of the year (Chart 7).

Chart 7: Balance of production and consumption of Turkey meat in Chile

	Tons Carcass	
Production	76,313	4% of the meat produced in Chile
Consumption	54,331	3.7% of the meat consumed in Chile
Exports	30,923	4.6% of the meat exports
Imports	8,942	

Market weights in Chile are 7-8 Kg hen and males at 18 – 22 Kg. Only 3 major poultry meat producer companies, which operate in a fully integrated system, from breeders to processing. Backyard production is very low and most of them, under official control.

PERU

Population is 34 million. 30% lives in the capital, Lima. Turkey consumption per year, is about 1.1 Kg per capita. There are some companies producing all year round, some others, producing only for end of the year. There is a market for small seasonal growers and back yard production. Some companies supply this demand. Same companies also export day old poults, to Ecuador, Bolivia and Colombia.

Still an immature market in terms of consumption. About 45% of the carcasses, are consumed at Christmas, but there have been important marketing initiatives to promote turkey demand during the rest of the year, such as companies that reward their workers with a turkey or the "*Jueves de Pavita*" ("Thursday of Turkey") and with special retail prices.

Peru is renowned for fantastic food. This is an attractive field that companies have been trying to capitalize upon and no doubt is one of the countries with more potential opportunities in the future.

Breeders at lay are 60,000, to process 3.3 million birds and export and sell 2.2 million day old poults.

This backyard market, is a big challenge since represents a problem to control and trace for biosecurity purposes.

Market weights for hens is 5 Kg and males in a range between 13 – 19 Kg.

Most of the turkey production in Peru is in the center of the country which is mostly desert, right in front of the coast. This location and special weather conditions, allows production in light, usually well-ventilated barns but they are managed with intense use of labor.

ECUADOR

Population is 18 million. Turkey production is located both at Andean mountain landscapes, this means, 1,800 Mt altitude, under mountain weather and at the sea level, in tropical areas, with high temperature and relative humidity.

1.6 million (70%) of the poults, are supply from abroad. One company has breeders in the country. 70% of the consumption is in December. The companies produce during the year and freeze the carcasses.

Consumption is 0.65 Kg per capita and the processing weights are 6 – 9 Kg hens and 9 – 13 Kg the males.

Turkey production in Ecuador, is done by 7 companies, most of them, having other business units besides turkeys.

COLOMBIA

Population is 52 million, but is one of the lowest turkey consumption, 90 grams. So, big opportunity looking forward. One turkey company in the country that operates in the Coffee Axis region. As is common in the region, many backyard birds, as part of the culture.

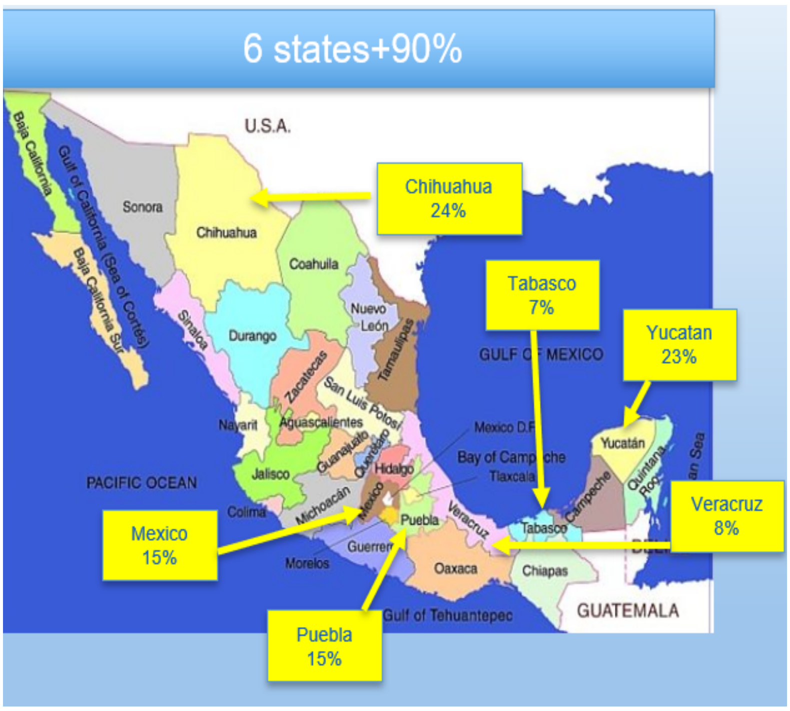
Market weight for hen is 8 Kg and toms 16 Kg.

MEXICO

Population is 129 million and the consumption is 1.3 Kg per capita. Yucatan, the place where turkeys are supposed to originate, the turkey consumption is 5 Kg.

Turkeys in Mexico are produced in 6 states, as shown in picture 1, being Yucatan and Chihuahua, the most important.

Picture 1: Turkey production in Mexico



Turkey production is 970,000 to 2,000,000 and is low in comparison with its imports. Importantly (90%) of whole turkeys imported by Mexico come from the United States. Chile and Brazil also supply Mexico.

Only one company in Mexico has breeders and supply growers, some of them, raise the birds until the processing age or brood them and sell after the brooding period.

Many poults are imported too. Good market for by products breeders. Backyard production is very important in the country.

CENTRAL AND SOUTH AMERICA: CHALLENGES AND OPPORTUNITIES.

The region has some important advantages, both as a supplier of turkey meat and to increase consumption. This will be done by supporting companies on the proper way to get a better position of the product for consumption on a daily basis. Colombia, Peru and Ecuador are countries with room to increase turkey consumption.

Brazil and Chile can still grow as reliable suppliers for North America and Europe, as long as their production meets the requirements of the importers.

Major worldwide subjects, such as Carbon footprint and animal welfare are part of the daily chores of the companies in the region.

The focus on performance as source of cost reduction and increased competitiveness is an important advantage to continue competing both internally and abroad.

Health challenges, such as Avian Influenza, Salmonella sp., Histomonas meleagridis, Pasteurella multocida, Ornithobacterium rhinotracheale and/or Avian Metapneumovirus (ORT/TRT), Mycoplasma gallisepticum and Clostridium perfringens, are permanent threats to the turkey industry, with potential impacts on the production, performance and/or commerce.

A separate comment is regarding political problems, corruption and bureaucracy, which sometime work in favor of agriculture and sometimes against.

On the other hand, the wide range of climates, some which are very favorable and good water availability are showing us that there is room for continued growth.

Vaccination of turkeys against blackhead disease: science fiction or science becomes reality?

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Blackhead disease (syn. histomonosis, histomoniasis, infectious enterohepatitis) was first described by Samuel Cushman in 1893 as a severe disease in turkeys. In this report, Cushman advised "stamp out the disease when it first appears" as the only effective method to reduce the substantial suffering and death of animals and further spread. Later on, the disease was also noticed in chickens and other *Galliformes* but turkeys always remained the primary host. Fundamental research on the aetiology of the disease and the morphology of the protozoan parasite *H. meleagridis* was performed by Tyzzer (1920).

Following the initial studies a subsequent period was characterized by intensive research mainly on drug development which was very successful. Research on the disease nearly ceased for decades after the introduction of medication in the 1960ties with hardly any publications until the 21st century (McDougald, 2005; Hauck and Hafez, 2013). However, the ban of all substances for prohylactic and therapeutic purposes led to a re-emergence of the disease (Hess et al. 2015). Considering the efforts needed to lisence new drugs (Clark & Kimminau, 2017) and the conflicting reports on herbal substances (Liebhart et al. 2017) a specific drug-based strategy to prevent histomonius losses is rather unrealistic. Furthermore, certain chemcial compounds do also not provide a reduction of lesions (Barros et al. 2020).

Similar to the initial studies on the pathogen it was again Tyzzer performed the first studies on the attenuation of *in vitro*-passaged histomonads demonstrated successfully *in vivo* (Tyzzer, 1934). However, in a later study he noticed that histomonas strains varied considerably in their ability to be attenuated with some strains showing substantial pathogenicity despite long term *in vitro* cultivation (Tyzzer, 1936). The concept of *in vitro* attenuation was further questioned by Lund et al. (1966). Such authors postulated that *in vitro* attenuation is not possible at all and the claimed loss of virulence of *in vitro* cultivated histomonads is based upon the co-existence of virulent *H. meleagridis* and non-pathogenic *H. wenrichi* within a culture, with the later being selected during long term passaging.

The need of a live vaccine is obvious as transfer of serum from surviving birds did not protect susceptible animals against infection (Clarkson, 1963). Consequently, an inactivated vaccine inducing mainly antibodies was not effective to protect turkeys (Hess et al. 2008; Bleyen et al. 2009). This indicated that a live vaccine is needed in order to induce robust immunity, if possible at all. However, there is no information on attenuated or non-virulent natural histomonas strains which can be used as a live vaccine.

In 2004, we established a clonal culture the first of its kind and implemented a completely new concept (Hess et al. 2006). Afterwards, the culture was passaged *in vitro* for about 3 years and used to infect turkeys. Surprisingly, turkeys infected with such long-term cultivated parasites did not die, instead they were also completely protected against a severe challenge (Hess et al. 2008). Subsequently, such results were confirmed in numerous other studies including trials in turkeys and chickens (Liebhart et al. 2010; Liebhart et al. 2013 and reviewed by Liebhart et al. 2017). It was also shown that such attenuated histomonads reside in the caeca and do not revert to virulence following backpassages in chickens or turkeys (Liebhart et al. 2011; Sulejmanovic et al. 2013). Furthermore, it could also be demonstrated that the attenuated parasites to be used as a vaccine protects against other virulent histomonads isolated from turkeys or chickens in different countries (Sulejmanovic et al. 2016) or even between strains representing different genotypes (Hatfaludi et al. 2022). Strain selection as earlier reported by Tyzzer (1934) is crucial with clonal cultures being somewhat advantageous as certain residual pathogenicity might be present in non-clonal cultures (Beer et al. 2021).

Clonal cultures were also used to investigate the immune response of host birds against such a complex organisms in different studies (Mitra et al. 2017 and 2018 and Kidane et al. 2018). However, the majority of experiments cited above were done by cloacal vaccination which seems less applicable for the poultry industry deserving further investigations.

An important, but very much unresolved, feature of *H. meleagridis* is the peculiar interaction with bacteria, needed for *in vitro* growth and to establish an infection (Bilic and Hess, 2020). Although *in vivo* data demonstrated high efficacy the presence of uncharacterized wildtype bacterial flora, so called xenic culture, was a severe constraint with regard to licensing and standardization of the vaccine. In this context it needs to be mentioned that histomonads do not grow in the absent of live bacteria and as a consequence a monoxenic culture, characterized by the presence of a single bacteria, was successfully established (Ganas et al. 2012). With this, a unique shuttle system was created allowing the targeted exchange of the bacterial strain. The actual composition contains an Escherichia coli isolate isolated from the gut of a healthy specified pathogen-free (SPF) chicken. Despite this, various challenges remain to be solved for registration of such a vaccine containing two live organisms. In fact, it needs to be considered whether the alternative procedure of "magistral formula" as laid out in the EU regulation for veterinary pharmaceuticals (regulation (EU)2019/6) could be an alternative approach until registration is achieved being the final goal of the current development.

Overall, vaccination against blackhead in turkeys is a novel strategy to combat the disease with a clonal monoxenic culture as the most well-defined antigen tested in various *in vivo* studies (Figure 1). The development of a live vaccine to protect against a flagellated protozoan parasite is a unique approach in medicine and directed by the complicated biology of the pathogen.

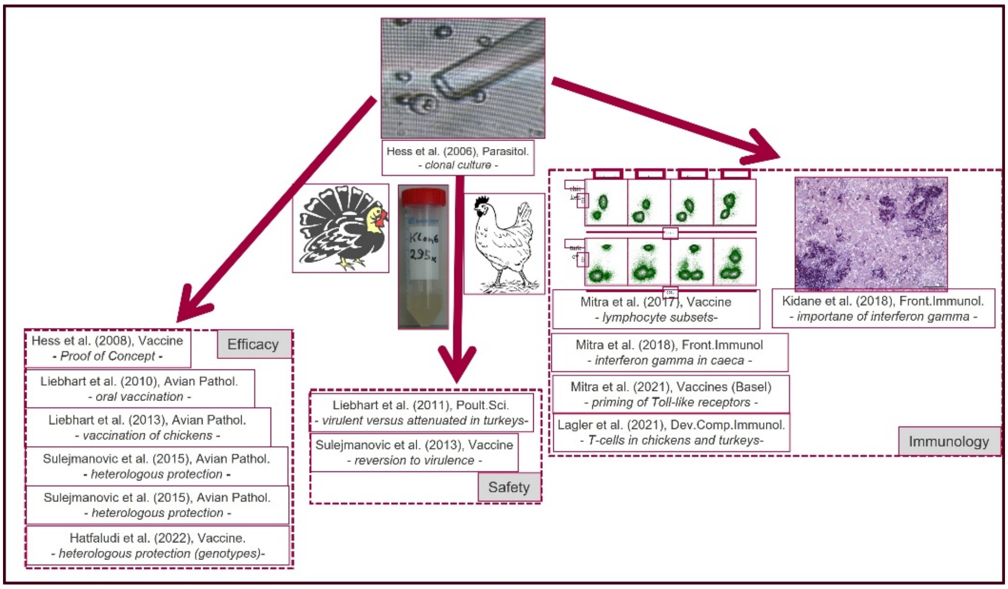

Figure 1: Summary of studies published on clonal cultures of *H. meleagridis* focusing on different areas of vaccine development.

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Absorption capacity – the critical link in the chain

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In today's challenging market, producers are constantly seeking improved efficiency in an attempt to increase productivity. In recent decades we have made huge strides. Havenstein *et al.,* (2007) demonstrated the improvement we have made in growth performance, when they compared a modern commercial strain of turkey with that of a 1966-type bird. The authors found that the modern strain birds were around twice as heavy as the 1966-type bird at all slaughter points, and that feed efficiency was around 50% better. It has been suggested that we may be nearing genetic potential in terms of growth rate, which means we need to identify alternative strategies for improving efficiency.

In recent years the use of feed additives has continued to make incremental gains in feed efficiency (Zuidhof *et al.,* 2014). Exogenous enzymes and emulsifiers (amongst others) have facilitated increased nutrient availability and reduced anti-nutritional factors, allowing the birds to achieve more growth from less feed. However, nutrient uptake is ultimately limited by the absorptive capacity of the gut (Croom *et al.,* 1998). Therefore, regardless of the nutrients made available by the various additives, unless the bird can absorb them, they will simply be excreted, contributing to environmental contamination and loss of investment in the diet components.

Furthermore, if we consider a modern stag (tom) turkey, in 20 weeks he can reach over 20kg. This is a growth increase of 12400%, requiring average daily gain of more than 142g (Aviagen, 2023). In this time, he will have eaten in the region of 55kg of feed. If we assume a rough digestibility rate of 90% that means we have faecal output of 5.5kg over the 20 weeks. Not only does this faecal matter amount to a lot of wasted nutrients, but it is also a vector for microbial disease and can associated with pododermatitis. Therefore, anything we can do to reduce this will ultimately improve the health, welfare, efficiency and of course productivity of the bird.

With this in mind, it is therefore logical that the primary consideration for improving nutrient utilization needs to be optimising the guts capacity for nutrient absorption, rather than purely increasing nutrient availability. The gut's absorptive capacity is impacted by numerous factors. The optimal absorption environment is one with maximum surface area, optimal membrane permeability to facilitate diffusion of nutrients across the intestinal wall, good muscle tone to facilitate retro peristalsis and a balanced and diverse microbiome (Roffler *et al*., 2003). In short, we need to reach a stasis in the gut and overcome or prevent issues that disturb that stasis.

Possibly the main common denominator that can impact all of these factors is the presence of disease, be that microbial, parasitic, chemical (toxin induced) or stress (Wickramasuriya *et al*., 2022). Historically, the use of antibiotics to both treat and prevent disease has masked many of the issues that we now recognize as factors that affect gut stasis. However, with ever increasing evidence of antimicrobial resistance, we need to dramatically reduce our reliance upon antibiotics and seek alternatives. Whereas an antibiotic is designed to kill microbes, probiotics directly supply microbes believed to be beneficial to the gut, manually supporting colonization of these potentially beneficial microbes. However, as with antibiotics we know that bacteria mutate, potentially altering it's function. We are already starting to see research demonstrating potential pathogenicity and antibiotic resistance to some common probiotic strains (Li *et al*., 2020; Jose *et al*., 2015), so maybe this is not the right approach either.

Eubiotics provide a new approach to maintaining a favourable gut environment. Rather than manually manipulating the microbes in the gut as with anti and pro-biotics, eubiotics support the favourable microbes in the gut, facilitating the establishment of a balanced, healthy intestinal microbiome (Santovito *et al*., 2018). By achieving this, eubiotics help the host to prevent the occurrence of pathogenic colonization and subsequent physical damage to intestinal structures, facilitating optimal functionality of the gut and therefore optimal absorption capacity.

Oregano essential oil (OEO) is a eubiotic that has widely been shown to support optimal gut function, due to its antimicrobial, antioxidant and antiparasitic activities (Nowak and Kasprowitz-Potocka, 2017). Essential oils (EO) form the basis of a plants defence mechanisms, acting as a type of immune system (Bakkali *et al.,* 2008). EO are comprised of numerous compounds that each offer a function to protect the plant from microbial or physical attack. Oregano essential oil contains over 100 different compounds including carvacrol, thymol, β-caryophyllene, linalool, γ-terpinene and ρ-cymene. The avian gastrointestinal tract is a complex and diverse organ, with both active and passive involvement in numerous systems and process. As such, no single compound is likely to improve every aspect. The complex composition of OEO facilitates it's multi factorial activity on multiple gut processes and immune challenges, therefore allowing the gut to function optimally, maximising absorption capacity and therefore optimal performance and minimal nutrient wastage.

Antimicrobial resistance often occurs more rapidly in compounds that use a single, or limited number of mechanisms for overcoming the microbe (Mittal *et al.,* 2019). For example, if an antibiotic targets cell wall permeability, it has been shown that the bacteria can adapt to overcome this mechanism quite rapidly. However, compounds that target multiple bacterial mechanisms are considerably more difficult for the bacteria to adapt to and resist (Yap *et al.,* 2014). It has been found that the multi factorial activity of essential oils makes them far less likely to confer microbial resistance (Yap *et al*., 2014).

Next, to determine the effect of OEO in the bird, a research study was performed at Southern Poultry Research Inc, Georgia, USA for a total of 9 days to examine the effect of OEO on birds challenged with *Eimeria* A total of 256 female turkey poults were obtained at day-old and reared in coccidia-free battery cages until 14 days of age. At 14 days, poults were assigned to treatment cage with ad libitum access to feed and water. At 16 days of age, each poult was challenged orally with a 1ml mixed coccidial inoculum. At 23 days of age, 8 poults per treatment were weighed and faeces collected from all cages and examined for the number of oocysts/g faeces (OPG).

Table 1. Treatment Groups

Treatment	**No. birds/cage**	**No. cages**	**Challenge**
Non-medicated, non-infected control **(NC)**	8	8	-
Non-medicated, infected control **(IC)**	8	8	✓
Orego-Stim (150ml/1000l) **(OSL150)**	8	8	✓
Orego-Stim (300ml/1000l) **(OSL300)**	8	8	✓

* Per dose, approximately 100,000 oocysts, comprising Eimeria meleagrimitis, E. gallopavonis and E. adenoides field isolates.

The study found that predictably, the infected control demonstrated significantly worse performance compared to the negative control. The birds supplemented with OEO however, were able to perform significantly better than the infected control for both weight gain and feed efficiency (Figures 2&3). Furthermore, the quantity of oocysts shed in the faeces was significantly lower for the OEO supplemented bird compared with the infected control (Figure 4)

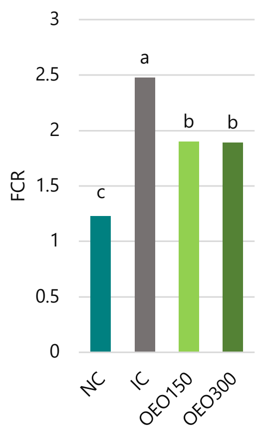

Figure 2. Comparison of feed conversion ratio (FCR)

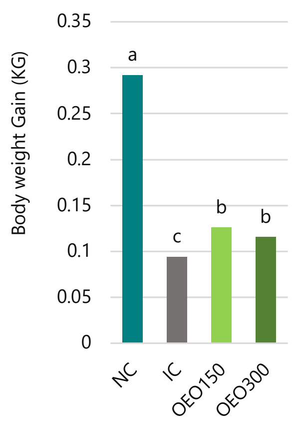

Figure 3. Comparison of body weight gain (kg)

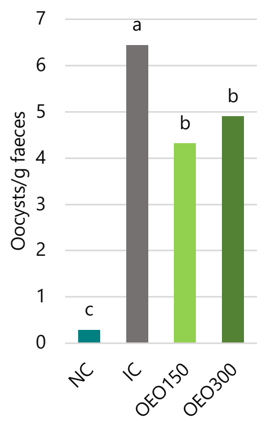

Figure 4. Comparison of the number of oocysts/g of faeces during the treatment period

In a research setting, the concept of eubiotics and eubiosis can be difficult to demonstrate without the use of a pathogenic challenge due to the legislated cleanliness of research facilities. As such the microbial challenges seen in commercial production can be difficult to replicate. To try and overcome this and assess the viability of OEO as an effective eubiotic, a large-scale commercial trial was performed in 2023 in collaboration with a turkey producer in Poland. Two groups of 9,000 BUT 6 stag turkeys were reared in brooding houses until 4 weeks of age, a control group and an OSP supplemented group. Each group were then moved to finishing houses until 20 weeks of age. OSP was supplemented at 450g per tonne from day old until slaughter.

The results found that the use of OEO significantly enhanced growth performance (Figures 5&6), and that OEO consistently reduced mortality compared to the control (Figure 7)

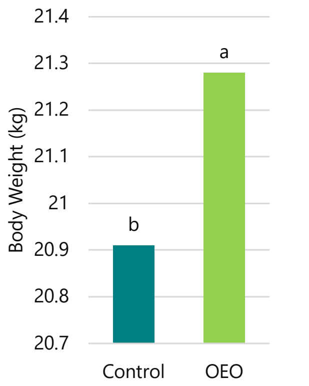

Figure 5: Comparison of body weight

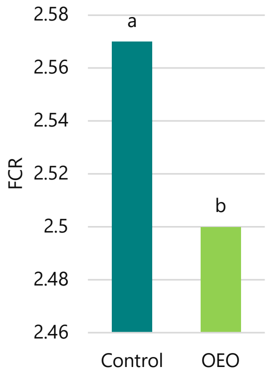

Figure 6: Comparison of FCR

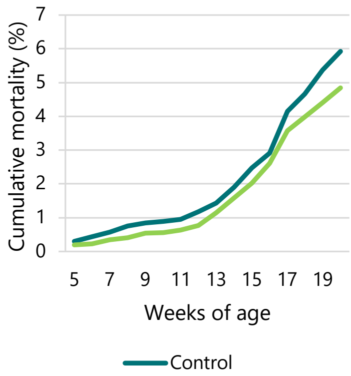

Figure 7: Comparison of mortality

Furthermore, meat yield was increased compared to the control and fewer carcass down grades were achieved meaning an additional 54 carcasses were processed per house (Figures 8&9). Overall, this resulted in over 3mt of additional saleable turkey meat (Figure 10)

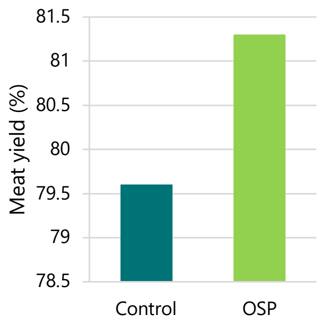

Figure 8: Comparison of meat yield

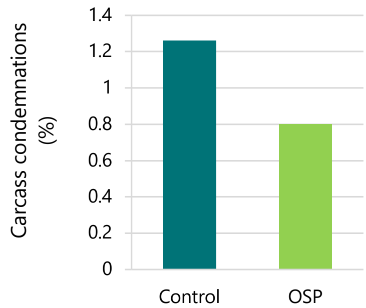

Figure 9: Comparison of carcass condemnations

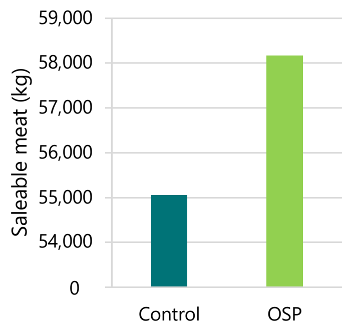

Figure 10: Comparison of saleable meat

In summary, OEO substantially enhanced performance and livability of the commercial flock. When considered in context with the coccidiosis challenge work, there is consistent indications that OEO offers an effective and holistic approach to optimising gut stasis and therefore reducing the need for antibiotics in a way that doesn't contribute to increased antimicrobial resistance.

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The EU legislation on Turkeys: EFSA mandate, challenges and opportunities

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The European Union (EU) plays a pivotal role in shaping legislation that governs various aspects of 27 member states' agricultural practices. The EU decision-making process serves as the foundation for the development and implementation of policies that impact member states.

European Commission

- Role: propose legislation, implement EU policies and manage the day-to-day affairs of the EU.
- Functioning: It starts with the proposal of new laws or the amendment of existing ones.

European Parliament

- Role: represents the EU citizens. Members of Parliament (MEPs) are elected for five years.
- Functioning: reviews and amends legislative proposals from the Commission. Adopt or reject legislative acts and co-decides with the Council.

Council of the European Union

- Role: represents the governments of member states.
- Functioning: coordinate national policies, reach compromises among member states, and adopt laws.

Decision-Making Process

1. ***Initiation***: The process begins with the European Commission proposing legislation based on identified needs or issues. This proposal is then submitted to Parliament and the Council.
2. ***First Reading***: The Parliament (first) and the Council (second) review the proposal. Each may accept, reject, or propose amendments. If both institutions agree, the proposal is adopted. If not, the proposal is returned for a second reading.
3. ***Second Reading***: Parliament examines the Council's position and either approves it, rejects it, or proposes amendments. The Council either approves all of Parliament's amendments or does not approve all amendments, leading to the Conciliation Committee.
4. ***Conciliation Committee***: tries to reach agreement on a joint text. If unsuccessful, the procedure is ended. If a joint text is agreed, it is forwarded for a third reading.
5. ***Third reading***: Parliament examines the joint text and votes in plenary. The Council does the same. If it rejects it, the act is not adopted, and the procedure is ended. If it is approved by Parliament and Council, the act is adopted.
6. ***Adoption***: Once both institutions agree on the final text, the legislative act is adopted.

EFSA Mandate on Welfare of turkeys on farm

EFSA is an agency of the European Union set up in 2002 to serve as an impartial source of scientific advice to risk managers and to communicate on risks associated with the food chain. EFSA cooperates with interested parties to promote the coherence of EU scientific advice. **EFSA provides the scientific basis for laws and regulations** to protect European consumers from food-related risks – from farm to fork.

The revision of the animal welfare legislation within the EU framework sets the stage for addressing contemporary challenges and aligning regulations with evolving scientific findings and societal values. EFSA has been mandated by the European Commission, to issue several scientific opinions on the welfare of animals on farms and during transport. Those will feed into the 4 legislative proposals the EU Commission will make on animal welfare (on farm, during transport, at the time of killing and animal welfare labelling).

In October 2023, the Commission formerly mandated EFSA to issue an opinion on the welfare of turkeys on farms. EFSA will deliver the opinion at the latest in December 2025. In scope for the review are common husbandry systems and practices for breeders, hatcheries and turkeys kept for meat production; evaluation of welfare risks, identification of welfare consequences, providing qualitative and quantitative recommendations and assessing and defining suitable animal-based indicators collected at the slaughterhouse.

Since then and in view of the heavily criticized latest EFSA opinions on broilers welfare and transport of poultry, EFSA has shown a true desire to better cooperate with the livestock sector. This has translated into an early stakeholder meeting (November 2023), where the intention was not only to ‘break the ice’ but also to create a platform for collaboration with various experts from the field as well as to identify key participants of the scientific review process.

It is important to acknowledge such an effort from EFSA, which is and shall remain an independent agency.

Challenges surrounding the future EFSA opinion on turkey welfare require careful consideration. Divergent national practices, economic implications for farmers/producers, and commercially confidential information are all aspects to consider when sharing and providing scientific information to EFSA. Although EFSA’s work solely focuses on animal welfare, the data monitored and collected within our sector is multifactorial and is the result of a thorough evaluation of multiple social, economic and environmental criteria in order to find the best point of equilibrium.

Nevertheless, the opportunity exists and is out there. With non existing EU legislation on turkey production, we are today, in a position to actively participate into shaping the future of the EU rules on turkey farming by sharing our knowledge and expertise on the topic. And it starts with EFSA! Highlighting innovative approaches, technological advancements, and best practices that can enhance turkey welfare on farms would show our willingness to cease the occasion.

Finally, and although EFSA’s review plays a key role into the legislative process, there are many other opportunities to collaboratively work with the EU institutions towards a future where the welfare of turkeys on farms aligns with the ethical and sustainable standards set by the EU.

Nadia Khaldoune - Senior Policy Advisor

AVEC – The Voice of the European Poultry Meat

The effect of slaughter age and bodyweight on turkey processing yields

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Introduction

Optimising production cost is a major challenge to remain competitive in turkey production, particularly given the current increase of heavyproduction. Utilisation of the whole carcass is a challenge to guarantee competitiveness but focus is often on the high value breast and the thigh, with little recent data available on the other meat parts.

The aim of the project is to portion turkeys slaughtered at different ages to determine the weight of different meat parts and study the effect of slaughter age and bodyweight on turkey meat yield.

Material and methods

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

162 Aviagen Premium male turkeys were placed in 18 pens with 12 turkeys per pen. Turkeys are fed ad libitum the same diet.

At 106, 113 and 120 days, 3 randomly selected turkeys are removed from each pen for slaughter (54 turkeys in total per slaughter). Feed was withheld for approximately 12 hours before slaughter. Weight of fasting live birds is measured.

Carcass measurements

Turkeys are conventionally processed, eviscerated and chilled overnight. Carcass was hand deboned and the weights of the following carcass parts are collected for each bird : skin, fat, neck and rump, full breast, breast and tenderloin, full wing, drumette, middle wing and wing tip, full thigh, thigh, drumstick and oyster.

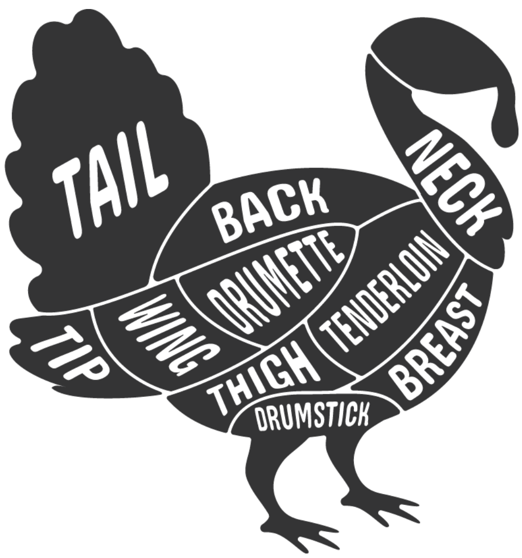


Figure 1 : Turkey meat cutting diagram

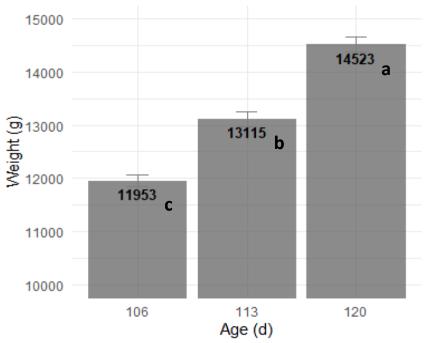
Determination of yields

Parts weight percentages are calculated using fasting live weight of birds.

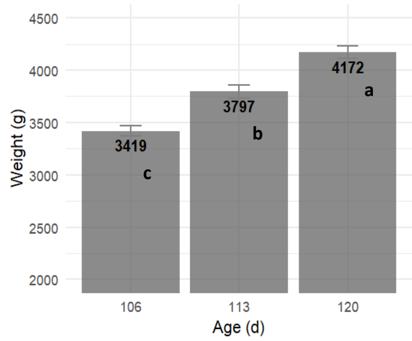
Results

As age increases there is an increase in overall carcass weight (Graph 1) and also an increase in breast part weights (Graph 2), we found no weight increase for fat, neck and rump (data not shown), between 106 and 113 days, but it did increase between 113 and 120 days (p-value < 0.05).

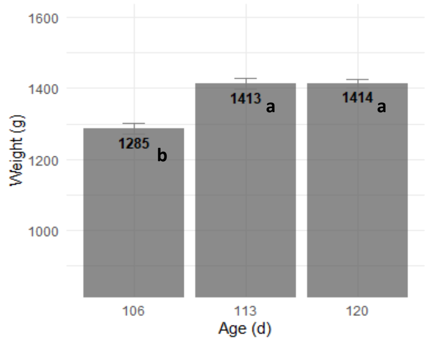
Full wing weight (Graph 3) increases up to 113 days but not to 120 days and we see drumette weight increase to 113 days and then reduce to 120 days (Graph 4) (p-value < 0.05).



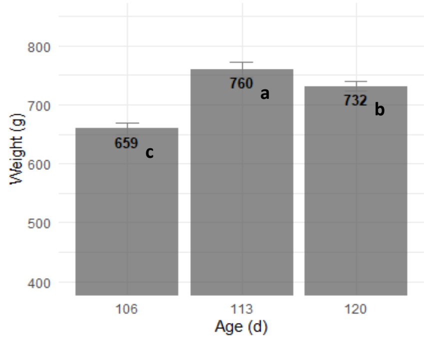
Graph 1 : Carcass weight according to slaughter age



Graph 2 : Full breast weight according to slaughter age



Graph 3 : Full wing weight according to slaughter age



Graph 4 : Drummette weight according to slaughter age

We can observe an increase, decrease or reduction in growth patterns of parts yields with an increase of slaughter age.

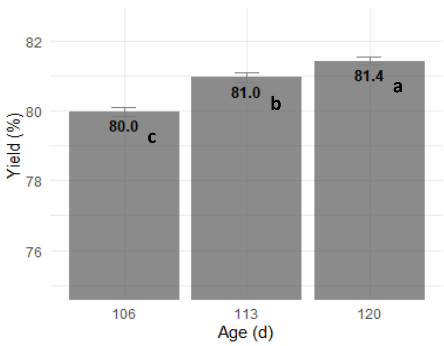
Firstly, carcass yield increases significantly with slaughter age (Graph 5), as do skin and neck yields (p-value < 0.05).

Fat yield does not vary with slaughter age (data not shown).

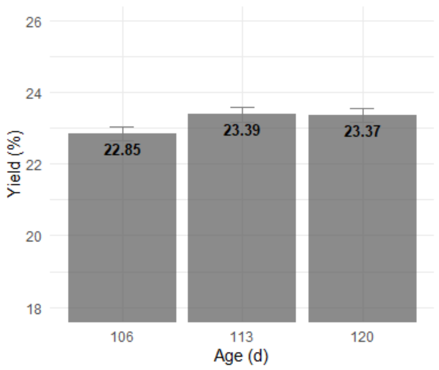
Full breast yield increases to a plateau (Graph 6), with the same yield at 113 days and 120 days.

Full thigh yield increases with slaughter age (Graph 7), particularly from 113 to 120 days (p-value < 0.05).

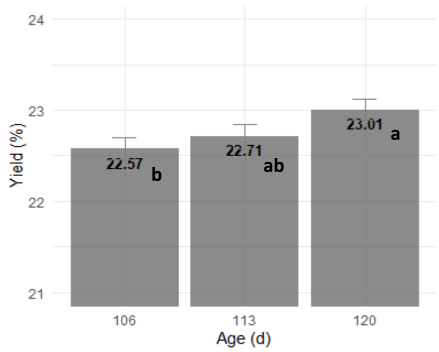
Indeed drumstick and oyster yields remain unchanged (data not shown).



Graph 5 : Carcass yield according to slaughter age



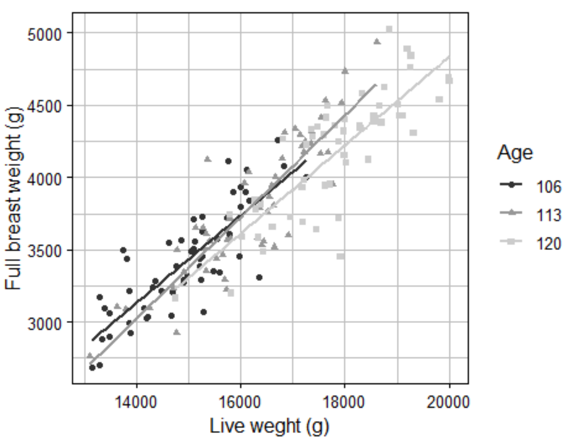
Graph 6 : Full breast yield according to slaughter age



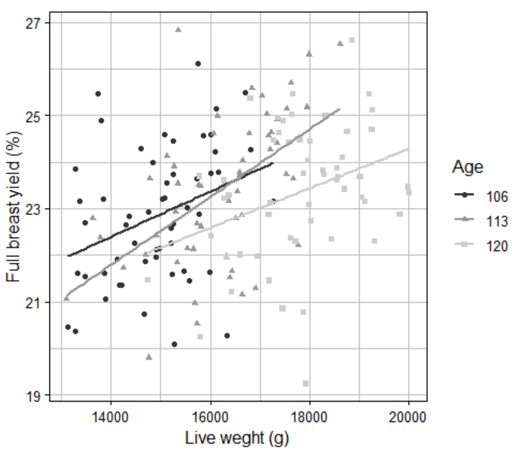
Graph 7 : Full thigh yield according to slaughter age

Discussion

An increase in the weights of turkey parts relative to an increase in slaughter age have also been observed by Peng et al. (1985) and Larsen et al. (1986) and the importance of body weight as the primary factor in predicting turkey yields was underscored by Leighton et al. (1961). However, Larsen et al. (1986) reported different growth patterns of parts, where fat yield increases with slaughter age, contrary to the present study. Also breast yield does not encounter a plateau but continues to progress in the same study. This could be explained by the use of female turkeys instead of males, and by the evolution of genetics and strains used. Wesley et al. (1981) and Warnick (1982) also reported increased percentage yields for breast muscle and fat with increased age of birds. Peng et al. (1985) observed in male turkeys that the percentage breast appeared to level off and percentage breast muscle seemed to decline from 25 weeks, which is consistent with the results of the present study although this was observed earlier at 17 weeks. The decreased percentage yields of wings and drums as a function of age have also been observed by other researchers (Dobson, 1969 ; MacNeil, 1969 ; Salmon, 1974; Wesley et al, 1981 ; Warnick, 1982). The increase of thigh yield observed in the present study is also made by Hasiak (1978) who found that age did not affect thigh meat yield.



Graph 8 : Evolution of full breast weight according to live weight, for 3 slaughter ages



Graph 9 : Evolution of full breast yield according to live weight, for 3 slaughter ages

In contrast, other studies (Wesley et al, 1981 ; Peng et al,1985) reported a decrease of thigh yield with age. Turkeys in the present study are slaughtered younger than in other studies which may explain some of the differences observed, in addition to several years gap and genetic evolution.

Effect of slaughter age and bodyweight on breast weight and yield

The results obtained provide information on the effects of age and slaughter weight on weight and yield of parts. Thus, the age/weight parameters for optimum breast weight can be determined as follows : for a given age, breast weight increases with live weight ; for a given weight, the younger the weight is reached, the higher the breast weight (Graph 8). Same applies to yield (Graph 9).

Valuable insights into the distribution of turkey parts

Muscle distribution is an essential factor in determining the most profitable size. This trial provides us with recent data on weights and yields of the various meat parts in turkey.

Breast and thigh are the parts that are usually of most interest when looking at meat value. These two cuts account for around 50% of live yield (57% of carcass yield). The remaining 50% includes parts such as the wing (8.5% average carcass yield in the trial) and the neck (4% average carcass yield in the trial). These parts may be valuable in some consumption patterns, as is the case with necks in Morocco, for example.

Economic outlook

From these results, an economic simulation has been achieved (Table 3). Two economic criteria have been evaluated : feed cost per ton of live weight and feed cost per ton of breast.

	106 days	113 days	120 days
Average live weight (g)	14 695	15 999	17 631
FCR	2.16	2.23	2.28
Cost of feeding program (€/t of feed)	350	349	348
Breast yield (%)	22.85	23.39	23.37
Feed cost (€/t of live weight)	755	778	793
Feed cost (€/t of breast)	3 305	3 325	3 395

Table 3 : Feed cost per ton of live weight and per ton of breast meat for the 3 slaughter ages

Feed cost per ton of live weight logically increases with slaughter age, following the increase of FCR. Feed cost per ton of breast meat follows the same pattern but has a higher rate of increase between 113 and 120 days due to reduced breast yield.

Conclusion

This trial gives references for weight and yield of meat parts in turkey, including usually less interesting parts such as the neck. The slaughter of turkeys at three different ages allows us to establish meat deposition parameters and found that reaching a given weight as early as possible optimises associated breast meat yield.

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Comparison of phytase efficacy in broilers and turkeys – is the broiler a relevant model?

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Summary

A google scholar search for scientific articles where the words “turkey” or “turkeys” coincides in the title with “phytase” yielded 69 papers in total whereas the equivalent for broiler yielded 1305 hits, representing an 18.9 fold difference. This is not only a reflection of the relative size of the industries and hence funding for research but also the relative expense of a turkey compared with an equivalent broiler trial. With such a limit in turkey relevant data it is not surprising that a great deal of extrapolation is made between the two species when considering phytase use but is this a valid assumption? As far as we are aware, a direct comparison of phytase effects between the two species has been undertaken in only 9 papers. The data suggests that whilst there are physiological differences which should be of some concern, fortunately to date the estimates of phytase efficacy transcribed from broilers to turkeys have not overestimated the value of this enzyme in turkeys. Regardless, the value of a phytase in turkeys should be investigated in turkeys if the full value of this enzyme is to be realised.

The value of a phytase in poultry (Turkey) nutrition.

Phytases have been shown to improve the digestibility of P, Ca, Na, amino acids, and energy and thus their application allows for a reduction in feed costs by reducing the concentration of these nutrients with no performance or welfare issues, provided the “matrix” is correct. At present most feed producers use the P, Ca and Na matrix with less frequent application of an amino acid or energy matrix, although with increasing ingredient prices this is changing. There is and has been a general assumption in the poultry industry that phytases release similar amounts of nutrients in all poultry species even though data is limiting for some. Indeed, limited early work suggested that the P release from phytase was marginally greater in turkeys than broilers (Applegate and Angel, 2003), which provided some comfort in this assumption given that there was far more data for broilers from which extrapolations had been made. Commercial experience at the time bore out these assumptions although it has to be noted that most phytase users employed (and still do) a significant safety margin in the matrix applied. However, the growth rate and efficiency of current poultry stock has improved markedly and the identity and dose rate of phytases in the market have also changed considerably, suggesting that this assumption needs re-investigation. With this in mind, and given the title of this paper, the question at hand is whether the estimates of nutrient release from use of phytase in broiler studies can be used to formulate turkey diets. This is not easily answered because it is not simply the species that differ but also their diets, so physiological and dietary factors that influence phytase efficacy need to be considered together.

Factors influencing phytate hydrolysis.

The extent and rate of phytate hydrolysis noted when an exogenous phytase is used is dependent upon many factors including pH, intestinal segment retention time, moisture, the concentration of Ca, P, and other metal ions, the ingredient source of the dietary phytate, the age of the animal as well as the identity and quantity of the phytase used. The key factors of interest with regards to this paper are the marked inhibitory effects that both Ca and P have on phytase functionality when present in quantity (Rodehutscord et al., 2022), a state of affairs far more likely encountered in turkey compared with broiler diets. Even in the absence of an exogenous phytase, phytic acid is degraded in the crop and small intestine by a combination of ingredient, microbial, and intestinal mucosa-derived phytases. If conditions are optimal, the extent of such hydrolysis can be considerable. The benefits of an exogenous phytase are therefore supplemental to this baseline level, and many of the factors that influence exogenous phytase have a similar effect on the “endogenous activity”, meaning an unknown mix of enzymes from the mucosa, resident microbiota, and feed intrinsic enzymes. It is not surprising, therefore, given so many factors influence the scale of response noted to an added phytase, that the estimated P matrix even for broilers varies so much between studies. Given the nutritional requirements for turkeys and broilers are divergent, and that Ca and P in particular influence phytase (endogenous and exogenous) efficacy, it is apparent that variance in efficacy of a phytase between species may be due to differences in diet, physiology or both.

Focus on digestible P effects

In a study of P utilisation, (Rodehutscord and Dieckmann, 2005) fed the same diets to same-age broilers and turkeys. While broilers had a higher P utilisation than turkeys when the basal diet without mineral P was fed, turkeys showed higher utilisation of the P added from monocalcium phosphate than broilers. These results indicated differences between the species in both endogenous phytate breakdown and P absorption. In 2016 (Kwakernaak, 2016) investigated the effects of varying the dose of an E coli and a Butiauxella derived phytase on mineral digestibility and tibia ash in turkeys and broilers fed species relevant but Ca and P depleted diets from 5-21 days of age. The turkey diets contained more Ca (8 vs 6.5g/kg), P (5.66 vs 4.4g/kg), protein (28.4 vs 21%) and phytate P (3 vs 2.5g/kg) than the broiler diets. The data from the E. coli derived phytase is presented graphically below and shows that on the unsupplemented diet, the broilers digested more phosphorus and laid down more tibia ash than the turkeys, but the response

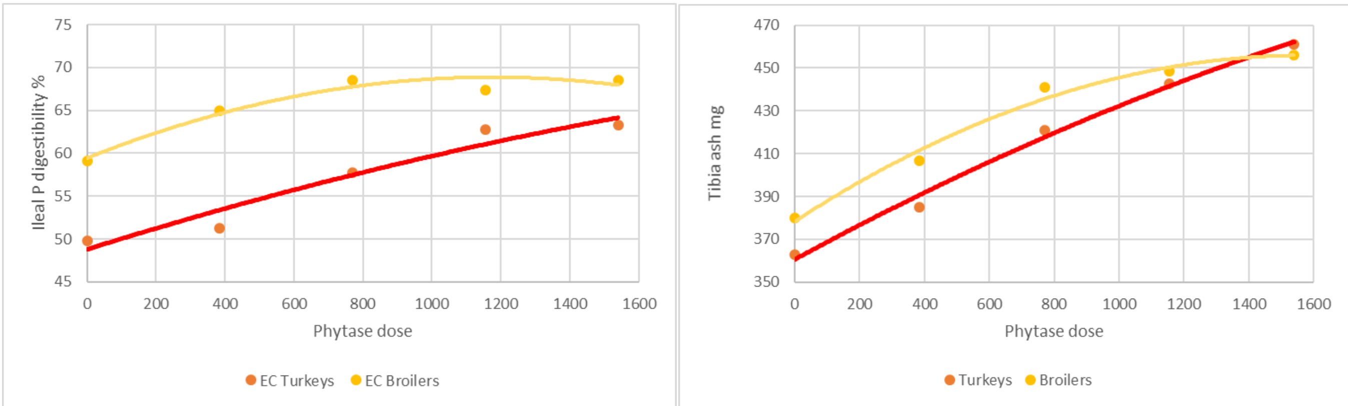

Figure 1. Effect of varying the dose of an E. Coli phytase on pre-caecal P digestibility and tibia ash in 21d old turkeys and broilers fed species-specific diets. Adapted from Kwakernaak (2016).

to added phytase continued in a more linear fashion for the turkeys resulting in the differences being reduced at higher levels of phytase utilisation. Consistent with the results of the study by Rodehutscord and Dieckmann (2005), a possible explanation of the above is that phytate utilisation would appear to be higher in broilers in the absence of exogenous phytase addition, especially as the turkey diets contained more phytate and inorganic P. However, it is still not clear whether this is a facet of broiler physiology or if it is due to the broiler diet being less constraining on endogenous phytases than the turkey diet because of the lower mineral concentrations. Indeed all work where species-specific diets were fed suffers from the same confounding effect between diet composition and species. For example, (Karadas et al., 2005) noted that increasing phytase dose from 0, 250, and 500 up to 2500 FTU/kg in species-specific but P-deficient diets resulted in a dose-dependent accumulation of Coenzyme Q in the liver of broilers at 28 d of age. In contrast, no such effect was noted in turkeys. In subsequent work conducted exclusively in broilers (Pirgozliev et al., 2010), administration of a P-deficient negative control resulted in significantly depleted hepatic concentrations of both Coenzyme Q and α-tocopherol compared with the P-adequate positive control and phytase addition lead to the same dose-dependent increment in Coenzyme Q (and α-tocopherol) as noted previously. This suggests that the response was related to P release and that the lack of a response noted in the Karadas et al. (2005) work in turkeys could just as likely be due to the P level of the diet as it could to species idiosyncrasies.

With a closer look at the substrate (phytate, $InsP_6$), more recent work, again using species-specific diets, reinforces the suggestion that broilers are more capable in degrading InsP6 from the diet than turkeys in the absence of phytase (~70 vs 10% respectively) and its presence (~90% vs 35%; (Ingelmann et al., 2019)). Although the response to phytase was proportionately greater in turkeys, InsP6 disappearance was still far greater in broilers (Ingelmann et al., 2019). Pre-caecal phosphorus digestibility likewise was greater in broilers than in turkeys in both the absence and the presence of phytase (Ingelmann et al., 2019, Olukosi et al., 2020). Turkeys not only were less able to degrade InsP6 in the presence or absence of exogenous phytase, but they were also less capable of degrading lower esters as well (Ingelmann et al., 2019) which explains their relatively poor utilisation of phytate P and P digestibility response.

Regardless, with incremental phytase dose the additional P digested in turkeys in g/kg diet were similar if not greater than that in broilers, suggesting any values derived from broilers could be applied to turkeys when they are fed species-specific (albeit P-deficient) diets (Figure 2). Note, however, that the P released per unit dose in both species was incredibly low (4 fold lower) compared with the suggested matrices (a typical E. coli phytase when used at 500 FTU/kg feed is expected to release 1.2g digestible P/kg feed). In the case of the broilers this is likely due to the very high degree of InsP6 and lower ester hydrolysis in the low Ca and P diets by endogenous phytases, leaving far less phytate P for the exogenous phytase to release. However, in the case of the turkey no such "pre-digestion" of InsP6 had taken place which theoretically should have left far more for the exogenous phytase to degrade. The expectation might be for a greater potential effect of exogenous phytase in turkeys, but this is not realised. It may well be that the conditions which constrain endogenous phytase activity equally constrain exogenous, hence there is a plausible reason for relative parity in P release between species. Whether such parity between species exists when identical diets and more commercially relevant concentrations of Ca and P are fed was unknown at this juncture.

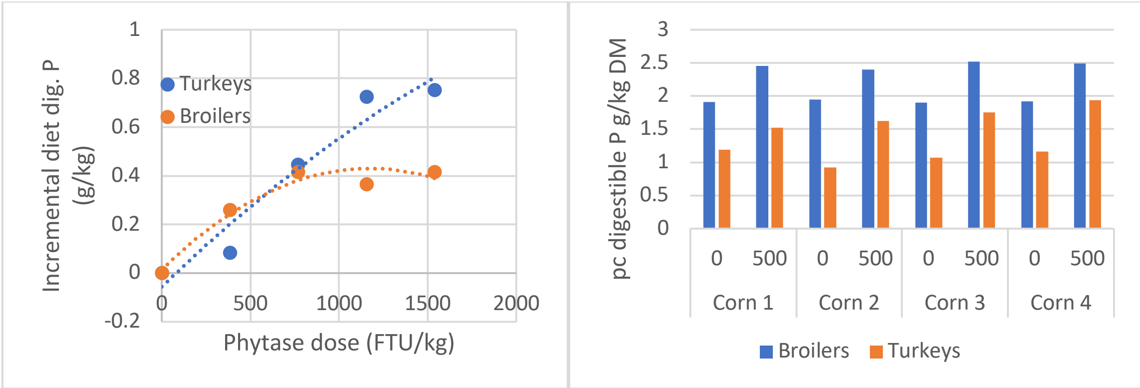

Figure 2. Panel A: Effect of increasing the dose of an E. Coli phytase on the increment in diet digestible P content (g/kg) compared with the control. Adapted from Kwakernaak, 2016. Panel B: Effect of 500 FTU of an E. coli derived phytase on pre-caecal digestible P content of the diet. Adapted from Ingelmann et al. (2019). Average pc dig P increment Broilers = 0.55g, Turkeys = 0.62g

More recently both turkeys and broilers have been fed identical diets which were either high (12g and 9g/kg Ca and P respectively) or low (5.5g and 4.1g/kg Ca and P respectively) in Ca and P in the absence or presence (1500 FTU/kg) of an evolved E coli phytase (Novotny et al., 2023a, Novotny et al., 2023b). This eliminated the confounding influence of species-specific diets. In the absence of exogenous phytase the jejunal mucosal phosphatase (including phytase) activity and pre-caecal InsP6 disappearance in 3 wk old birds was similar between species when the low CaP diets were fed, but the higher CaP diets (which would be considered excessive in broilers and marginally deficient in turkeys) reduced InsP6 disappearance more so in broilers than turkeys. Addition of phytase increased InsP6 hydrolysis in both species but markedly more so in broilers than in turkeys regardless of the CaP level of the diet. This would suggest that the increment in P digestibility would be greater in broilers than turkeys but this was not entirely true. In fact P digestibility was significantly higher in turkeys fed the high CaP diets whether phytase was fed or not (as was also noted by Rodehutscord and Dieckmann, 2005), and in the low CaP diets P digestibility was higher in broilers only when exogenous phytase was added. Nevertheless, the estimates of digestible P release by the enzyme in both species suggests that turkeys on average retrieve half of the P matrix of broilers in low CaP diets and a quarter in the higher CaP diets (Table 1) which is clearly a problem from the perspective of using broiler data to estimate responses in turkeys.

From the perspective of the commercial user this is proof that the one should not be used to predict the P matrix of a phytase for the other. However a more pragmatic approach is to consider what will be the diet digestible P content on the use of the enzyme.

Species	Diet	Increment in digestible P (g/kg) 3wks of age due to addition of 1500 FTU /kg phytase	Increment in digestible P (g/kg) 6wks of age due to addition of 1500 FTU /kg phytase
Broiler	Low CaP	1.22	1.59
	High CaP	0.45	2.12
Turkey	Low CaP	0.68	1.16
	High CaP	0.12	1.78

Table 1. Effect of adding 1500 FTU/kg diet of an evolved E coli phytase on the incremental digestible P content of low or high Ca and P diets. Adapted from Novotny et al, 2023a and 2023b

Figure 3 highlights an interesting conundrum; if the broiler were used to predict the digestible P content of a diet when phytase was used, it would marginally overestimate it in the low CaP diet and underestimate it in the high CaP diet (which is closer to commercial reality for turkeys).

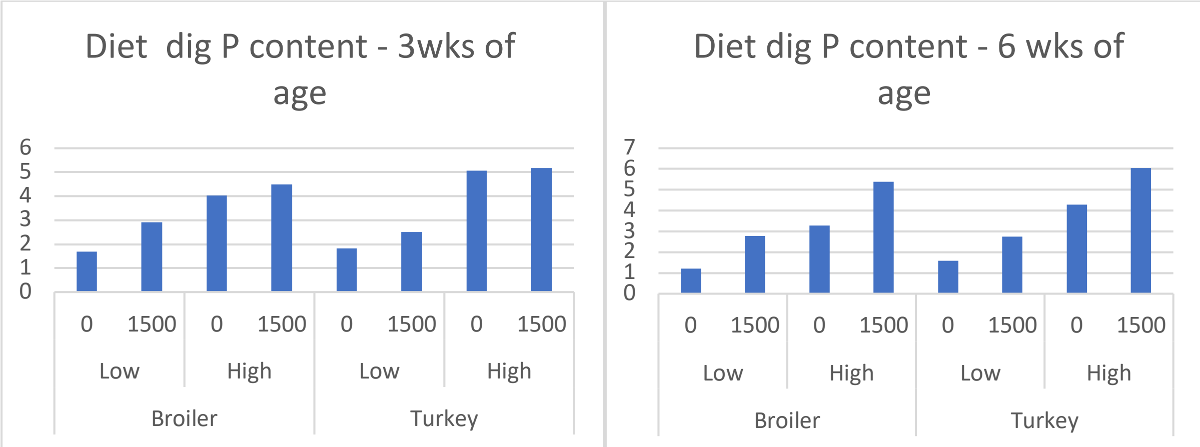

Figure 3. Calculated diet digestible P content in high and low CaP diets (in g/kg) with and without 1500 FTU phytase/kg feed. Adapted from Novotny et al, 2023a and 2023b

In the second study Novotny et al (2023b) focussed on 6 week old birds and noted that pre-caecal InsP6 digestibility increased with age in turkeys but not broilers and this also coincided with a relative increase in pre-caecal P digestibility in turkeys compared with broilers. Thus, if broiler data is used to estimate P digestibility of the unsupplemented diets then it will, on average underestimate this for turkeys. When the effect of the phytase is then considered, the net effect is the predicted P digestibility of the phytase supplemented diet is not significantly different from that determined in the turkeys. The phytase effect is larger in the broiler but the absolute P digestibility in the phytase supplemented diets is largely similar between species. This is particularly true in high CaP diets which normally would be offered to turkeys. It is clearly flawed to rely on such an approach going forwards, especially as targeted Ca and P levels are falling and thus turkey specific data, in diets relevant to the phase of feeding are needed.

Focus on myo-inositol effects

The release of myo-inositol following the complete dephosphorylation of InsP6 is thought to play some part in the "extra-phosphoric" performance benefits of broilers noted when high doses of phytase are deployed (Sommerfeld et al., 2018, Lee et al., 2017). Thus, when phytases are used to model the response in turkeys it is important to consider not only the incremental P release, but also the incremental myo-inositol produced in the intestines and ultimately absorbed into the blood. Inositol plays many roles in metabolism and is the backbone for inositol phosphate esters and phospholipid synthesis which are essential for cell functionality (Whitfield et al., 2022, Gonzalez-Uarquin et al., 2020b, Gonzalez-Uarquin et al., 2020a) which may underpin the performance benefits attributed to this molecule. As noted above, the efficacy of exogenous phytases in degrading InsP6 is markedly greater in broilers compared with turkeys and as a result the determined intestinal myo-inositol concentrations were also found to be much higher in broilers in the presence of the exogenous phytase regardless of the Ca and P content of the diet (Novotny et al., 2023a, Novotny et al., 2023b). Differences in gastrointestinal conditions were cited for such an effect with the crop and gizzard being significantly divergent to the point that the broiler presents a significantly better environment for successful dephosphorylation of InsP6 to inositol. Such an observation would tend to suggest that the benefits in using a phytase to deliver inositol in turkeys could and should not be modelled by broilers. However, in both of the most recent comparative studies (Novotny et al., 2023a, Novotny et al., 2023b), the authors also measured plasma levels of inositol and surprisingly found that they were comparable between the two species in absence of phytase at 3 and 6 weeks of age, and in the presence of phytase they were equivalent if not higher in the turkey, regardless of Ca or P concentration (Figure 4). Whilst this by no means suggests that the broiler can be used to model inositol production in the turkey, from a practical viewpoint it could be argued that the broiler data does not overestimate the effect of phytase on plasma inositol levels.

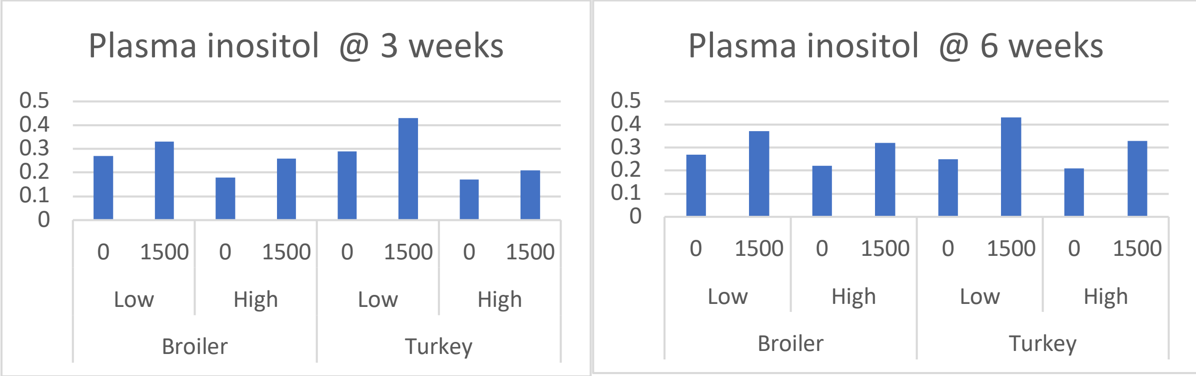

Figure 4. Plasma myo-inositol level (µmol/ml) in high and low CaP diets with and without 1500 FTU phytase/kg feed. Adapted from Novotny et al, 2023a and 2023b

Focus on amino acid digestibility effects

Many nutritionists also credit phytases with an energy and amino acid release matrix as well and the value of these spared nutrients can equal or even surpass that of phosphorus. As such the energy and amino acid matrices derived from broilers need to be confirmed as being representative of those achieved in turkeys if they are to be used in prediction of response. Although there are many papers investigating the effects of phytase on energy and amino acid digestibility in broilers or in turkeys, the benefits noted are not universally consistent. Intuitively one would expect the opportunities in turkeys to be greater given the greater protein and phytate content of their diets, but given the discussion in the present paper above this expectation may be ill conceived. There is only one study where the effect of phytase on amino acid (not energy) digestibility in the two species has been evaluated on the same diets (Novotny et al., 2023b). Digestibility of all amino acids were subject to a 3 way interaction whereby the use of the phytase increased amino acid digestibility in broilers fed the low CaP diets whereas the effect was only a trend in the high CaP diets. In turkeys, however, phytase addition tended to and in some cases significantly *reduced* the digestibility of amino acids in the low Ca and P diets but increased it markedly in the high CaP diets. Moreover, there was a negative effect of feeding the high CaP diets on amino acid digestibility in turkeys but not broilers in the absence of exogenous phytase. Dietary effects on the digestibility of cysteine as an example amino acid and the average of all amino acids are shown in figure 5 below.

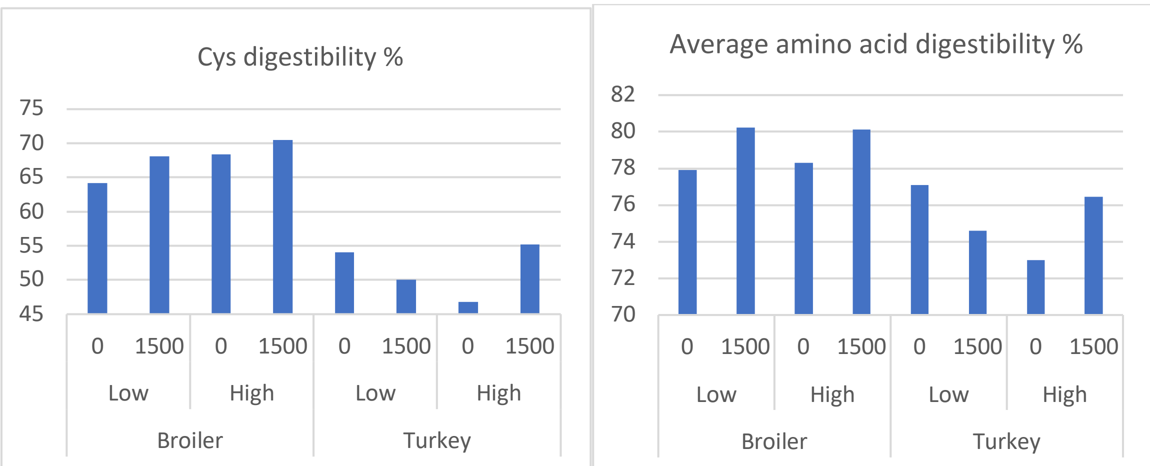

Figure 5. Amino acid digestibility (%) in high and low CaP diets with and without 1500 FTU phytase/kg feed. Adapted from Novotny et al, 2023b

Clearly broilers and turkeys respond differentially to phytase addition with regards to the responses noted in amino acid digestibility. As such it cannot be considered reasonable to use broiler data to predict the responses in turkeys which mirrors the conclusions for both P and myo-inositol release. Nevertheless, looking at the data pragmatically, in diets which are close to their Ca and P requirements (ie the high CaP diet), turkeys respond to phytase addition to greater extent in absolute and proportionate increment terms than broilers. As a result the “matrix” generated from broiler work would underestimate that if turkeys were used instead. This does not mean the data are reliable, but the consequences of using broiler data from this one study at least errs on the side of caution.

Conclusions

The manner in which the efficacy of both endogenous and exogenous phytases respond to changes in dietary Ca and P clearly differs between turkeys and broilers and the pattern of differences between species does not seem to be consistent or predictable. This means that the nutrients attributed to phytase application in turkey diets should be determined in turkey and not broiler trials moving forwards if more precise nutrition is the goal. It is fortuitous that the likely effects of phytase on P, inositol and amino acid uptake by the turkey has not been significantly overestimated to date through application of broiler data but clearly this practice should not continue.

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Unravelling the intestinal microbiota within the turkey selection environment

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Introduction

Poultry production is ubiquitous globally meaning that birds are placed in a diverse range of environments. As such, it is essential that the commercial bird has the ability to thrive in a wide range of production settings where they may experience different management approaches, geographical and environmental conditions including feed, and diverse microbial challenges. The adoption of multi-environment selection by primary breeders helps to achieve this as part of a balanced breeding strategy. This approach involves rearing pedigree selection candidates in an optimal high hygiene environment (*ped farm*) to allow for the expression of full genetic potential for biological efficiency and growth. Simultaneously, siblings of the selection candidates are reared in environments more akin to commercial poultry production (*sib test*) with commercial levels of biosecurity, stocking density, and supplied with diets with poorer feed form and reduced nutrient density. A fundamental difference in the two environments is the use of built up litter in the sib test (a common commercial practice in certain global regions) whereas the ped farm uses fresh litter with every flock placement. The use of built up litter acts as a tool to select for gut and immune robustness as the birds are exposed to a greater diversity of commercially relevant microbes. This approach allows for genetic improvements in bird health, robustness and performance holistically by enabling the selection of candidates in the ped farm that offer the desired trait improvements, whilst at the same time ensuring that they have the innate ability to thrive in more commercial like environments through the data obtained from their siblings in the sib test. Ultimately, this strategy allows accounting for the Genotype x Environmental interaction allowing the prediction of genetic merit across contrasting environments.

Intestinal health of livestock is an essential component of health, welfare and biological efficiency. One of the key constituents of intestinal health is the intestinal microbiome: this is a community of microbes found within the intestinal tract of all animals. Decades of research has shown that the intestinal microbiome plays a key role in the health and wellbeing of its host by promoting the development and maintenance of the immune system, enhancing intestinal function, aiding digestion and inhibiting the activity and growth of intestinal pathogens. Research carried out in a wide range of animal species has shown that the composition of the microbiota is dynamic, and there can be changes in the presence or abundance of species in relation to a range of factors such as host genetics, environmental conditions, nutrient intake, dietary formulation, and different health states. In addition to its role in promoting host health, the composition and activity of the intestinal microbiome have been associated with feed efficiency and animal performance.

Even though the intestinal tract of the developing poult *in ovo* is not completely sterile, the majority of bacterial species that colonise the intestinal tract of the poult originate from the feed, the litter and the farm environment in which they are placed. It has been shown through longitudinal studies that early exposure to different species of bacteria can influence subsequent gut function and composition of the microbiome. It is common for young birds to peck and ingest litter substrate as part of normal behaviour; consequently the contrasting environments used in multi-environment selection will expose the poults to different environmental bacteria that may influence intestinal health. The environmental microbiome may play a role in influencing the contrasting growth and performance of the birds across the difference environments

The advancements in sequencing technology and improved affordability of microbial community profiling has allowed for larger scale exploration and utilisation of the microbiome within commercial operations. Within the animal breeding sector, understanding its relationship with animal performance is proving to be a new frontier to better understanding traits associated with intestinal function and biological efficiency. Data from laying hens, cattle and pigs have shown that not only are components of the microbiome heritable, but also the relative abundances of bacterial species within the microbiome account for a proportion of the phenotypic variance for growth and efficiency traits. This has led to proposals that animal breeding should include selection for the microbiome to improve intestinal health and function. However, before achieving this a better understanding of the natural variation and succession of the composition of the microbiome throughout the life of the bird in relation to age, diet and environment is needed. Presented here is a preliminary investigation into the faecal and litter microbiome of multiple genotypes of pedigree turkeys and different ages reared in multiple environments within the Aviagen turkey breeding program in the USA and UK.

Materials and methods

Ten fresh faecal samples were collected randomly from each of the above groups displayed in figure 1 at three different ages (USA: 3, 8 & 13 weeks; UK: 5, 11, 17 weeks). Additionally, at the same time, four litter samples were collected from different areas of each pen. Samples were frozen immediately to ensure preservation of the bacterial composition at the time of sampling.

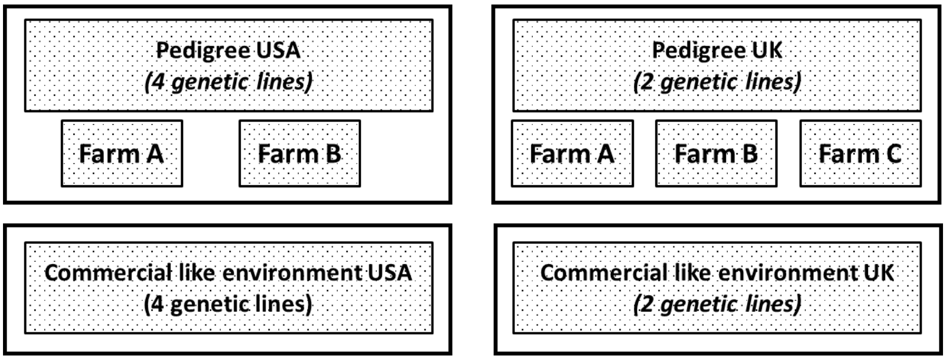

Figure 1. Trial design showing where samples were collected

DNA was extracted from all the samples using Qiagen Stool Mini Kit as per the manufacturer's instructions. The DNA was quantified using Qubit dsDNA quantification assay kit (Thermofisher, UK) and then pooled for each sample group and type for PCR and microbiome profiling. Each pool was analysed using 16S rRNA microbiome profiling using the Oxford Nanopore GRIDion platform. Sequence analysis and alignment was performed using OpenGene-fastp for filtering reads, Kraken 2 for taxonomic alignment with the Greengenes 16S database, and Braken for abundance correction. Microbial community analysis was then performed using the R statistical environment. Microbial communities were compared and contrasted across the different genotypes in the different environments at the different ages for both litter and faeces

Results and discussion

The data analysis demonstrated that there were very few significant differences in the microbial profiles when the respective litter and faecal samples were compared from each group. As the faeces is expelled into the litter it is only logical that the profiles would be similar. This is a useful finding as it means that litter microbiota can be used to obtain insights on the intestinal microbiota at the flock level where individual bird samples are not required.

In all genotypes across both the ped farms and sib tests in the UK and the USA breeding programs there were significant differences in the microbiota across bird age. For example, there are significant changes in the lactobacillus population as the birds age with species like *Lactobacillus salivarius* and *Lactobacillus reuteri* increasing in abundance with age. Similarly, members of the genera clostridium and ruminococcus can also be seen to be increasing. This is not unexpected as the microbiome becomes more complex with age with an increase in the level of bacterial fermentation. Furthermore, through the life of the flock there are changes in diet that will also contribute to a change in the microbiota as the birds age.

Comparisons of the microbiota across the different genotypes show that there are a small number of significantly different species and genera between some of the genetic lines. Many of the differences are members of the lactobacillaceae and the clostridiaceae; it is not clear whether these are biologically significant as the different bacterial species could be fulfilling the same biological role within the intestinal tract. Host genetics has been shown to influence the microbiome as such significant differences in microbiota between genotypes are expected. Further investigations are needed to understand the significance of these bacterial differences in relation to bird performance and intestinal function.

One of the key comparisons within this data set is the comparison of the microbiota between the optimal ped farms and the commercial like sib tests within the multi-environment selection program. The data shows that there are significant differences between all the environments indicating that each farm has its own distinct microbiome; this is a common finding across the published literature. However, the extent of the differences between farms varies (Figure 2.), for example, the least number of significant differences in the microbiome is between the ped farms both within the country of origin (i.e. UK vs UK, and USA vs USA) and when comparisons are made between the *ped* farms of the UK vs USA. This is likely due to the enhanced level of biosecurity on these farms and the consistency in management factors including the use of fresh litter with every flock. There are more significant differences in the faecal and litter microbiota when the *ped* farms are compared to the *sib test* farms. More fermentative bacterial groups (e.g. lactobacillaceae and the clostridiaceae) can be found in the *ped* farms which is indicative of better intestinal function; this likely has an association with the better growth and performance of the birds in this environment. Conversely, in the *sib test* environment there is an increased level of potentially pathogenic bacteria such as *Clostridium perfringens* and a range of potentially pathogenic staphylococci species. The nature of the *sib test* is to expose the birds to the normal challenges (e.g. environment, feed, microbial) that a bird may experience in the field to enable selection for robustness. This data shows that this environment does indeed expose the birds to commercially relevant bacterial challenges to allow for selection for robustness. The greatest number of significantly different bacterial species and genera is seen when the *sib test* farms in the UK and the USA are compared. This demonstrates the large diversity of bacterial species and genera that can be present in the environment of the turkey.

Further work is needed to investigate the biological and physiological significance in the changes in microbiome across bird age, genotype and environment. However, this data set demonstrates how the microbiome can be investigated on a large scale within a commercial landscape. This methodology provides the ability to monitor the abundance and composition of the microbiome within the breeding environments and gain more insight into the dynamics of the microbiome over time and the impact of farm hygiene practices. This study provides empirical evidence that multi-environment selection provides exposure to relevant environmental bacteria to continue to ensure the selection of traits associated with robustness, environmental adaptability and immunocompetence. In addition, this approach can be expanded to characterise the microbiome of commercial turkey farms and contribute to the expression of genetic potential from across the global industry.

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Connecting Gut Microbiome To Turkey Performance In Commercial Conditions

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I. Introduction

The gastrointestinal tract of poultry hosts a complex microbial community with hundreds of bacterial species and up to 10^{11} CFU per gram of digesta. The gut microbiome confers several benefits to broilers and also turkeys including exclusion of pathogens, breaking down polysaccharides to provide energy as short chain fatty acids and promoting development of a tightly controlled gut epithelial barrier and immune system. So establishment of a balanced microbiome is key to preserve gut health. Extensive research in broilers has learnt that the development of the microbiota includes rapid successional changes, developing from a simple, to a more complex and diverse composition based on gradual colonisation with microbiota. Defining the core gut microbiome and relation with health and disease is critical to understanding how this baseline may be modulated. In broiler chickens, the core microbiome has been difficult to define, due to high variability between birds and flocks, dynamic changes of core players across age and because the relation between bacteria and between bacteria and factors impacting them may also be non-linear. For that reason, Cargill developed a practical non-invasive microbiota assessment tool GALLEON® to define the core gut microbiota and extract actionable insights. In practice application of GALLEON® involves (1) collection of swab samples, (2) quantification of microbial biomarkers using a microarray chip with previously selected DNA populations which are then (3) analysed using statistics and non-linear AI models. With this combination of technologies GALLEON® provides poultry producers practical, actionable insights on the gut microbiome health of their flocks to help improve animal health, performance, preharvest food safety, and ultimately return-on-investment. Large scale field trials with GALLEON® have identified that high performing broiler flocks have a more stable microbiota at earlier age which is characterised by relative high abundance of lactate producing bacteria such as *Lactobacillaceae* in ileum during the first 14 days and consequently due to cross-feeding mechanisms higher abundance of short chain fatty acids (SCFA; butyrate, propionate) producing bacteria (*Lachnospiraceae*, *Ruminococcaceae*) since these bacteria use lactate as a substrate to produce SCFA. High performing broiler flocks are typically also characterised by lower abundance of *Proteobacteria* which contain several opportunistic pathogens. Further, several case studies with GALLEON® have shown that these microbiome insights allow to design targeted dietary interventions to change the gut microbiome towards a better developed profile resulting in better performance and health.

Growth performance and sustained flock health is of major economic importance to turkey producers too. Therefore the gut microbiome is assumed to play a critical role in overall health and performance of turkeys as well but the composition of bacterial species within the turkey gastrointestinal tract is less studied when compared to broilers. However several studies have learnt that also in turkeys development of the gut microbiota is age-dependent and includes rapid successional changes based on gradual colonisation with microbiota. Danzeisen *et al* (2013) proposed a model for the succession of bacteria in the turkey ileum where the core microbiome involves early colonisation with *Proteobacteria* which rapidly decline while birds mature. At the same time, consistent and dominant colonisation occurs with different lactate-producing bacteria (*Lactobacillus species*) similar as what we see in broilers. A shift occurs at weeks 3–4 of age when some birds are colonized heavily but variably by *Candidatus division Arthromitus (Lachnospiraceae)*, which is accompanied by consistent colonisation by *L. aviarius and L. johnsonii*. At weeks 5–7 of age, *Clostridium group XI* organisms and *L. ingluviei* begin to consistently colonize the ileum. This is accompanied with an increase in short chain fatty acid producing bacteria (*Bacteroidaceae, Ruminococaceae and Lachnospiraceae*) in ileum and mainly ceca when birds become older probably due to cross-feeding mechanisms similar as in broiler chickens. Another important shift of community transition is around weeks 10-12 with higher abundance of *Proteobacteria* (eg *Campylobacter*) which is hypothesised to be a time of increased susceptibility to colonisation by pathogens. The objective of this study was to correlate microbiota composition and development to performance in turkeys.

II. Method

Two high and two low performing farms were selected based on historical performance of the last four flocks – High performing flocks (Good farms): 5.27 kg body weight at 9 weeks and 9.07 kg at 12 weeks whereas low performing flocks (Bad farms): 4.74 kg at 9 weeks and 8.48 kg at 12 weeks. All chicks originated from the same breeder flock (Premium™, Aviagen) and hatchery. All 4 four farms used the same diet.

Cloaca swabs were collected from 24 turkeys in each farm at 9 and 12 weeks and were analysed using GALLEON® to assess differences in gut microbiome.

Microarray generated fluorescence readings passed data quality control and were standardised. Relative intensity for each bacteria DNA probe was submitted to ANOVA in a factorial arrangement with fixed effect of Age (9 and 12 weeks), Performance (Good vs Bad farms), and their interactions. Pairwise comparisons between standardised LS-means were made for each bacteria and variable combination adjusting for FDR (False Discovery Rate) test with P = 0.05.

III. Results

At 9 weeks the Good farms had significant higher numbers of fibre degrading bacteria (*Bacteroides, Alistipes*) and butyrate producing bacteria (*Faecalibacterium*) while Bad farms were associated with high abundance of *Clostridium perfringens* and *Enterobacteriaceae* which include many pathogens (Figure 1).

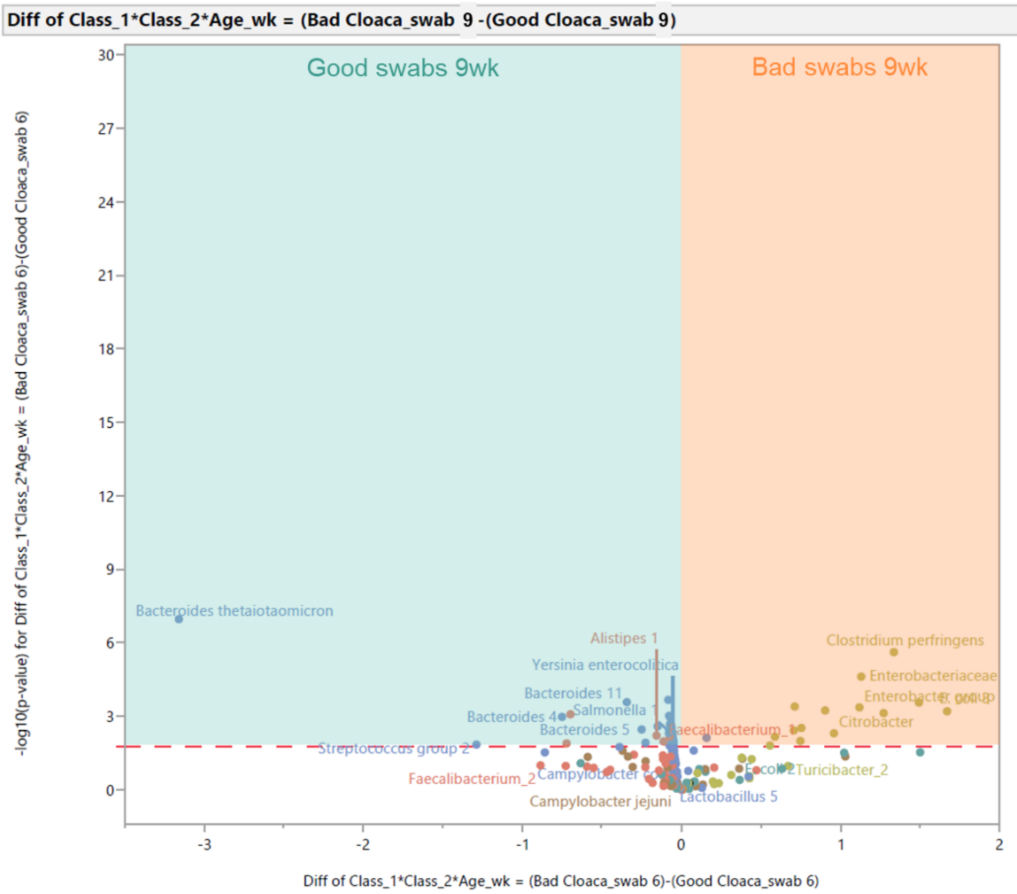

Figure 1 – Volcano plots for the pairwise comparison of microbiota differences between Good and Bad farms at 9 weeks.

At 12 weeks the Good farms showed high presence of different butyrate producing bacteria (*Ruminococcaceae, Lachnospiraceae*) while the Bad farms showed high numbers of several undesirable bacteria such as *Campylobacter coli*, *Turicibacter* (associated with impaired fat digestion) and *Listeria*. In addition, higher abundance of lactate producing bacteria (*L. crispatus, L. 5)* in the bad farms is referring to a delay in cross-feeding butyrate producing bacteria when compared to Good farms (Figure 2).

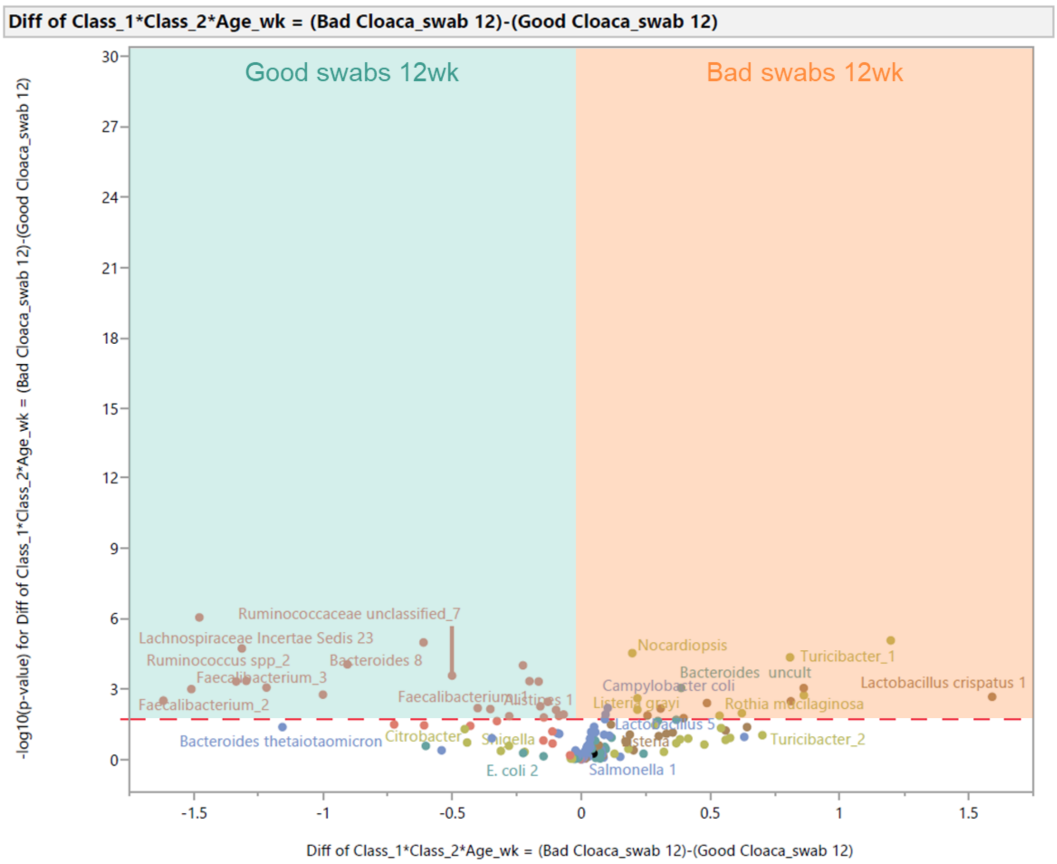

Figure 2 – Volcano plots for the pairwise comparison of microbiota differences between Good and Bad farms at 12 weeks.

IV. Discussion

The objective of this study was to evaluate any correlation between microbiota composition and performance in turkeys. Fibre degrading and short chain fatty acid producing biomarkers were consistently more abundant in Good performing farms while Bad performing farms were associated with higher abundance of pathogens, especially at 12 weeks.

Scupham et al (2006) investigated trends in microbial colonisation of the ceca of male turkeys between 9 and 14 weeks. *Faecalibacterium prausnitzii* pre-dominated at week 9 in both trials and was followed by a large transformation to principally *Bacteroides*. Also in our trial *Faecalibacterium* and different *Bacteroides* species were more present in the Good farms. *Faecalibacterium prausnitzii* belongs to the Family of *Ruminococcaceae* and is a predominant butyrate producing microorganism in the intestines of many mammals as well as poultry. *Bacteroides* have a very broad saccharolytic potential and some *Bacteroides* species are associated with good performance in broilers.

Weeks 11 and 12 were a time of significant changes and Scupham et al (2006) hypothesised that this period may be associated with increased susceptibility to colonisation by pathogens. This was confirmed with the detection of *Campylobacter coli* during that period. Also in our trial we identified higher abundance of *Campylobacter coli* at 12 weeks in the Bad farms.

Danzeisen et al (2013) compared the bacterial communities of young turkeys of different average flock weights using 16S rRNA microbiome analysis and observed a clear trend where the flocks classified as light contained greater proportions of OTUs classified as *Lactobacillus* species. *Lactobacillus crispatus* and *Lactobacillus 5* numbers were also higher in the Bad farms in our trial. This may refer to lagging cross-feeding mechanisms in Bad versus Good farms.

Further research is planned to evaluate how dietary interventions including nutrition and feed additives can modulate microbiome composition and dynamics resulting in improved performance and health in turkeys.

V Conclusion

The intestinal microbiome plays a crucial role in turkey health and production performance. Through GALLEON®, a practical non-invasive microbiota analysis platform, it was possible to compare gut microbiota composition between high and low performing farms and establish the correlation between microbiome and performance in turkeys. Results showed that high performing farms had higher numbers of fibre degrading and short chain fatty acid producing bacteria and were associated with lower pathogen risk compare to low performing farms. Further research is planned to evaluate dietary interventions including nutrition and feed additives to modulate turkey gut microbiome composition and dynamics and how this correlates with performance.

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Farming the microbiome to deliver returns.

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Introduction

The gut microbiome of a Turkey is the collection of all microoganisms (microbiota), such as bacteria, protozoa, fungi, viruses, and their genes, that are contained within the gastrointestinal tract of the bird. The microbiota can have both symbiotic and harmful interactions with the bird, the relationship and interactions between the bird and the microbiota is essential for bird production, health, and welfare, as the microbiota affects the nutrition, physiology, and gut development (Aruwa *et al*., 2021). A diverse and complex microbiota can act as a protective barrier (Jandhyala, 2015), by reducing the attachment of pathogenic bacteria to the gut cell wall through competitive exclusion, enhancing the resistance to colonisation and producing metabolites such as antimicrobials, organic acids, short chain fatty acids (SCFA) and vitamins (Yadav and Jha, 2019). These have important roles in the metabolism, nutrient digestion and absorption, immune system, and performance of poultry (Aruwa *et al*., 2021). Consequently, an unbalanced microbiota can lead to gut health issues including inflammation and leaky gut, that will negatively affect the bird's health and its performance (Clavijo et al., 2022). Therefore, focusing on managing gut health and the microbiota by supporting microbial diversity is key to disease control, performance, and maximising returns.

Helping to establish a healthy gastrointestinal microbiome.

Farming to help establish and maintain a balanced and functional gut microbiome will drive improved welfare, production and returns (Corrigan *et al*., 2023). Many factors affect the intestinal microbiota composition of poultry, including feed, environment, age, breed, temperature, use of antimicrobials, litter, stress etc (Kers *et al*., 2018). One of the main environmental factors that affects the microbiota is the diet (Corrigan *et al*., 2023). Several feed additives have been reported to influence the microbiota balance and diversity of the gut, including prebiotics, probiotics, and organic acids, helping to reduce dysbiosis (Corrigan *et al*., 2023). Prebiotics are non-digestible feed ingredients, such as oligosaccharides, that can alter the composition and metabolism of the gut microbiota (Yadav and Jha, 2019). Prebiotics containing mannan rich fraction, such as Actigen (MRF; Alltech Inc. USA), are one of the most promising feed supplements to alter the microbiome diversity and composition, decrease pathogens and support immunity (Corrigan *et al*., 2018; McCaffrey *et al*., 2021; Corrigan *et al*., 2023). MRF has a consistent impact on caecal microflora diversity, with Phylum Bacteroidetes replacing Phylum Firmicutes with supplementation (Figure 1, Corrigan *et al.,* 2018).

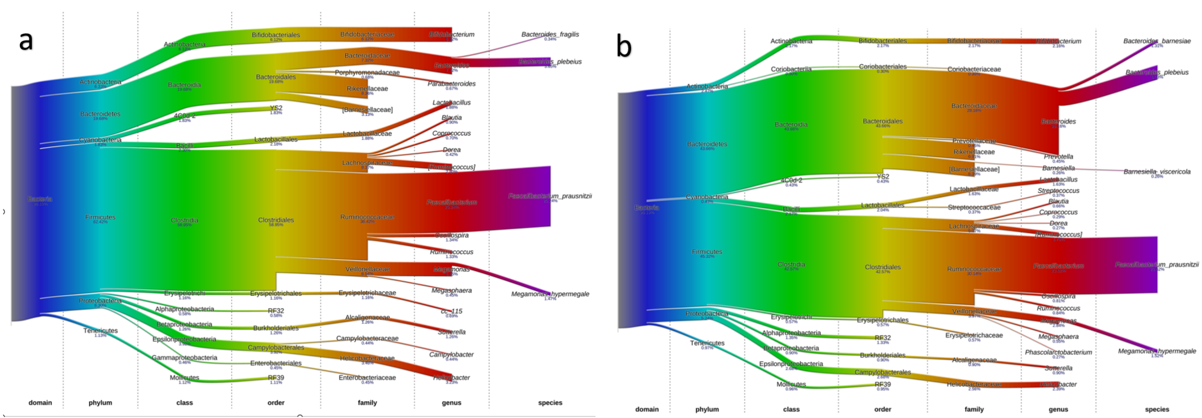


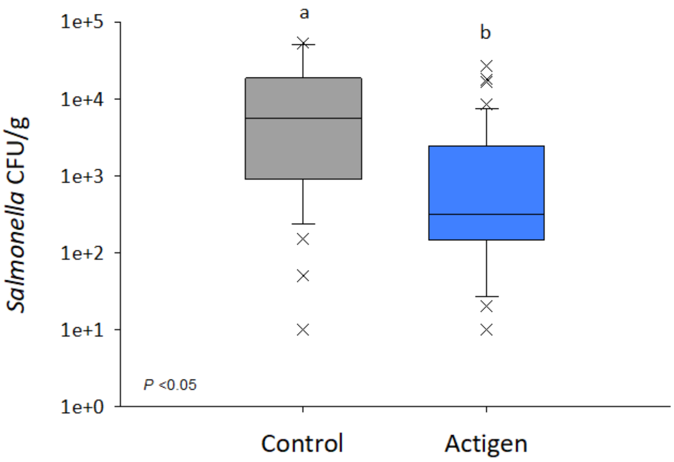
Figure 1. Bacterial phyla distributions (percentage relative abundance) at day 35 post hatch in control (a) and MRF-supplemented (b) groups for the four broiler trials combined (Corrigan *et al.,* 2018).

MRF has particular efficacy in binding to type-1 fimbriae in Gram-negative bacterial pathogens, allowing commensal microbiota like Lactobacillus to flourish. Increases in the complexity of the microbiome have been associated with improved health, with increases in the phylum Bacteroidetes being associated with favourable outcomes. These beneficial bacteria in the microbiome also produce compounds that can be used by the gut such as SCFA, vitamins and antimicrobial compounds. MRF stimulates the production of SCFA through influencing the microbiome (Corrigan *et al.,* 2019, Leigh *et al.,* 2022).

Excluding pathogenic bacteria from the gastrointestinal tract

When farming the microbiome, we not only need to increase the microbiome balance and diversity but also reduce the load of the pathogen in the bird through – vaccination, biosecurity, and competitive exclusion of pathogenic bacteria with commensal non-pathogenic bacteria. Historically antibiotics were used in poultry production to prevent and treat diseases, as well as promote growth (Roth *et al*., 2018). However, the effect of antibiotics is non-specific on the gut microbiome and results in the reduction of the overall gut microflora diversity (Vrieze *et al*., 2014), and can lead to increased antibiotic resistance within bacteria. Therefore, the goal is to have an intervention that will promote the beneficial bacteria whilst reducing the pathogenic load. A number of pathogenic bacteria including *E. coli* and *salmonella* colonize and infect the gut through attaching to mannose receptors on the gut cell wall via type 1 fimbriae. MRF can adhere to these type 1 fimbriated bacteria, which prevents them from attaching to the gastro-intestinal cell wall, preventing pathogen colonisation. This reduces inflammation and tissue damage and the overall pathogen load within the bird (Corrigan *et al.,* 2017; Figure 2, Girgis *et al.,* 2020).

Figure 2. Box plot of the mean concentrations (log10 CFU/g) of Salmonella in cecal contents from 17 week-old layers 1 week after a challenge with S. Enteritidis as influenced by dietary treatment. Means designated by different letters are significantly different (Girgis *et al.,* 2020).



Reducing the issue of antibiotic resistance

Antibiotic resistance is a substantial threat to global health, it can occur naturally, however the use of antimicrobials in the production of poultry has accelerated the rise in antibiotic resistance. With antibiotic resistance, infections become challenging to treat and lead to longer hospital stays and increased mortality in humans. In a recent report by the European Food Safety Authority, multidrug resistance was reported to be at high levels in Salmonella spp. recovered from fattening turkeys (EFSA, 2023), making antibiotic resistance a food safety issue. The increased awareness and pressure around antimicrobial resistance has led many countries to ban the use of antibiotic growth promoters in animal feeds. However, this alone is not enough to stop the growth of antimicrobial resistance which is a persistent issue because pathogens harbour the resistant genes. With rates of resistance still high despite restrictions on antibiotic use, it is critical to find ways to reduce resistance and increase bacterial sensitivity to antibiotics.

Recent published work by Smith *et al* 2022, has shown that MRF can impact bacterial susceptibility to antibiotics. MRF significantly reduced the growth of antibioticβesistant E. coli relevant to the control culture, and when MRF was used in combination with ampicillin there was a larger reduction in the growth of antibioticβesistant E. coli relevant to the control culture (Figure 3). Smith first reported that resistant organisms became more sensitive to antibiotics in the presence of MRF in 2017. Smith *et al.* (2017) observed that *Salmonella*-carrying plasmids that conferred antibiotic resistance had decreased growth when MRF were present. Smith *et al.* (2020) went on to report that the metabolism and growth of resistant *E. coli* changed when grown in the presence of MRF, resulting in an increased sensitivity to antibiotics. This increased effectiveness of the antibiotic used with MRF could result in a reduction in the level of antibiotic-resistant organisms in the bird and environment, as well as the development of a diverse and balanced microbiome.

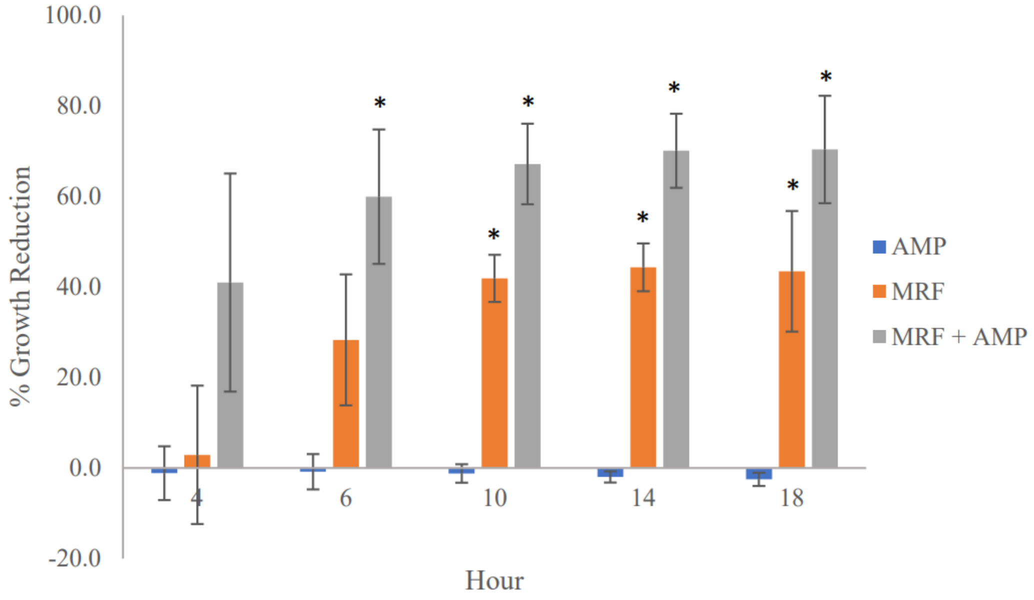


Figure 3. The effect of MRF and antibiotic treatment on the growth of antibiotic-resistant E. coli relevant to control culture. Standard error is represented by error bars. Means that were significantly different to one another within each time point are marked with an asterisk (*), p≤ 0.05 (Smith *et al* 2022).

Farming the microbiome to deliver returns.

The connection between the gut microbiome and health and efficient growth of poultry is well-known (Wei *et al*., 2013; Wilkinson *et al*., 2017). Indicating if you farm in a way to promote the microbiome balance and diversity of Turkeys then returns will be delivered. Support the gut microbiome and it will support the bird's performance. Having good gut health allows the bird to digest and absorb nutrients from its diet efficiently, meaning that it will have good performance. The overall performance benefits of MRF in Turkey diets are highlighted in a published Meta-analysis looking at 17 turkey trials, showing improvements in body weight, mortality and FCR (Table 1, Hooge , D. 2004).

Parameter	Control Average	MRF Average	Relative Improvement	P Value
Body Weight	5.643	5.770	+2.25%	0.006
FCR	2.019	1.988	-1.55%	0.125
Mortality	10.329	7.784	-24.64%	0.049

Table 1. Meta-analysis - The effect of MRF on turkey performance parameters compared to antibiotic-free control diet (Hooge , D. 2004).

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Muramidase improves gut functionality resulting in improved growth performance, feed efficiency and breast meat of turkeys

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Summary:

This study assesses an overlooked stressor to gastrointestinal functionality, namely the presence of Peptidoglycans (PGNs) in the gut. PGNs, integral components of bacteria cell walls, stay behind in the lumen after bacterial cell death or division. Here, PGNs reduce the effectiveness of the enterocytes and reduce nutrient absorption. Muramidase specifically selected to target PGNs of dead bacteria, facilitate the hydrolysis of PGNs and thereby reduces the burden on the intestinal cells. This results in higher availability of nutrients and energy for growth. The supplementation of broiler feeds with muramidase has been shown to be highly effective in a dose dependant manner. This paper reviews three in vivo turkey trials investigating the impact of muramidase on turkey performance and discusses the mechanisms by which muramidase improves gastrointestinal functionality and turkey performance.

Introduction:

Turkey production is a significant contributor to the global poultry industry, and with a projected consumption of nearly 7 million tonnes by 2025 (Johnson, 2018), turkeys are the second most crucial source of poultry meat worldwide (USDA, 2022). To meet the increasing demand for animal protein driven by the expanding world population, the poultry industry continuously optimizes bird growth performance. One way to contribute to improved growth is the application of feed additives. Feed additives, like enzymes, play a pivotal role in optimizing the digestibility of nutrients present in the feeds provided to the birds. Other additives, like pre- or probiotics and organic acids modulate the gut microbiota thereby improving gut functionality and facilitating optimal nutrient absorption.

An overlooked facet of poultry gut health involves the proportion of dead bacterial debris, constituting up to 30% of the total bacterial biomass in the intestinal tract (Frederiksen et al., 2021). Peptidoglycans (PGNs), an integral part of the bacterial cell wall, are released in the lumen of the gastrointestinal tract after cell death or multiplication. Accumulation of PGNs along the epithelial surface may impede nutrient absorption and diverts energy away from growth. The potential impact on the immune response further emphasizes the costliness of immune activation in terms of nutrient and energy partitioning.

PGNs, serving as the structural backbone of bacteria, trigger immune system receptors when in proximity to epithelial cells (Sorbara and Philpott, 2011). Consecutively, an immune response is initiated to prevent the infiltration of live bacteria across the brush border and into the bloodstream, mitigating potential damage to host tissues (Strober et al., 2008; Sorbara and Philpott, 2011). However, when bacteria die or multiply, PGNs are left behind in the lumen of the gastrointestinal tract. Recognition of these PGNs by immune system receptors initiates an unnecessary immune response, diverting nutrients and energy away from growth processes.

The immune system's response is delicately balanced between activation and downregulation. While PGNs upregulate the immune system, the ultimate end-product post-hydrolyzation, muramyl dipeptide (MDP), downregulates the immune system, signalling that these bacterial components no longer pose a threat (Hedl et al., 2007).

Muramidases contribute to the growth performance of the birds by hydrolysing PGNs of dead bacterial cell debris (Wang et al., 2020), facilitating improved nutrient absorption by epithelial cells. Subsequently, this hydrolysis induces a shift from immune system upregulation to downregulation (Wang et al., 2020), ensuring that the immune system responds to live bacteria while conserving nutrients and energy by refraining from reacting to dead bacteria. The net result is a more efficient and improved growth performance in poultry. Previous research on broiler chickens has demonstrated improved growth rates, feed efficiency, and nutrient digestibility with the inclusion of muramidase in their diet (Goodarzi Boroojeni et al., 2019; Sais et al., 2019). However, the influence of these enzymes in turkeys remains unexplored.

Bridging the gap: Assessing the Impact of Muramidase on Turkey Performance

Different studies address the existing gap in knowledge regarding the influence of muramidase on turkey performance, meat yield and welfare from hatch to market age (d126). Additionally, we have compared a full dose (45,000 LSU(F)/kg) of muramidase with a step-down dose from 45,000 to 25,000 LSU(F)/kg after d 56 post-hatch. This step-down dose is explored as a potential economic alternative, balancing the benefits with cost considerations. Finally, we evaluated the supplementation of muramidase in two different step-down schedules to turkeys reared under semi-commercial conditions.

The initial study investigated the effect of supplementing a full dose of muramidase to turkey feeds. A negative control diet was compared to the same diet including 45,000 LSU(F)/kg feed muramidase. A total of 1144 Aviagen BUT Premium female poults were divided over 26 replicates with 22 poults/pen in 2 consecutive runs, spanning an 84-day trial period. Feeding phases were as follows: period P1: days 1-21, P2: days 22-42, P3: days 43-63, and P4: days 64-84. The diet was based on maize, wheat and soybean meal, containing increasing metabolizable energy (ME) levels: from 2750 kcal/kg in P1 increased by 100 kcal/kg per phase to 3050 kcal/kg in P4. Crude protein (CP) levels decreased from 27.6% in P1 to 21.4% in P4. Besides growth performance, footpad dermatitis was measured as an indication for welfare.

Significant improvements due to muramidase were observed in feed conversion ratio (FCR) during P1, decreasing from 1.450 in control to 1.403 ($P<0.001$) in muramidase treatment. No significant differences were found in the other phases or in the overall trial. Muramidase significantly improved body weight (BW) in P1, P2, P4 and the overall trial. At d84 at the end of the trial, the birds fed muramidase were 2% or 145g heavier ($P<0.05$). Performance data from day 1-84 can be found in Table 1. Footpad dermatitis, assessed on a 0 to 4 scale (Picture 1), indicated a trend towards improved footpad health in muramidase-fed birds, characterized by reduced scores of 1 and 2 and increased scores of 0, although these results were not statistically different. Despite control birds exceeding breed standards, muramidase was able to give the poults a better start according to better BW and FCR. Moreover, the potential correlation between footpad health and gut functionality suggests an overall performance improvement with muramidase supplementation, even in comparison to high-performing control birds.

Building upon the insights gained from the initial trial, the second trial further investigated the effect of a muramidase step-down approach alongside a high dose of muramidase and a control treatment. A maize, wheat, soybean meal control diet (Ctrl) was either supplemented with 45,000 LSU(F)/kg muramidase throughout the entire 126-day trial period (MUR45), or 45,000 LSU(F)/kg from d0-56 and 25,000 LSU(F)/kg from d56-126 (MUR45-25). A total of 768 male BUT6 poults were divided over 24 pens, 32 poults/pen and 8 replicates per treatment. There were 6 feeding phases: P1: days 0-21, P2: days 21-35, P3: days 35-56, P4: days 56-70, P5: days 70-105, and P6 from d105-126. ME levels ranged from 2775kcal/kg in P1 to 3190 kcal/kg in P6 and CP from 27.15% in P1 to 18.54% in P6.

Significant improvements in FCR ($P=0.043$) were observed in MUR45 compared to Ctrl in P1, P2, P3, and the overall study, with a 4-point improvement at day 126. MUR45-25 exhibited improved FCR compared to Ctrl in P3 and the overall study, demonstrating a 2.7-point improvement over the control at day 126. FCR comparisons between MUR45 and MUR45-25 were not statistically significant. BW of MUR45 was significantly higher in all phases and overall study apart from P1 ($P=0.014$). BW of MUR45-25 was significantly higher than Ctrl in P3, P6 and the overall study. Again, there were no significant differences on BW between the MUR45 and MUR45-25 groups. At the end of the trial, the BW of MUR45 was 8.3% higher than Ctrl and BW of MUR45-25 was 5.5% higher than Ctrl. Conversion of BW and FCR results into the European poultry efficiency factor (EPEF, formula = (survival (%) x average weight (kg) / trial period (days) x FCR) x 100). EPEF score was significantly improved in MUR45 vs. Ctrl ($P=0.025$) and intermediate in MUR45-25. Results into carcass yield assessments showed higher breast proportions as a percentage of live weight for both muramidase treatments compared to Ctrl: 22.4% in Ctrl, 23.4% in MUR45, and 23.3% in MUR45-25 ($P=0.012$). Performance and meat yield data from day 1-126 can be found in Table 1. Similarly to the first trial, footpad dermatitis responses for both muramidase treatments, measured at d56 showed a significantly higher amount of score 1 and lower score 2 compared to Ctrl (Graph 1). This study shows the effectiveness of the step-down muramidase supplementation approach for turkeys, providing an optimal return on investment. The findings further emphasize that enhancing nutrient availability through the hydrolysis of PGNs and subsequent improvements in gut functionality result in higher performance, meat yield, and overall welfare of turkeys.

In the last step-down study described here muramidase efficacy was evaluated under semi-field conditions using 1485 BUT6 male poults allocated to 18 replicates with 81 animals each, spanning a trial duration of 21 weeks (147 days). P1: wk 1-2; P2: wk 3-5; P3: wk 6-9; P4: wk 10-13; P5: wk 14-17; P6: wk 18-21. Three treatments were tested: T1, the control, comprised of a wheat, corn, and soybean basal diet. T2, low muramidase: control including 45,000 LSU(F)/kg muramidase in P1 and P2 and 25,000 LSU(F)/kg muramidase in P3 and P4, with no muramidase in P5 and P6. T3, high muramidase: 45,000 LSU(F)/kg muramidase in P1-3 and 25.000 LUS(F)/kg muramidase in P4-6.

BW was significantly higher for T3 ($P<0.05$), while T2 and control diet showed no significant difference. FCR exhibited a numerical advantage for T2 and T3 vs. T1, however, statistical significance was not observed. The EPEF score was found highest in T2, followed by T3 and the lowest for control. Both muramidase treatments performed significantly better than the control treatment ($P<0.05$). Performance and meat yield data from day 1-147 can be found in Table 1. There were no significant differences in footpad dermatitis, litter scores and meat yield. Considering performance, efficiency, mortality, and treatment costs, T2 resulted in the highest ROI. This aligns with previous trial outcome, where the step-down muramidase approach demonstrated superior economical performance compared to the high muramidase treatment. These findings underscore the economic viability and effectiveness of a step down muramidase supplementation in enhancing turkey performance under semi-field conditions.

Conclusion:

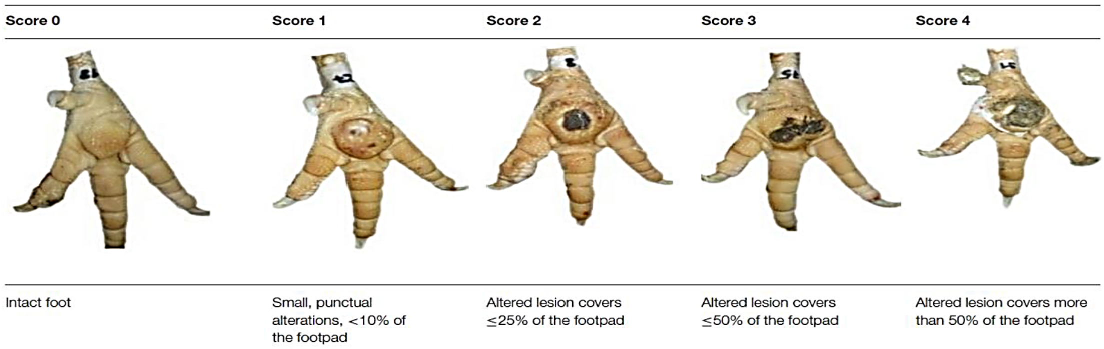
This comprehensive series of studies confirmed the capacity of muramidase to hydrolyse PGNs, thereby alleviating the burden on intestinal cells and enhancing nutrient availability, resulting into higher growth performance in turkeys. The initial study showed significantly improved FCR and BW in specific feeding phases, indicating a positive impact on turkey growth performance. Consequently, muramidase improved footpad health, suggesting a potential link between enhanced gut functionality and overall performance, even surpassing high-performing control birds. The second trial further explored the efficacy of a step-down muramidase supplementation approach, comparing it with a high-dose supplementation and a control diet. Results demonstrated that the step-down approach significantly outperformed the control in terms of FCR, BW, and carcass yield. With the highest performance in the high-dose treatment. The study reinforced the economic viability of the step-down muramidase supplementation, showing higher returns on investment compared to the high-dose treatment. The efficacy of a step-down approach was validated in the third trial under semi-field conditions. Treatment 2 (low muramidase) resulted in the highest body weight and EPEF. This aligns with the results of the second trial, confirming the interests in the step-down muramidase approach in turkeys. The findings underscore the economic effectiveness of this supplementation strategy in enhancing turkey performance under semi-field conditions. Collectively, these studies contribute valuable insights into the mechanisms by which muramidase improves gastrointestinal functionality and turkey performance. The step-down muramidase supplementation approach seems to be a particularly promising strategy, resulting in superior performance and economic efficiency in comparison to both high dose muramidase and control treatments. These findings hold significant implications for the poultry industry and provide a begin to understand the potential benefits of muramidase in optimizing turkey production.

Table 1: Performance data of all three trials described.

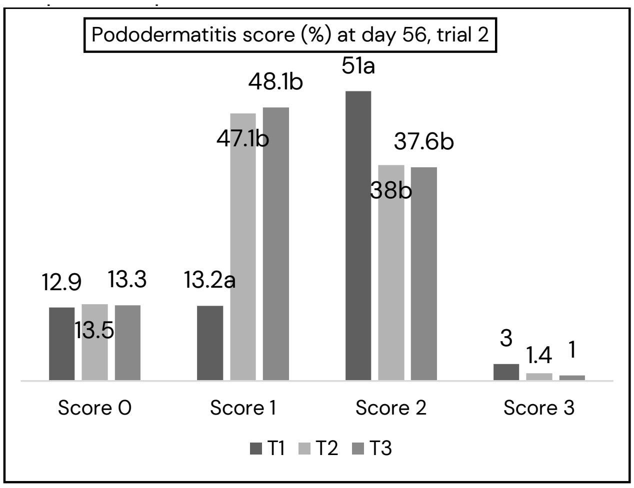
		BW end trial (kg)		FCR		EPEF		Carcass weight (% of live weight)		Breast weight (% of live weight)	
Trial 1	Control	7.369b	*	2.030							
	45k Muramidase	7.531a		2.026							
Trial 2	Control	12.856b	***	2.54a	****	393b	**	70.6b	*	22.4b	*****
	45k Muramidase	13.927a		2.12b		453a		71.6a		23.4a	
	45-25k Muramidase	13.573a		2.27ab		418ab		71.5a		23.3a	
Trial 3	Control	21.81a	*	2.550		531.9a	*	75.63		23.9	
	Low Muramidase P1-4	21.94a		2.510		548.6b		74.03		23.18	
	High Muramidase P1-6	22.14b		2.530		544.3b		74.09		23.08	

*P<0.05
**P=0.025
***P=0.016
****P=0.014
*****P=0.012

Picture 1: Score system used to measure footpad lesion severity.



Graph 1: Footpad dermatitis scores from trial 2, measured at day 56.



P=0.021

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The first extensive bio-monitoring mycotoxin exposure survey in turkeys reveals significant exposure to multi-mycotoxins

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Introduction

Mycotoxins are toxic fungal secondary metabolites which contaminate agricultural commodities during cultivation, harvesting, transport, processing, and storage. Many filamentous fungi are toxigenic, and the most important producing genera are *Aspergillus*, *Fusarium*, *Penicillium* and *Alternaria* (Marin et al., 2013). Despite several prevention strategies on the field and adequate grain storage and transport, the presence of mycotoxins cannot be avoided as reflected in recent large field feed surveys (88% prevalence with at least one mycotoxin; Gruber-Dorninger et al., 2019). Even low mycotoxin concentrations in the feed, below the limits described by the European commission (EC) (EC 2002, EC 2006, EC 2013), can impact animal health and performance significantly (Kolawole et al., 2020). Therefore, it is of crucial importance to establish reliable tools to elucidate the real exposure. Until now, feed analysis has been the main tool for monitoring mycotoxin exposure in animals which however suffers inherently from the large variability associated with the overall testing procedure, mainly due to feed sampling, as this accounts for >75% of the analytical error (Whitaker, 2006). As feed analysis presents important limitations to perform an accurate and precise risk assessment, biomarker analysis has been proposed as the effective tool to assess the risk of exposure through an analysis of their metabolites in biological fluids (Vidal et al., 2018). However, due primarily to sample collection difficulties, mycotoxin biomarker analysis has not yet been widely adopted. To this, recently a UHPLC-MS/MS method targeting 36 mycotoxin biomarkers via dried blood spots (DBS) was validated by our group (Lauwers et al., 2019). The methodology enabled analysis of mycotoxin biomarkers at an industrial scale using minimal blood volumes with the aim to establish a more precise mycotoxin exposure in animal production under field conditions.

Objective

The main aim of this study was to elucidate for the first time a more realistic mycotoxin exposure in turkey from farms all over the globe by combining feed and blood analyses. The secondary objective was to evaluate possible correlations between this combined (feed and blood) mycotoxin assessment with turkey clinical symptoms observed under real field conditions.

Material and methods

Sample collection and analysis

From May 2020 to January 2024, >200 blood samples (with the use of FTA cards) from individual turkey and 30 feed samples were collected from 42 farms. Both, feed and blood were analysed using highly sensitive chromatography. Thirty-six different mycotoxin biomarkers were analysed in blood and sixteen different mycotoxins in the feed. Additionally, animal clinical symptoms and performance were recorded in an attempt to establish possible correlations with mycotoxins exposure.

Statistical Analysis

Statistical analysis was carried out with Microsoft Office Excel 2007 (Redmond, WA, USA) and SPSS® 15.0 (Chicago, IL, USA). The paired t-test ($r < 0.05$) was applied to explore probable mycotoxin concentration variances in the stability trial.

Results and Discussion

The combined analysis of feed and blood analysis via DBS demonstrated a persistent and widespread exposure to multi-mycotoxins in turkey farms around the world. Specifically, combined feed and blood analysis revealed

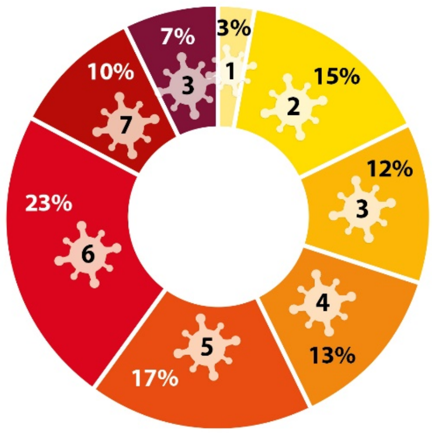

Figure 1. Distribution of total number of mycotoxins detected in feed + blood analysis after analysing >200 turkey animals.

that nearly all cases studied (97%) demonstrated simultaneous exposure to two (2) or more mycotoxins, 70% of the total cases demonstrated simultaneous exposure to four (4) or more mycotoxins, 57% of the total cases to five (5) or more mycotoxins and, 40% of the cases to six (6) or more mycotoxins (Figure 1). The co-exposure to different mycotoxins is of concern as simultaneous exposure result in a synergistic or additive toxic effect (Alassane-Kpembi et al., 2017). The blood analysis via DBS specifically, revealed that mostly neglected emerging mycotoxins produced by *Alternaria* and *Fusarium* spp. were predominant in blood alongside ochratoxin A, zearalenone and deoxynivalenol. More specifically, tenuazonic acid was the most prevalent mycotoxin found in blood accounting for 67% of total cases and followed by enniatins (65%; cumulative sum of enniatin A, enniatin A1, enniatin B, enniatin B1), ochratoxin A (45%), aflatoxins (33%), zearalenone (30%; all forms and their metabolites) and deoxynivalenol (15%; all forms and their metabolites). As these emerging mycotoxins are as toxic as the legislated mycotoxins, the predominant prevalence of them confirms the need to include emerging mycotoxins in the routinary mycotoxin control analysis as they suppose a significant part of the total mycotoxin risk. The concentrations in blood ranged from trace level up to 144 ng/mL. Moreover, the blood biomarker analysis uncovered additional risk which was ignored by feed analysis as in 72% of the cases, blood analysis identified mycotoxin exposure missed by the latter. Interestingly, some tendencies between mycotoxin exposure and health status were also seen. For example, reported necrotic enteritis cases were consistently exposed to deoxynivalenol and fumonisins or kidney lesions were exposed to ochratoxin A.

In conclusion, the first of its kind extensive mycotoxin exposure survey elucidated that turkey farms under real farming conditions are dominantly exposed to multi-mycotoxins and that biomarker analysis can be key in optimizing animal health and performance and it an essential tool to optimize the mitigation strategies.

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Metabolisable energy of rapeseed meal for turkeys does not vary by batch

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Introduction

With the constant rise in the price of imported soybean meal (SBM) and its high environmental footprint, attention has been redirected toward the need to develop alternative protein sources for modern turkey production, such as rapeseed meal (RSM) (Watts et al., 2021). Due to its relatively high-protein content (36% to 40%) over the past decade the use of RSM in UK turkey rations has become commonplace. However, there is limited recent data on its nutrient value to turkeys and the variability that exists between RSM produced from different rapeseed cultivars, leaving tabulated data as a key source of information (Olukosi et al., 2017). Variation in the nutrient composition, available energy and digestibility of feed ingredients can be substantial and is associated with biological and environmental factors as well as regional crop management practices (Azhar et al., 2019). Co-products such as RSM are particularly prone to increased variability in nutritional values due to differences in the manufacturing practices between crushing facilities (Adewole et al., 2016). This experiment was designed to investigate the influence of rapeseed batches received in crushing plants on the range of variation in the nitrogen corrected apparent metabolisable energy (AMEn) for young turkeys.

Animals and Experimental design

All RSM samples were obtained from a single UK manufacturer (ADM, UK). The sampling interval was between 14 and 21 d during a period of 90 d from January to April 2018, yielding 7 samples in total. All samples were stored in bags at ambient air temperatures in a dry store. The stored RSM samples did not experience any freezing temperatures during this storage. A representative sample was taken from each of the seven batches and the major chemical components were measured. Although the manufacturer followed the same procedures during the oil extraction and RSM production, different batches of rape seed were used.

Nutrient availability was examined in a turkey poults experiment from 12 to 21 d age. An experimental diet with the main ingredients being wheat (42%), maize gluten meal (5%), RSM (5%) and SBM (32%) was produced (Table 1). Each of the seven RSM samples were incorporated into a nutritionally complete diet in meal form at 200 g/kg (800 g of the basal feed + 200 g of each RSM sample). The nutrient specification of the diets met the breeder's recommendation (Aviagen Ltd.). An eighth dietary treatment was also fed that was the basal feed only. Female BUT Premium turkeys were obtained from a commercial hatchery (Faccenda Foods Ltd., Dalton, UK) at day old and were placed in a single floor pen and fed on a proprietary wheat-SBM turkey feed until 12 d of age. During the first eleven days, all birds were fed the basal feed only. Two birds were randomly allocated to one of 48 cages with 0.36 m^2 floor area and given the experimental diets *ad libitum*. There were six replicates for each diet. Standard temperature and lighting programmes for turkeys were used (Aviagen, Turkeys Ltd.). At 17 d of age, after 5 d given to adjust to the diets, the total excreta were collected for 4 d until the end of the study at 21 d age. Feed intake for the same period was recorded.

Ingredients	**%**
Wheat	47.76
Prairie meal	5.00
Rye	2.0
Rape seed meal (RSM)	5.00
Soya ext hipro	32.00
L-Lysine HCl	0.50
DL-methionine	0.40
L-threonine	0.14
Soya oil	2.00
Limestone flour tru.270	1.00
Dicalcium phosphate flour	3.50
Salt	0.30
Turkey premix	0.40
Calculated provisions %	
Oil	3.57
CP	26.52
ME	11.85
Lysine av	1.59
M+C	1.22
Ca	1.395
P av	0.7786

Table 1. Composition of experimental turkey diet.

[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 49 g/kg bird and the body weight at 21 d age was 492 g/bird, as no difference was detected ($P > 0.05$; data not in tables). The control diet had higher AMEn compared to the rest of the RSM diluted diets, but no differences in NR were detected (Table 2). This may be explained by the relative dilution of the diets with fibre and reduced available energy of the RSM samples compared to the control diet. There was no difference in NR between diets and between AMEn of the studied RSM samples ($P > 0.05$). Watts et al. (2021) also reported no significant effect of cultivar on AMEn or protein digestibility coefficients when fed to broiler chickens. However, the values of RSM metabolizable energy were slightly lower than those obtained with broilers (Watts et al., 2020, 2022), presumably due to the differences in gastrointestinal tract development at this stage between the two species. The lack of an effect of batch on the AMEn was likely due to the similarities between most of their major proximate components including EE, NSP and neutral detergent fibre (NDF) (data not in tables). Variations in their glycosylate contents did not reduce the energy values of the RSM, which agrees with research with broilers (Olukosi et al., 2017; Watts et al., 2021). Based on the results of the correlation analysis there is some suggestion that NDF could be a good predictor of the AMEn of RSM (Table 3). This agrees

Table 2. Nitrogen retention coefficients and AMEn in diets and rapeseed meal.

	NR diets	AMEn diets (MJ/kg DM)	AMEn rsm (MJ/kg DM)
Basal	0.623	13.63[a]	-
RSM 1	0.546	12.39[b]	7.36
RSM 2	0.520	12.01[b]	5.50
RSM 3	0.539	12.39[b]	7.44
RSM 4	0.532	12.20[b]	6.43
RSM 5	0.540	12.52[b]	8.09
RSM 6	0.557	12.60[b]	8.53
RSM 7	0.539	12.45[b]	7.69
SEM	0.0158	0.223	1.023
P	0.003	<0.001	0.440

NR = nitrogen retention in diets; AMEn diets = nitrogen corrected apparent metabolisable energy in diets; AMEn rsm = nitrogen corrected AME in rapeseed meals; SEM = Pooled standard error of the mean.

Table 3. Selected correlation coefficients between determined AMEn and compositional profile of rape seed meal samples when fed to turkeys.

	AMEn	*N*	*Oil B*	*GLS*
N	0.549			
Oil B	-0.285	-0.601		
GLS	0.231	0.589	-0.588	
NDF	-0.864	-0.511	0.067	0.165

$P < 0.1$ ($r \geq 0.669$; $0.753 \leq r$); $P < 0.05$ ($r \geq 0.754$; $0.873 \leq r$); $P < 0.01$ ($r \geq 0.874$)

AMEn, nitrogen corrected apparent metabolisable energy (MJ/kg DM) in RSM; N, nitrogen in rapeseed meals (g/kg DM); Fat, as oil B in rapeseed meals (g/kg DM); GLS, glycosylates in rapeseed meals (umol/g); NDF, neutral detergent fibres in rapeseed meals (g/kg DM).

With previous research with broiler chickens (Adewole et al. 2017; Watts et al. 2021). There were no other significant correlation coefficients to describe AMEn values of RSM cultivars. The greater fibre content could have accelerated digesta transit time and also limited the activity of the gut microbiota leading to greater excreta output (Adewole et al. 2017). Clark et al. (2001) obtained higher AMEn values for low-fibre, tail-end dehulled RSM compared to proprietary RSM suggesting that a lower fibre content can improve energy utilisation in RSM.

CONCLUSION

The reported results showed that there is no significant difference in AMEn obtained from the same processing plant. There was a correlation between AMEn values and NDF content in RSM. Experiments involving more samples and obtained from different crushing plants may provide a wider and robust data set.

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A new approach to evaluating enzyme combinations in turkey feeds

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Introduction:

Exogenous enzymes are widely used in poultry production (broilers, layers, ducks, and turkeys). Individually, phytases, carbohydrases, and other enzymes are added to feed based on their isolated effects upon specific substrates within the feed and ultimately, the direct and indirect effects on animal performance. Although these enzymes are combined to take advantage of the individual activities, the efficacy of each of the enzymes is not additive. In the turkey industry, various enzymes are utilized; however, their use is predicated on broiler, not turkey research.

Considering the substrate-enzyme relationships within a practical formulation, the following turkey trial was conducted to better define the potential nutritional uplift when a multi-carbohydrase and alpha galactosidase were used in the presence of phytase. The results of this study were used in an arabinoxylan-AME prediction model from similar broiler trials (Cozannet P. et al., 2017); to define if the response in turkeys and broilers is similar or different.

Trial:

The turkey research was conducted at Applied Poultry Research (APR) in collaboration with Adisseo NA. In total, 1200 Nicholas tom poults were split across 4 treatments, with 15 replicates x 20 tom poults/pen, blocked over 2 rooms. The dietary treatments within each phase were based on a common feed (corn/ wheat/ SBM/ phosphate) that followed industrial ingredient and nutritional specifications, except for AME (less 120 kcal/ kg feed). It is important to note that the diets contained a 6-phytase that was dosed at 1000 FTU/ kg feed. The treatments were: T1: Control diet; T2: Control diet + a multi-carbohydrase (xylanases, beta- glucanase and arabinofuranosidases) at the recommended dose; T3: Control diet + alpha- galactosidase at the recommended dose; T4: Control diet + multi-carbohydrase and alpha-galactosidase at the recommended doses. Five dietary phases were fed over 87 days, with AME in Control diet ranging from 2900 to 3220 kcal AME / Kg feed and dig Lys ranging from 1,65 to 1,21%. All diets were fed *ad libitum* throughout all phases.

Results:

The main highlights from the trial: In the presence of 1000 FTU phytase, the multi- carbohydrase (Trt 2) significatively improved the feed conversion ratio (FCR) by 0,02 points when compared to the control (Trt. 1). The use of alpha-galactosidase resulted in significantly lower BW and feed intake, and no improvement in FCR. See results in Table 1.

Table 1. Performance results, from 0-87d of age

Dietary treatment	Body weight kg/bird	Body weight gain kg/bird	Feed conversion ratio adjusted mortality	Feed Intake kg/bird
1. Control diet	**10.61 ± 0.02 [a]**	**10.55 ± 0.03 [a]**	**2.06 ± 0.003[a]**	**23.67 ± 0.05[a]**
2. Control diet plus Multi carbohydrase	**10.73 ± 0.02 [a]**	**10.67 ± 0.03 [a]**	**2.04 ± 0.003[b]**	**23.62 ± 0.05[a]**
3. Control diet plus alpha-galactosidase	**10.42 ± 0.02[b]**	**10.36 ± 0.03[b]**	**2.06 ± 0.003[a]**	**22.39 ± 0.05[c]**
4. Control diet plus Multi carbohydrase and alpha-galactosidase	**10.61 ± 0.02 [a]**	**10.55 ± 0.03 [a]**	**2.06 ± 0.003[a]**	**22.69 ± 0.05[b]**

Discussion:

Based on nutrient values from the Adisseo NIR database, the dietary total Arabinoxylan (AX) ranged from 2,04 to 2,05%. Using the AX-AME prediction equation of Cozannet, et al. (2017), the level of AX in the current trial (y-variable) was used to solve for AME (X-variable). The AME release was calculated to be 37 kcal/ kg feed (for broilers). Using the linear response equation for ME in broilers to predict the ME effect on the FCR of broilers (Marx F.O. et al, 2023), it was determined that 0,02 points in FCR corresponds to 44 Kcal/Kg of broiler feed. Based on the two separate equation sets and AME responses, the predictions indicate that similar responses between AME and FCR can be observed between turkeys and broilers, especially when a multi- carbohydrolase is used and the effect on AME is predicted based on AX content. More studies should be conducted to confirm the use of total dietary AX content in precisely predicting the improvements expected in AME for the multi-carbohydrase in turkeys.

Conclusion:

A multi-enzyme complex containing xylanases, beta-glucanase, and arabinofuranosidases can improve the FCR in turkey feeds on top of 1000 FTU phytase. A new approach to better predict enzyme effect on turkey diets based on the AX content level in feed can bring higher precision to the use of enzymes in turkey feed. Future studies are necessary to include a variety of enzymes in turkey feeds to further understand their commensal or antagonistic effects.

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Efficacy of herbal and aromatic components in vitro suggests prevention of histomonosis and enteritis problems in turkeys.

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Abstract: Histomonosis is the main disease facing all turkey producers since the ban on specific antihistomonal drugs in 2003. Moreover, coccidiosis and non-specific enteritis are diseases that can exacerbate blackhead disease because of the intestinal health problems they cause. Therefore, it appeared pertinent to develop a solution that can prevent all these issues at the same time. Recent publications underlined the reemergence of the blackhead disease not only in turkeys but also in chicken parent stock and free-range laying hens. Because of the stop or the limited access to specific therapeutics and the difficulty in manufacturing a vaccine, it appeared that a plant-based solution would be a possible way forward. Different *in vitro* experimentations, in collaboration with some research institutes, indicated that a pertinent choice of plant extracts and essential oils can lead to the design of an effective product capable of preventing all these gut disorders at the same time.

Introduction

The main challenge for the turkey gut health is to fight against the main parasitic threats, mainly histomonosis and coccidiosis, against bacterial infections like *E. coli* and *Clostridium* and at the same time preserving the positive microflora. The right balance of this microflora is very valuable for the preservation of the gut epithelium integrity and consequently for a positive digestibility trend.

Anti-histomonosis drugs were banned for a long time and the turkey producers are facing since that time regular black-head diseases outbreaks with different levels of mortality and with degraded performances in all cases of outcome (clinical or sub-clinical courses of the disea).

Coccidiosis prophylaxis relies for more than 50 years on the use of anticoccidials. Subsequently, the emergence of parasite populations resistant to anticoccidials was unavoidable due to their widespread use as a feed additive in poultry feed.

The research works carried out to design a phytoproduct for the chicken coccidiosis prevention and the dysbiosis that occurred at the same time will be also presented here. For this purpose, we worked with INRAE team which is able to cultivate Eimeria parasites on specific cells culture.

Together with INRAE (National Research Institute for Agriculture, Food and Environment), we evaluated 150 active ingredients, mainly of natural origin (plant extracts or essential oils) but also synthetic aromatic compounds naturally present in the environment. These compounds were selected for their direct cytotoxic effect on the dissemination stage of the parasite (oocyst) or for their ability, alone or in combination, to limit invasion and/or development in epithelial cells.

In another side, we have studied with another research partner (Clinic for Poultry and Fish Medicine, University of Veterinary Medicine, Vienna) a way to test specific bioactives, in the same spirit than the previous study. The goal of this study was to investigate the effect of plant substances on the growth and viability of *in vitro* cultivated *Histomonas meleagridis.*

Finally, some bioactive mixtures were *in vitro* tested against a wide range of bacteria, *Clostridia, Enterococcus and Lactobacillus* in particular, in collaboration with the University of Lille (France).

Besides these three experimental studies with scientific partners, we have added the specific research results coming from the R&D department from IDENA (final choice of compounds, choice of adjuvants, choice of emulsifiers to make the microemulsion).

Specific experimental designs for each part of the project

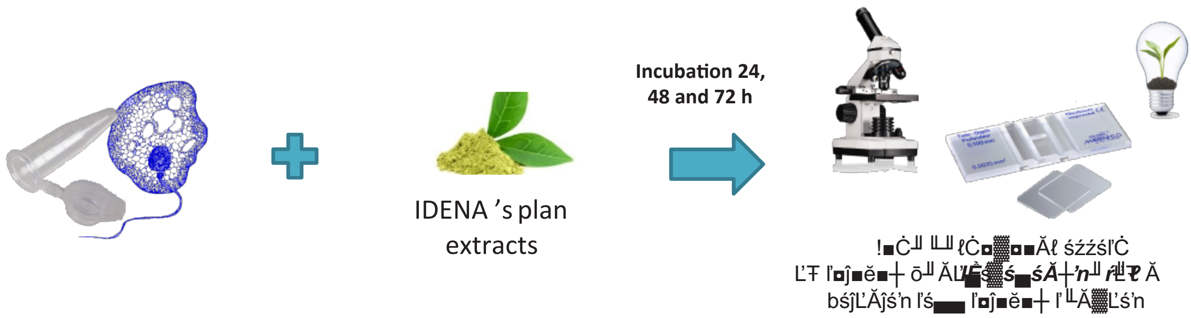
Histomonads study part

The aim of the project was to investigate the effect of plant substances on the growth of *H. meleagridis in vitro*.

For that, ten substances (aromatic and synthetic substances and mix of different substances) with two different concentrations (0.5% and 2.5%, respectively 100 and 500 ppm for bioactive concentration) were selected and provided by IDENA (Sautron, France).

The *in vitro* tests were performed at the Clinic for Poultry and Fish Medicine, University of Veterinary Medicine Vienna.

The research process was the following:



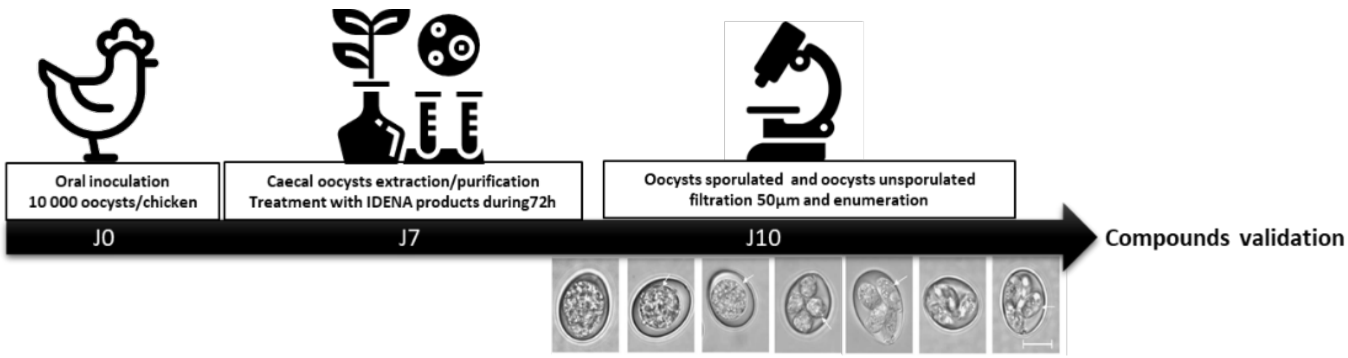
Coccidia study part

In collaboration with INRAE, IDENA evaluated a wide range of bioactives against avian coccidiosis agents by carrying out an *in vitro* screening. It aimed at evaluating their capacity to limit the invasion and or the replication of the parasites in intestinal epithelial cells.

For the adaptation of this study to the specificity of turkeys Eimeria, we consider that the mode of action of the bioactives on Eimeria in this study (*E. tenella* here) is not specific to one Eimeria species but common to the Apicomplexa phylum and thus those affecting the turkey species.

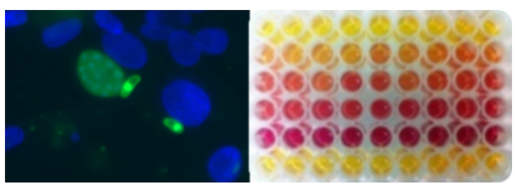
Effect of IDENA compounds on sporulation of Oocysts:

Infected animal release unsporulated oocysts in their feces, in order to be infectious for other congeners, need to sporulate. Several IDENA compounds significantly reduced 90% of sporulation of *Eimeria tenella* oocysts with dilutions up to 30 ppm.



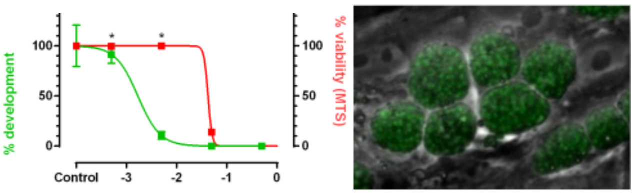
Effect of IDENA compounds on the invasion of sporozoites in cell culture:

Transgenic parasites expressing β-galactosidase (β-Gal) were developed by INRAE team to facilitate the evaluation of this process. A range of doses of IDENA compounds were preincubated with sporozoites (invasive stage) and their ability to invade monolayers of epithelial cells was quantified by β-Gal activity. Some compounds have been further selected for their ability to reduce cell invasion by 50%.



Effect of IDENA compounds on the parasite development in cell culture:

Specific *E. tenella* transgenic INRAE strain were produced to investigate parasite development *in vitro* and precisely determine the half maximal inhibitory concentration (IC50) for each compound.



Bacterial control part

To design effective bioactives, we carried out an extensive screening of active ingredients in partnership with the University of Pharmacy in Lille (France).

The aim was to determine the Minimum Inhibitory Concentration (MIC) using agar dilution, in the same way as antibiotic molecules.

These trials enabled us to test around thirty combinations of active ingredients for their ability to inhibit bacterial strains.

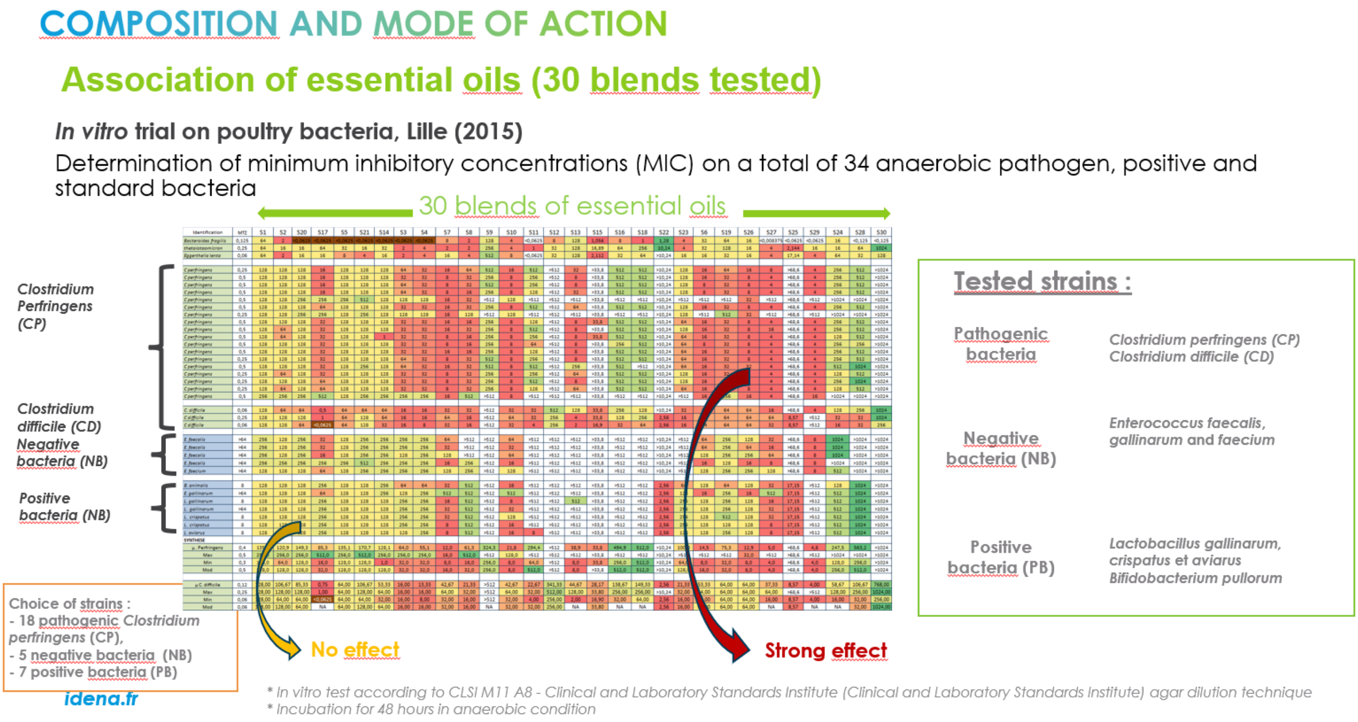
We tested several active ingredients (essential oils, plant extracts or mixtures of active ingredients) on different strains of Clostridium and on positive flora such as lactobacilli.

The study generated 4 main results.

Thanks to these tests, we were able to select 3 interesting essential oils and calculate their effective incorporation dose.

Gelose diffusion: in vitro test on poultry germs, Lille (2015)

The determination of the Minimum Inhibitory Concentrations (MICs) on pathogenic and beneficial anaerobic bacteria was performed at the University of Lille (France). These different strains were tested according to this way:



Part of the other component properties:

Because we know that the infection and infestation processes always generate inflammation as part of the infection, we have added some well-chosen plant extracts that have famous anti-oxidant and anti-inflammatory properties.

Other processing ways to improve the efficiency of the components:

To decrease the drawbacks of the essential oils present in the final product, a specific technology was implemented based on the choice of emulsifiers and the application of a high-shear emulsion. That specific process leads to an optimal bioavailability of active ingredients, a better penetration of coccidia (*Eimeria*), *Histomonas* and bacteria by active ingredients and a longer product stability without demixing.

Results:

Efficacy on *Histomonas*:

In this *in vitro* study, the MLC (Minimum Lethal Concentration) showed a significant reduction ($p<0.05$) of live *H. meleagridis* cells without re-growing in a further culture with fresh medium. That was noticed by adding FORKEY and substance #6 at respectively a concentration of 500 and 50 ppm of active ingredients.

RESULTS

FORKEY LA SOLUTION NATURELLE POUR LA SANTÉ DIGESTIVE idena

		x 10³ histomonas					
Mix	Bioactive conc.(ppm)	**0H**	**24H**	**48H**	**72H**	**Mann- Whitney U**	**Regrowth**
1	100*	100	169	223	343		
	500	100	149	226	284		
2	100	100	36	16	8	p< 0,05	
	500	100	3	0	0	p< 0,05	
3	10	100	151	175	241		
	50	100	96	117	138		
FORKEY 1 Kg/T	100 (Incl. 10 EO)	100	32	103	241		
	500 (Incl. 50 EO)	100	0	0	0	p< 0,05	NO
6	10	100	89	167	236		
	50	100	0	0	0	p< 0,05	NO
7	10	100	80	191	151		
	50	100	12	34	67	p< 0,05	
8	100 (Incl. 10 EO)	100	94	97	99		
	500 (Incl. 50 EO)	100	0	0	0	p< 0,05	
9	10	100	128	198	339		
	50	100	102	367	574		
10	60 (Incl. 10 ppm EO)	100	9	55	147		
	300 I(Incl. 50 ppm EO)	100	0,6	0	0	p< 0,05	
DMZ	0,4	**100**	**0**	**0**	**0**	p< 0,05	
Negative control		100	154	163	301		

No regrowth of the parasite in a further incubation in fresh medium

Table 1 : List of substances with the applied concentrations and mean count *of H. meleagridis* before and after 24, 48 and 72 H of incubation

* mean number of live cells of three independent experiments, each performed in triplicate

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Efficacy on coccidia

Three recombinant *E. tenella* parasite strains were generated to screen the efficacy of the 150 IDENA library compounds against all stages of the parasite life cycle.

Six compounds were able to restrict *E. tenella* oocyst sporulation by more than 90%. Three compounds limited invasion by at least 50% with a Selectivity Index (S.I. =efficacy/toxicity ratio) up to 3.5. Finally, 15 compounds were capable of inhibiting parasite development in epithelial cells with an efficacy ranging from 50% to 100% and a S.I. (Selectivity Index) that can be as high as 8.

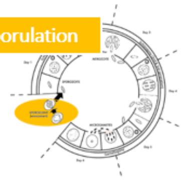
Results on invasion, development and sporulation stage are summarized in the table 1 below:

RESULTS OF FORKEY BIOACTIVES ON EIMERIA

Invasion

Development phase

Sporulation



	Invasion					Development phase					Sporulation	
Ingredients	sub toxic concentration[a]	%max efficacy[b]	TC_{50}[c]	IC_{50}[d]	SI[e]	sub toxic concentration[a]	%max efficacy[b]	TC_{50}[c]	IC_{50}[d]	SI[e]	IC50	%max efficacy[b']
Essential oil A	0,0005	1	0,008	0,050	0,2	0,0005	74	0,003	0,0004	7,9	<0,03	97,0
Essential oil B	0,0150	50	0,040	0,011	3,5	0,0150	50	0,009	0,003	3,5		4,9
Plant extract	-	0	5,670	no IC50	N/A	-	50	0,360	0,051	7,1	-	-

The SI ratio(Selectivity Index = efficacy/toxicity ratio) is used to select the most effective active ingredients and the least toxic for the epithelial cell

Table 1 : effects of Idena Compounds (alone or mixed) on invasion, development, and sporulation of Eimeria tenella on epithelial cells

Antibacterial effects:

The table below summarises the results of the screening carried out at the University of Lille, showing the inhibitory activities (in MIC) on selected strains of *Clostridium perfringens*, "negative" bacteria (*Clostridium, Enterococcus*) and positive bacteria (such as lactobacilli), leading to a relevant choice of a mixture with the active ingredients.

In conclusion, we select a mix of ingredients (blend #4) with a strong antibacterial effect against *Clostridum spp.* and weak detrimental effect against beneficial bacteria, with average minimum inhibitory concentration of 6.2 ppm and more than 512 ppm.

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**

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

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

INGREDIENTS	Blend A	Blend B	Blend C	Blend D
STRAINS OF C. PERFRINGENS (CP)				
CP s1	>512	8	32	4
CP s2	>512	4	8	4
CP s3	>512	4	8	4
CP s4	>512	4	32	4
STRAINS OF « NEGATIVE » BACTERIA (NB)				
NB s1	>512	32	>512	8
NB s2	>512	16	>512	8
NB s3	>512	64	>512	8
NB s4	>512	8	>512	8
NB s5	>512	128	>512	8
STRAINS OF « POSITIVE » BACTERIA (PB)				
PB s1	>512	32	>512	>512
PB s2	>512	512	>512	>512
PB s3	>512	16	>512	>512
PB s4	>512	32	>512	>512
PB s5	>512	32	>512	>512
PB s6	>512	8	>512	>512
PB s7	>512	8	>512	>512
	A	B	C	D

Conclusion:

All these experimental studies indicate that it is possible to formulate an alternative and specific solution able to prevent at the same time the following threats that affect the turkey health: an anti-parasitic effect to stop development of *H. meleagridis*, to break the parasitic cycle of Eimeria, and an intestinal antiseptic effect to fight bacterial infections (mainly *C. perfringens*).

By using this product for several years, some turkey production companies have demonstrated the efficiency of feeding turkeys with alternative in-feed solutions rather than the old anti-histomonosis drugs that are now banned, without encountering any clinical cases of histomonosis.

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Optimizing the nutritive value of turkey wheat-based diets through enzyme strategies containing beta-mannanase and endo (+xylo)-glucanase: a growth performance study

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Introduction

European turkey diets are heavily dependent on the use of high-quality protein sources, where soybean meal (SBM), rapeseed meal and sunflower seed meal may be included in formulations in order to facilitate the protein metabolism and growth of these animals (Drażbo et al., 2019). It is widely accepted that turkeys may be more adversely affected by specific complex carbohydrates due to the high inclusion levels of SBM (Odetallah et al., 2002), specially during early growth. Yet diets for turkeys modulate largely to adapt to the nutritional requirements as these animals grow, whilst keeping in with the principles of sustainability, local sourcing and minimum cost of feed. The objective of this study was to evaluate the effectiveness of a beta-mannanase and endo-(xylo)glucanase enzyme, together with an experimental carbohydrase, on the growth performance of female turkeys over a 16-week period.

Materials and Methods

A total of 960 one-day old female turkey Hybrid Converter sourced from a commercial hatchery were allocated into 4 treatment groups and replications, upon placement in the experimental farm, following a randomized complete block design. Birds were kept in a total of 60 pens (2 m x 2 m) with straw pellet as bedding material. Therefore, each treatment group consisted of 15 replicated pens, with 16 birds each. The length of the study was 16 weeks, following a 4-phase feeding program with each phase lasting 4 weeks. A positive control group [T1] was formulated with corn, wheat, SBM, rapeseed and sunflower seed meal to meet nutrient requirements following Hybrid recommendations. A lower energy density diet (negative control, T2) was formulated by forcing a down specification of 100 kcal/kg across all feeding phases. The remainder treatments were based on the negative control diet with the supplementation of a beta-mannanase and endo-(xylo)glucanase [T3] or an experimental carbohydrase [T4], both dosed at 100 g/MT. Body weight (BW) and feed consumption was determined every 4 weeks, coinciding with the change of feeding phase. Animals were observed, and mortalities or culled animals were recorded and weighted, on a daily basis. Mortality corrected feed conversion ratio (FCR) and European Production Efficiency Index (EPEF) were determined on a feeding-phase basis, and overall. Data was statistically evaluated by one-way analysis of variance (ANOVA) using Statistica 13.3.

Table 1. Composition of experimental diets used in a 16-week study evaluating the effect of novel enzyme strategies on the growth performance of female turkeys fed varying energy density diets

	Phase 1 (1-28d)		Phase 2 (29-56d)		Phase 3 (57-84d)		Phase 4 (85-112d)	
	PC [T1]	NC [T2]	PC [T1]	NC [T2]	PC [T1]	NC [T2]	PC [T1]	NC [T2]
Ingredients, %								
Corn	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Soybean Meal	44.00	43.37	39.04	39.27	30.83	30.19	14.34	13.69
Wheat	20.62	23.08	23.21	25.66	31.95	34.40	48.59	51.05
Sunflower seed meal	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Rapeseed meal	3.0	3.0	4.0	4.0	5.0	5.0	6.0	6.0
Soybean oil	4.24	2.43	5.44	3.62	5.87	4.06	5.33	3.52
Salt	0.326	0.326	0.329	0.329	0.284	0.284	0.269	0.269
Limestone	1.43	1.43	1.46	1.47	1.30	1.31	0.96	0.97
MCP	2.01	2.00	1.53	1.52	0.97	0.96	0.57	0.56
Arginine	-	-	-	-	-	-	0.014	0.019
DL-Methionine	0.33	0.33	0.26	0.25	0.12	0.11	0.11	0.10
L-Lysine	0.50	0.51	0.40	0.41	0.26	0.27	0.40	0.41
L-Threonine	0.126	0.126	0.069	0.069	0.010	0.011	0.055	0.056
Choline Chloride	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Phytase	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Coxidin	0.05	0.05	0.05	0.05	0.05	0.05	-	-
VTM	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Nutrients								
ME, kcal/kg	2,800	2,700	2,900	2,800	3,000	2,900	3,100	3,000
CP, g/kg	265	265	250	250	220	220	170	170
dLys, g/kg	16.37	16.34	14.74	14.71	11.77	11.74	9.38	9.35
dM+C, g/kg	10.29	10.26	9.23	9.21	7.33	7.30	6.15	6.13
Ca, g/kg	13.27	13.28	12.58	12.59	10.83	10.84	8.40	8.41
Av P, g/kg	7.29	7.29	6.18	6.18	4.90	4.91	4.03	4.04

Results

Over the entire evaluation, both enzyme strategies had a positive effect on final BW and ADG of turkeys (P<0.05; Table 2), particularly when compared with the corresponding non-supplemented lower energy density diet [T2]. Although of no statistical relevance (P=0.148), FCR was improved with both enzymatic treatments, with turkeys displaying feed efficiencies equivalent to that of the positive control (T1). Finally, a significant treatment on EPEF indicated that a beta-mannanase and endo-(xylo)glucanase and experimental carbohydrase restored productivity in low energy density diets for turkeys (P<0.05; Table 2).

Table 2. Effect of enzyme strategies containing beta-mannanase and endo-(xylo)glucanase or an experimental carbohydrase on the growth performance of female turkeys over a 16-week study

	BW112	**ADG**	**FI**	**FCR**	**EPEF**
T1	11.00^{b}	98.3^{ab}	250	2.557	380^{ab}
T2	10.92^{b}	97.5^{b}	251	2.571	375^{b}
T3	11.07^{ab}	98.9^{ab}	254	2.557	387^{a}
T4	11.22^{a}	100.1^{a}	249	2.535	391^{a}
SEM	0.031	0.272	0.090	0.006	1.750
PV	0.0049	0.0112	0.2142	0.1482	0.0031

Conclusions and Implications

The nutritive value of turkey diets can be optimized by using novel enzyme strategies, particularly a beta-mannanase and endo-(xylo)glucanase, which constitutes an alternative and flexible solution to improving growth performance of turkeys. Based on the feeding strategies of the current study, significant savings on feed costs could be realized using this enzyme.

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Can Cannabis-products make turkeys happy?

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Introduction

Poland is currently the leader in the European Union in terms of turkey meat production. For this reason, a big challenge for feed producers is to ensure the appropriate quantity and quality of feed raw materials (mainly sources of feed protein) to cover the growing demand in turkey production. One of the protein raw materials that can be used to feed turkeys is hemp seed cake. In recent years, due to the growing interest in products derived from hemp, their production has been growing dynamically in the country and around the world. Hemp seed cake (HSC) is a product obtained in the process of pressing oil from hemp seeds, so it is a by-product that often has no further use and is waste for producers. However, research on broiler chickens indicates the possibility of its use in rations. However, there is a lack of nutritional studies using the turkey model, and due to significant differences in "feeding physiology" between chickens and turkeys, results obtained from chickens cannot be directly extrapolated to turkeys. For this reason, there is a need for nutritional studies to determine the nutritional value of hemp products for turkeys.

Hemp (*Cannabis sativa L.*) is an annual plant belonging to the rose order of the hemp family (*Cannabaceae*). Over the years, hemp has been traditionally cultivated for its seeds and fiber production. In terms of chemical composition, hemp seeds are a valuable source of nutrients; they contain approximately 25% of crude protein (with a profile of amino acids with a high digestibility coefficient, especially limiting amino acids), fat with a high proportion of essential polyunsaturated fatty acids, they contain a high content of minerals, antioxidants (mainly tocopherols) and phenolic compounds. For this reason, their nutritional value for poultry is similar or higher than that of soybeans. Table 1 and Table 2 provide the approximate chemical composition and amino acid composition of hemp seeds.

Table 1. Chemical composition of hemp seeds of the Finola variety (Callaway, 2004)

Item	Concentration, %
Crude protein	24.8
Oil	35.05
Carbohydrates	27.6
Dry matter	93.5
Ash	5.6
Energy (MJ/100 g)	2.2
Fiber	27.6
Digestible fiber	5.4
Non-digestible fiber	22.2

Table 2. Comparison of the amino acid composition of hemp seeds and soybeans (g/100 g) (Callaway, 2004)

Amino acid	Hemp seeds (protein content 25%)	Soybeans (protein content 32%)
Alanine	1.28	1.39
Arginine	**3.10**	**2.14**
Aspartic acid	2.78	3.62
Cysteine	0.41	0.54
Glutamic acid	4.57	5.89
Glycine	1.14	1.29
Histidine	0.71	0.76
Isoleucine	0.98	1.62
Leucine	1.72	2.58
Lysine	1.03	1.73
Methionine	0.58	0.53
Phenylalanine	1.17	1.78
Proline	1.13	1.65
Serine	1.27	1.54
Threonine	0.88	1.35
Tryptophan	0.20	0.41
Tyrosine	0.86	1.14
Valine	1.28	1.60

Until recently, the cultivation of hemp was prohibited in Poland due to the presence of cannabinoid compounds, of which delta 9-tetrahydrocannabinol (THC) has psychoactive effects. Currently, thanks to the breeding of new varieties of hemp plants, the so-called "Industrial hemp" can be grown provided that the obtained seeds contain less than 0.3% delta 9-THC, but there are still many restrictions on this issue. Among the biggest obstacles to limiting the use of hemp products is the fact that these products (hemp seeds, hemp flour, hemp seed meal and hemp oil) have not yet been registered (approved) as "safe" poultry feed ingredients due to their potentially adverse effects on animal health and consumer safety. However, legal regulations regarding the use of hemp products in the feeding of farm animals, including poultry, may undergo major changes in the near future to increase the possibilities of using these products. This is due to the increasing number of scientific studies carried out aimed at assessing the safety of using hemp seeds in poultry nutrition (Fallahi et al., 2022; Tufarelli et al., 2023). The results of various researchers indicate that the use of hemp seeds at the level of 12%, hemp seed meal at the level of 10% and hemp oil at the level of 5% in the diet of broiler chickens had a positive effect on the results of rearing broiler chickens. However, in the case of laying hens, the use of hemp seeds in the amount of 25%, hemp seed meal in the amount of 15% and hemp oil in the amount of 7% in the diet did not have a significant impact on egg production, did not negatively affect the overall quality of eggs, but resulted in favorable changes. (in terms of functional properties) in the fatty acid profile (Stastnik et al., 2020; Shariatmadari, 2023).

Cannabidiol

Another product obtained from hemp seeds that may have practical use in turkey nutrition is cannabidiol (CBD). CBD is a metabolite of tetrahydrocannabinol and has no psychoactive properties, but has antioxidant, immunosuppressive and anti-inflammatory effects. Moreover, this compound has an effect that supports the functions of the digestive tract (by influencing mechanically, humorally, neurologically and immunologically on the digestive system, including the bacterial microflora inhabiting it) (Iffland and Grotenhermen, 2017). Although research on the potential of CBD has not yet been fully elucidated and mainly concerns human medicine or in vitro conditions, our previous research conducted in a broiler chicken model showed that CBD has a beneficial effect on the health status of birds (Konieczka et al., 2020), integrity gastrointestinal tract, as well as on the sensory properties of meat (Konieczka et al. 2022).

The use of HSC and CBD in turkey rations

In order to verify the possibility of using HSC and CBD in the nutrition of turkeys, a feeding experiment was carried out in which the birds were given experimental diets containing 10% or 20% of HSC (as a respective replacement for soybean meal) and diets with CBD (0.01% or 0.02% of supplemental oil). The experiment examined the results of performance and selected indicators of the functional status of the digestive tract. The turkeys were kept until 20 weeks of age.

Impact on turkey performance

The preliminary results obtained indicate that both HSC and CBD extract had a significant impact on selected indicators of birds' performance. The body weight of turkeys on the 56th day was significantly higher in the group with HSC (20%) and in the groups with the addition of CBD compared to the control. Similar results were found for the average daily weight gain of birds. The feed conversion ratio in the examined period was the best in the group receiving the HSC at the level of 20% compared to the control and other experimental groups. However, the performance results analyzed for the entire experimental period (1 - 140 days) indicate a significant improvement in final body weight, average daily body weight gain and feed conversion rate in the CBD groups and in the group with HSP at the level of 20% compared to the control group.

Impact on the functional status of the digestive tract

The analysis of selected indicators of the gut functional status of turkeys indicates that neither the addition of HSC nor CBD resulted in deterioration of gut functioning. However, beneficial effects were observed when both additives were used which were: (i) lowering the pH of the contents of the ilium and cecum, which is associated with a potentially reduced proliferation of pathogens in the gastrointestinal tract, (ii) lowering the activity of selected bacterial enzymes, including β-glucuronidase, which is responsible for the release of carcinogenic compounds in the digestive tract, (iii) reducing the concentration of putrefactive short-chain fatty acids in the contents of the cecum, which are an indicator of disorders in the process of fiber and protein fermentation in the digestive tract, and (iv) increasing the level of mRNA expression of selected genes responsible for maintaining the integrity of the intestinal barrier (tight junction proteins; TJP).

Summary

To the best of our knowledge, these preliminary studies conducted on turkeys are the first in which HSC and CBD extract were used. This is primarily due to the high costs of conducting research on turkeys due to their long rearing period and the large amount of feed consumed during the 20-week rearing period. For this reason, it is difficult to compare the obtained results with studies conducted in other researchers, however, the preliminary results obtained (together with studies on broiler chickens) indicate that hemp products did not have a negative impact on the health status of turkeys, hence they can be preliminary considered as a potentially safe and valuable source of protein and bioactive substances in the nutrition of turkeys. The beneficial effect of CBD on performance and selected indicators determining the health status of the gut may result from the effect of CBD on cannabinoid receptors (CB1 and CB2), which regulate many physiological processes in the birds' body. However, in the case of HSC, the fiber fractions contained in it may have very interesting properties. Turkeys, unlike broiler chickens, can better use of feed fiber because it increases the secretion of enzymes and thus stimulates intestinal peristalsis, increases the acidity of the stomach contents and prevents pathological changes in the epithelium and inflammation of the intestinal mucosa, which results from their natural adaptation to food intake rich in fiber.

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