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Living with Avian Influenza – Options to control this challenge

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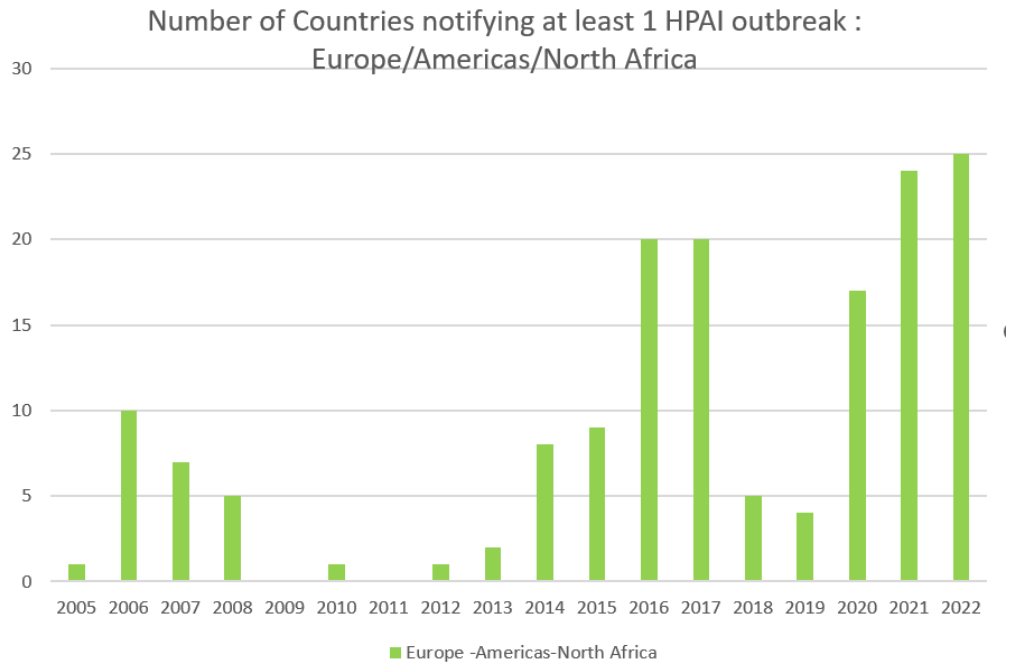
Although Avian Influenza is now at the forefront of the industry, Avian Influenza is not a new event as the first cases reported were in 1878 in Italy, even if at that time the agent was not clearly identified. Before the end of the 20th century, Avian influenza outbreaks were kept as rare events, and when it turned into an epizootic situation, it was always in relatively limited geographic areas. Since the 1990's, the number of epizootic situations has slightly increased, with a particularly high increase in the past three years:



Graph designed from analytics published on the WOHA website

For the past few years, Europe and North America, who historically succeeded to contain the virus by zoning and euthanizing the contaminated flocks, had to face very severe epizooties resulting in a huge impact on the economy.

Even if economy often comes first in our mind, we also have to consider that:

- Animals affected by a disease means suffering before dying in most cases
- Euthanasia to reduce the spread of a diseases can be ethically debatable
- When food security is a major issue for many parts of the world, food waste related to large numbers of animals dying or being euthanized must prompt us to question our actions
- Repeated outbreaks in their environment (when not in their own farm) can only stress the farmers and reduces the number of farmers willing to continue producing

Up until recently, we considered that the risk was mostly related to wild birds' migration flows and more specifically during the autumn migration period (North to South in the Northern hemisphere).

This changed in 2022, with outbreaks related to the 2 migration flows, and in the end, we faced a new situation with a virus that became endemic in the local wild bird's population.

When considering the slightly increased risk on one side, and the evolution in the societal perception on the other side, it seems clear that it is now time to think about new strategies to control Avian Influenza.

Biosecurity is still a must

Even when wild birds are presented as the source of the outbreak, the main route for contamination remains the indirect contamination through shoes, material or litter soiled with droppings: the biggest risk for outbreak is right outside the barn door.

If the virus became and remains endemic, ensuring that we will never have any outbreaks will be challenging, but we should avoid the spread from the initial outbreak to other facilities driving the situation out of control: animals, material, and staff movements must be reasonable and secured anytime it is possible by testing and cleaning/disinfection.

We should re-assess or production models

Multispecies associated with a high concentration of farms in the wetlands creates regions with a high risk for Influenza to spread: should we reduce farm concentration in these regions?

Birds' movement is the main risk for spread: should we stop production systems based on moving birds in phases to different locations?

Is vaccination an option?

- Vaccination has obvious weaknesses:

  Most scientists expect vaccination to reduce shedding when a flock is contaminated more than protect a flock against contamination.

  It is expensive in terms of vaccine cost and vaccine application

  Extensive sampling protocols will be associated with vaccination (with additional costs and constraints)

  It has been traditionally used as a reason for trade restriction

- Is the poultry food chain sustainable without a vaccine for the long term?

  Will the authorities pay compensation (adding up to billions of euros) for years?

  How can we build an efficient business with such uncertainty? (Processors without supplies, hatcheries without customers, from one day to the next).

  When will society strongly protest it?

  How to encourage vocation for the next generation of farmers with such a threat

Vaccination: Do it well or not at all

We do not rely on vaccination for flock protection but on spread control through the excretion reduction: a flock protection depends much more on the correct application of vaccination by its neighbors than on its own immunity. Vaccination can't be left to individual initiative and choice. It must be part of a collective approach based on scientific reflection. Once the strategy and framework are defined, vaccination cannot be optional for defined populations, it must be mandatory.

Virus circulation is pointed to as a major risk for promoting virus mutations, which may result one day in a virus adapted to humans with the potential to create a new pandemic. This was the motivation to ban countries notifying HPAI from exports. For human health and for international trade, it is very clear that vaccination has to be associated with thorough testing protocols designed to detect a low virus prevalence in a flock. It is also very clear that there will still be some vaccinated flocks which will turn positive and that these flocks will have to be euthanized.

Conclusion:

High Path Avian Influenza is major threat for the poultry industry, and likely this risk is even greater for turkeys than for any other poultry because of their high susceptibility to the virus and their long-life span.

It is our responsibility to strengthen all elements of biosecurity and to have the willingness to sometimes question our production systems and to be open to adapting them.

If the trend observed over the last few years is confirmed and the virus unfortunately becomes endemic in the local wild birds' populations, it becomes difficult to imagine a sustainable production without the use of vaccine solutions.

Vaccination should not be expected as a silver bullet. It can only help when used in very strict conditions, in a clearly defined strategy.

As High Path Avian Influenza is gradually reaching all the major poultry producing countries, health certificates governing international trade will have to be adapted to lift excessive and unjustified specifications. Additionally, it will be necessary to strengthen confidence based on efficient tools such as Zoning and Compartmentalization and to open the possibility to implement vaccination in a strictly defined framework according to the international standards (WOHA).

Based on the data available to us, it seems that High Path Avian Influenza is here to stay. For this reason, the poultry industry must think critically and potentially adapt standard practices for controlling Avian Influenza. To ensure a sustainable turkey industry, we must collaborate to find new solutions to address this health risk.

Surveillance as a main key for implementing a vaccination system against AI

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Avian Influenza has become endemic in many countries all over the world. The strategy just to have strict biosecurity measures and to cull infected flocks does not seem to be effective when wild birds in the environment remain latent virus carriers.

By collecting data from infected and uninfected flocks through the AIV-periods of 2020 to 2022 we learned a lot about the epidemiology of the virus. Because we did not have any sequencing data of virus isolates from the different cases, we established our own system and subdivided the flocks in primary and secondary infections. Infections that occurred temporarily and locally close to a first outbreak were classified as secondary infections. During the winter 2020 - 2021, 70 % of the infected turkey flocks were identified as secondarily infected.

Subsequently we decided to focus on developing a tool to detect infected flocks earlier to stop the lateral spreading of the virus.

In November 2021 we started the "Tränkenmonitoring". This means we collected swabs of drinkers and of "good dead birds" from each turkey house in a specific area of Lower Saxony with a high risk of AI infection.

What did we learn…?

- Early detection of infection events:
  Studies by LAVES (local food safety authorities, data winter 2021/2022) confirm that drinking trough monitoring identifies infections earlier than sampling dead birds in a flock.
- Very easy handling
- High statistical certainty:
  At least 100 animals visit a drinker within 2 hours, samples from 10 drinkers per swab possible, a pool of 10 swabs possible in the laboratory, means: 10.000 turkeys examined in one PCR sample
- High accuracy:
  Samples are taken over the entire barn area
- Sensitizes the worker on the farm and is good for the psyche of the supervisor and farmer
- Excellent as a monitoring tool in the DIVA-concept

Discussion:

Samples from drinkers are very effective to find an infected flock before it is spreading. For cost reasons it is impossible to sample all receptive birds and flocks through the Influenza season. But if there is an infected area we need this tool to stop the infection from spreading. On the other hand it will be very helpful if we start to vaccinate. And I am sure we will have to start vaccinating in the short term. I cannot see any arguments standing against it from the veterinarian, animal welfare and sustainability aspect.

The EU-commission gave the order to the member states to start field studies on vaccination. Italy is working on broiler and turkey vaccinations, France works with ducks, the Netherlands work on vaccination programs for layers. For all these groups of poultry commercial vaccines (vector, mRNA and subunit) are available and already used in some countries. The Ceva vector vaccine is based on a turkey herpesvirus, which is already in use against ND. The first vaccination can be easily done with the Poultry Service Processor (PSP) of NovaTech on the first day of life. Field-trials must show if we need further applications with the same or different vaccines.

As soon as possible all people involved with trading contracts have to start to change them. Having a safe and effective vaccine does not mean we can start using it, when vaccinated flocks cannot be marketed. Best example are the United States of America, they have a registered vaccine in stock but cannot use it.

People on the judicative side have to work on the surveillance papers. We need quicker and more effective sampling systems. We have to spread our knowledge to prevent lateral spreading. First of all our "Tränkenmonitoring" needs to be checked under lab conditions with experimental AI-virus challenges. If this confirms our results we have to establish it in the terrestrial code and it has to become part of the samples the veterinarians collect in the surroundings of outbreaks.

Every AI-vaccine, which will be used in the future, has to be a DIVA vaccine. The very effective "Tränkenmonitoring" will then be a great tool to sample vaccinated flocks. However, we need to be aware that if a flock is positive for field strains we have to continue culling! It has to be our goal to get back to a time without AI vaccination. In addition, we are not allowed to lower the biosecurity level when we start to vaccinate.

Finally we have a responsibility for the wild birds! Losing whole populations of birds while playing ping-pong with the virus between wild and domestic birds is no longer acceptable.

Innovative selection methods to drive product performance

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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. Recent examples of innovation in breeding and selection include RFID technology, CT scanning technology, and testing through Combined Crossbred & Purebred Selection (CCPS).

RFID Technology

In March 2021, we announced the implementation of RFID technology at our pedigree facilities. RFID technology aids in enhanced genetic progress via more accurate and real-time data, in addition to offering improved animal and worker-friendly methods of data collection. Since RFID implementation, R&D has been utilizing this technology on a greater scale to monitor feed efficiency, live body weights and in the newest trial - egg production.

Feed Efficiency

Feed stations use RFID technology to capture individual feed consumption when an animal enters a station. The use of this technology allows an increase in the number and accuracy of data records collected. It also provides a view on individual animal feeding behavior and habits. These efforts result in sustainable progress in feed efficiency which is known to address the high cost of feed while simultaneously lowering the industry's carbon footprint.

Live Body Weights

To improve the method of capturing body weights on live animals, we have developed an Automated Weigh System that utilizes RFID technology. The system captures individual bird weights each time they step on a scale allowing for the natural collection of data while limiting the need for handling. As genetic progress has seen continued growth in turkeys, this system allows for improved worker conditions with less requirement for lifting animals. Additionally, birds can hop on and off the scales as many times as they like which increases the number of data points collected. More data ensures the continued acceleration of genetic progress.

Individual Egg Collection

In the most recent application of RFID technology, there is a small trial focused on the development of an automatic nest to measure individual egg production in group housing. This trial also includes individual video recordings to observe behavior and validate nest occupancy and egg production. The automated collection of individual egg production has great potential for downstream application in order 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.

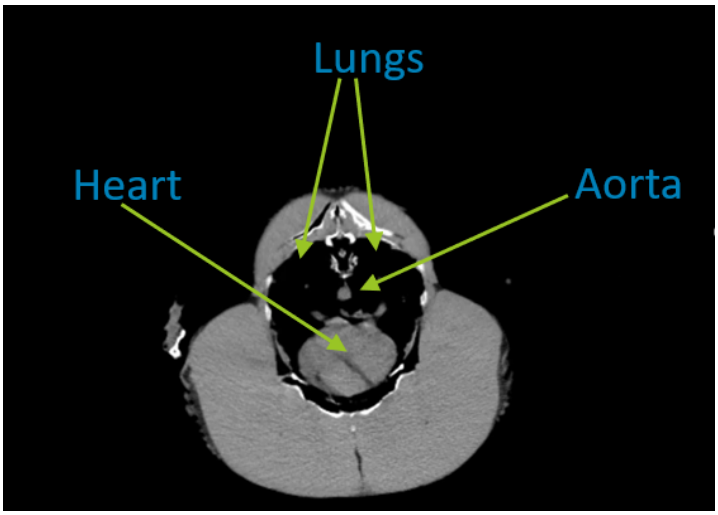
CT Scanning Technology

CT technology is a diagnostic imaging system that uses X-ray technology to produce multi-dimensional images of the inside of a body. We began using CT technology at our pedigree facility in 2022 to get in-depth information on the various aspects of a turkey's body such as skin, bones, body composition, airways, and organs.

Delivering economic sustainability

The first stages of application for CT scanning surround body composition, such as breast and thigh yield as these are recognized as the driving factors for economic performance in the market. To analyze the yield information of individual birds scanned, we use the 3D models created to estimate the total grams of breast meat per kilo of live weight. This information is used to analyze and compare birds and aid in the selection process. The use of CT technology comes as an enhancement, not a replacement to the current data collection efforts in our breeding program. This new technology will accelerate progress on these highly desirable yield-based traits.

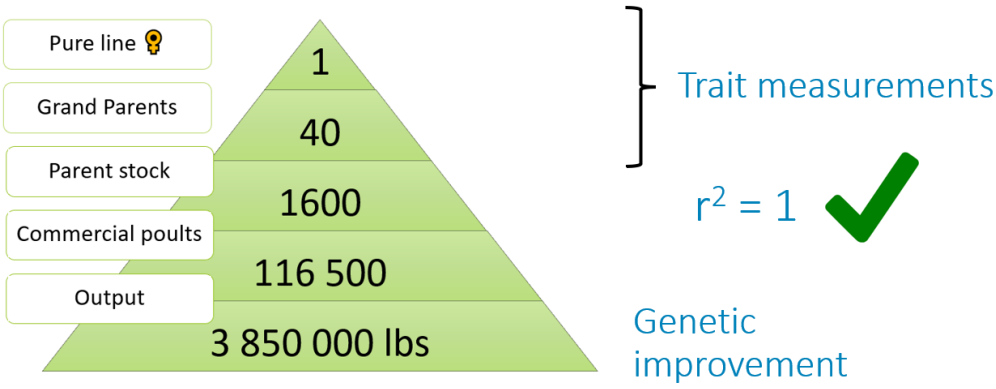
Although meat yield is a popular trait, we are collecting much more information on attributes like skin, bones, airways and organs. This additional data addresses key performance factors such as livability and overall health and welfare of a turkey. For example, assessing heart size and shape and how it contributes to blood flow to the muscles can provide interesting insights on livability. Or considering bone angulation and density can indicate the ability of a bird to support increased yields. From the data available through CT scans, there are several new possibilities to improve performance, efficiency and welfare traits. In the end it is about utilizing this technology to create a more sustainable turkey with enhanced economic, social, and environmental gains while still meeting the needs of the market.



Measuring performance in commercial environments

CCPS (Combined Crossbred & Purebred Selection) is another way we can improve livability through testing the performance of our products under commercial conditions. We take the data from these trials and roll this information back into our breeding program so that we select birds that perform well under conditions that closely match our customers' operations. With the information from these trials, we are able to breed stronger, more resilient birds and can better predict performance at the commercial level.

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. We will continue to maximize the use of some of these innovative technologies as well as continue to explore the use of new developments to deliver excellent performance at all levels of the turkey value chain.



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HENDRIX GENETICS

Assessing the effects of turkey stocking density on productivity, health and welfare.

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Introduction

Recommendations for maximum turkey stocking density (SD) vary significantly across the world. In Canada, requirements limit the maximum density, which varies by market weight. The National Farm Animal Care Council's Canadian Code of Practice for Meat Birds states that maximum densities for birds marketed at 6.2 kg and under be 40 kg/m^2, over 6.2 -10.8 kg at 45 kg/m^2, over 10.8-13.3 at 50 kg/m^2 and over 13.3 kg at 55 kg/m^2. Some allowance is made for conditional approval with excellent management at slightly higher densities. However, producers are interested in understanding the effects of increasing density on bird productivity, health and welfare, as often increasing density increases profits unless financial incentives are provided for lower density production.

The majority of research projects supporting these decisions are not current, and body weights and genetic advancements could mean bird response to density stressors may vary. This project focused on determining the effect of varying final stocking density on a wide variety of response criteria to give an overall picture of effects.

Materials and methods

Two experiments were conducted (Nicholas Select hens to 11 wk of age, or toms to 16 wk of age). Each experiment was repeated twice in a randomized complete block (trial) design, to allow 4 large-room replications (see Table 1) per estimated final stocking density treatment (30, 40, 50 or 60 kg/m^2 at market age, based on Aviagen predicted weights). Birds were fed ad libitum commercial hen or tom feed phases, housed on wood shavings in a completely environmentally controlled facility. Rooms (eight per trial) allowed individual control of ventilation and temperature. Ventilation was adjusted (room dependent) to ensure temperature, carbon dioxide and ammonia levels were similar across all eight rooms. Data were analyzed with regression analyses to determine the relationship between stocking density and dependent variables. Differences were considered significant when the probability of difference value was less than 0.05, and a trend noted when this value was between 0.10-0.05.

Table 1. Experimental bird numbers

	Number of birds placed per room (room size 67.5m^2)			
Final estimated density	30 kg/m^2	40 kg/m^2	50 kg/m^2	60 kg/m^2
Exp. 1 Toms	122	161	198	236
Exp. 2 Hens	295	388	482	571

Results

Graphs for some productivity and health/welfare data are shown below. Statistical significance is noted in the graph/chart title (linear or quadratic relationship, or NS (no significant difference).

Productivity: SD significantly impacted a number of productivity variables. Market body weight decreased with increased SD in a linear fashion for both toms and hens (Figure 1). Although gender differences were not tested, Figure 1 shows that the response was similar for both genders. Feed to gain ratio (mortality corrected) did not differ based on SD for hens, but for toms, increasing SD resulted in poorer feed conversion (Figure 2).

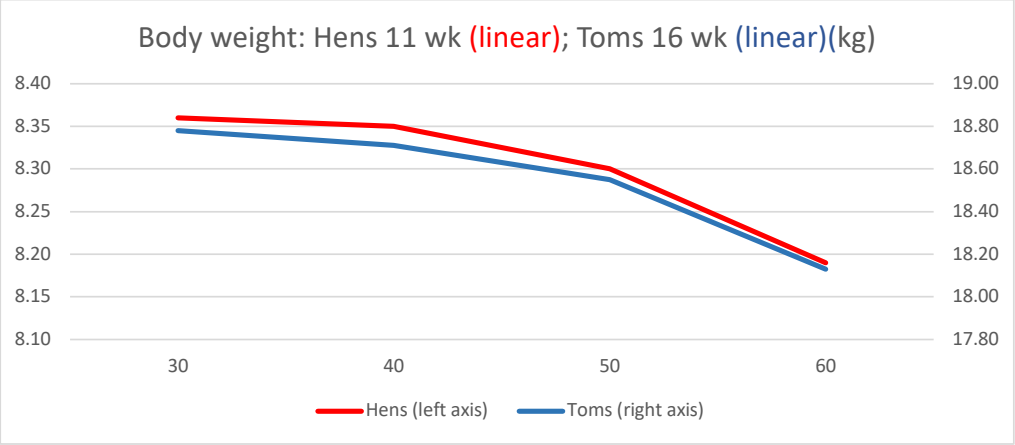

Figure 1. The effect of estimated final stocking density on market body weight (kg) of hens (11 wk – weights on left axis) and toms (16 wk – weights on right axis).

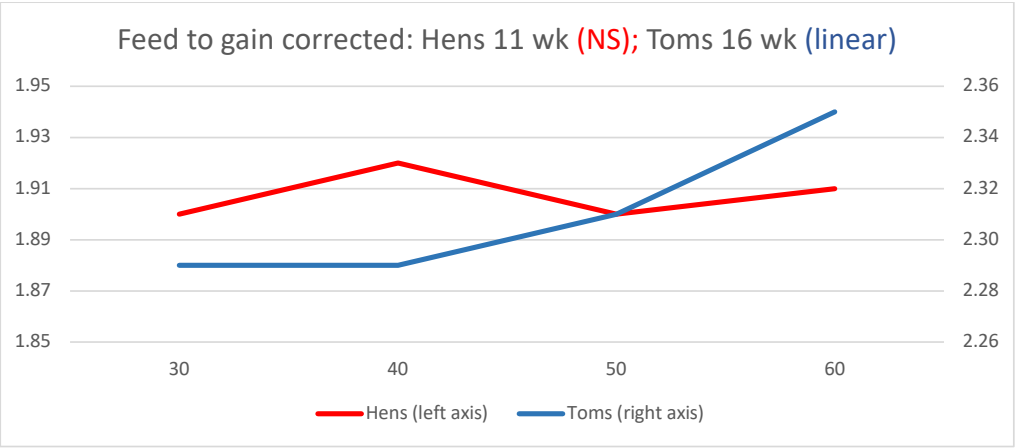

Figure 2. The effect of estimated final stocking density on feed to gain ratio (mortality corrected) for hens (0-11 wk – left axis) and toms (0-16 wk – right axis).

Health and welfare

Despite ventilation rates being adjusted to ensure rooms did not differ in carbon dioxide and ammonia with higher SD, some differences were noted in health and welfare parameters. Footpad scores worsened with higher SD for hens at 8 wk (Figure 3a) and toms at 10 and 16 wk (Figure 3b) of age (linear). This at least may partially have related to differences in litter moisture, as those values increased at 11 wk for hens (linear). However, litter moisture did not differ based on SD for the tom experiment. This may have related to methodology. Litter moisture was determined by sampling at various spots throughout each room, with the sample measured from the top to the bottom of the litter bed. Visually, it appeared that rooms with higher densities had a wet layer directly on the top of the litter material that may not have absorbed to lower levels, although this was not quantified.

Mobility, measured by gait scoring methodologies, identified no differences based on SD treatment for hens at 8 or 11 wk of age, nor toms at 12 wk of age (Figure 4a, b). At 16 wk of age, however, increasing SD resulted in higher gait scores, indicating that mobility was poorer for those birds.

Injuries from aggression were impacted for hens (0-11 wk) in a linear manner (Figure 6), with more injuries occurring at low SD than at high SD. However, there were no statistical differences noted in the tom flocks, although numerically, the percentages were higher for the highest and the lowest SD.

Chronic stress (measured by the ratio of heterophils to lymphocytes) was affected by SD. In the hen flock, it appears that age resulted in varying effects (Figure 7). At 5 wk of age, stress was the highest at the low SD. However at both 8 and 11 wk of age, higher SD resulted in higher stress levels. For the tom flocks, a linear effect was noted, with higher SD resulting in higher stress (Figure 8). A trend was noted for a similar response at 12 wk of age.

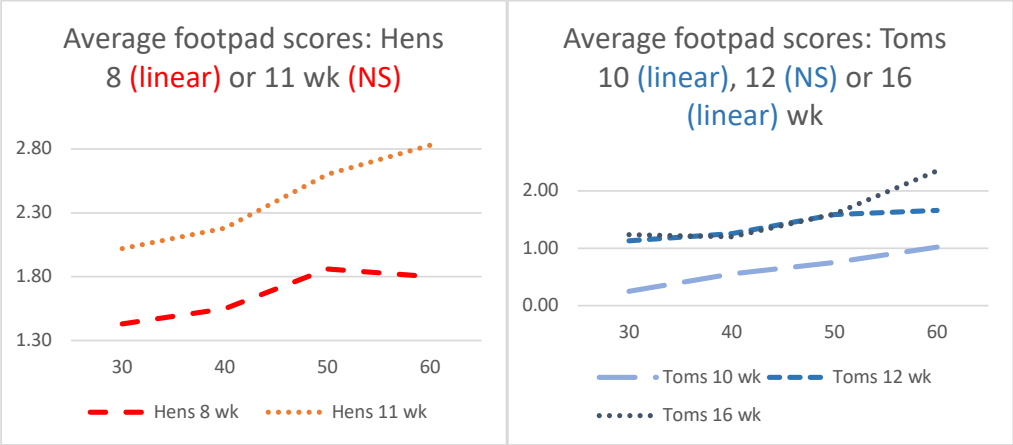

Figure 3a, b. The impact of stocking density on foot pad scores (0=no external signs of dermatitis; 4=over half the pad covered by necrotic cells) for hens at 8 or 11 wk of age (3a) or toms at 10, 12 or 16 wk of age (3b).

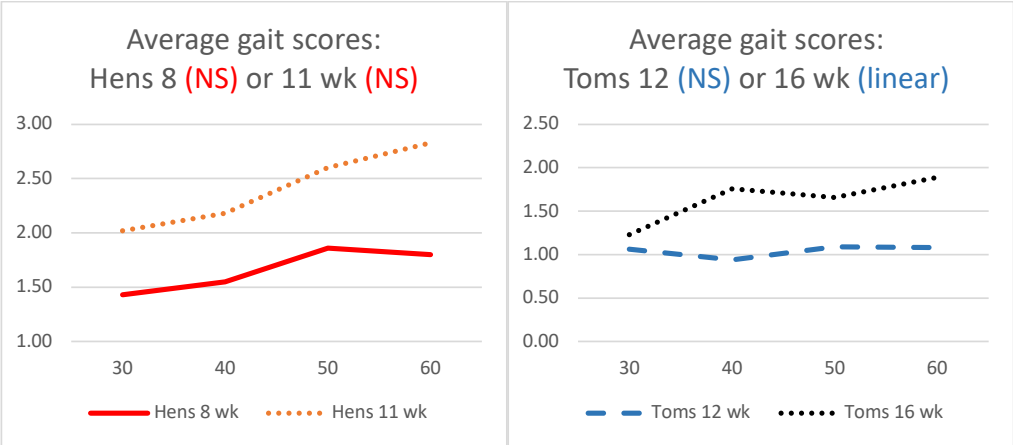

Figure 4a, b. The impact of stocking density on gait scores (0=no deformity; 5=complete lameness) for hens at 8 or 11 wk of age (4a) or toms at 12 or 16 wk of age (4b).

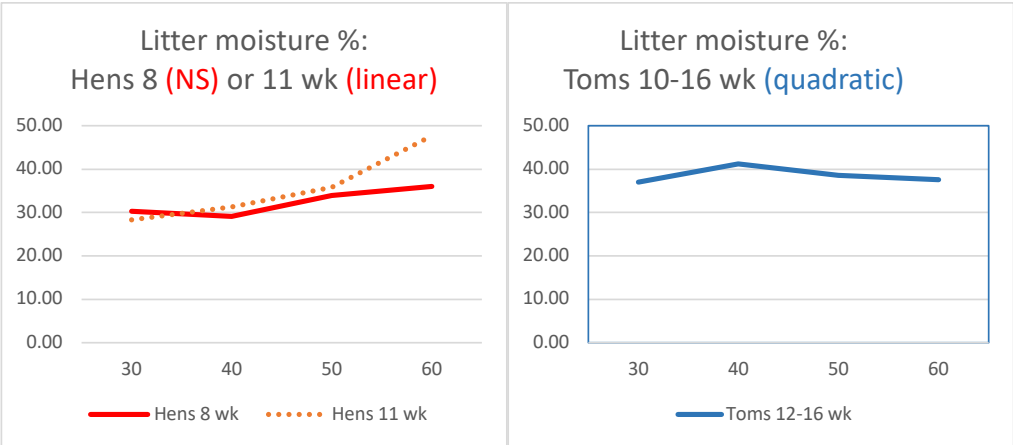

Figure 5a, b. The impact of stocking density on % litter moisture for hens at 8 or 11 wk of age (5a) or toms at 12 or 16 wk of age (5b).

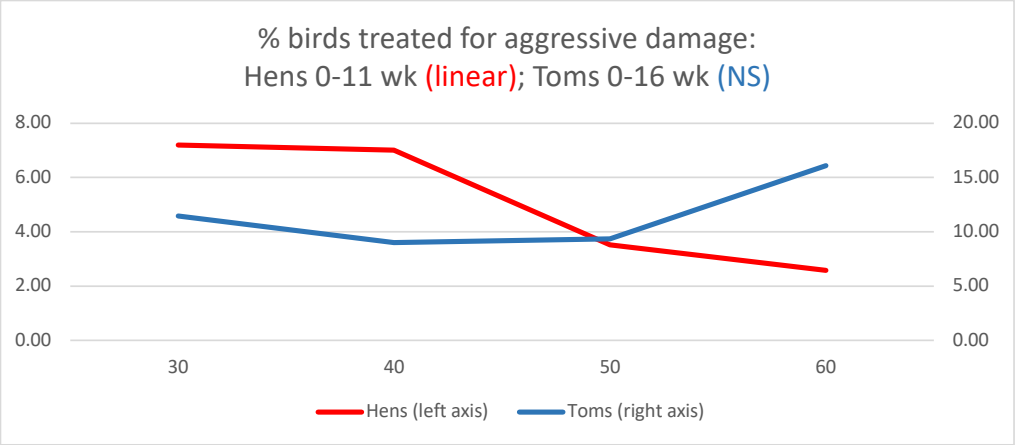

Figure 6. The percentage of birds treated with an aggressive pecking deterrent

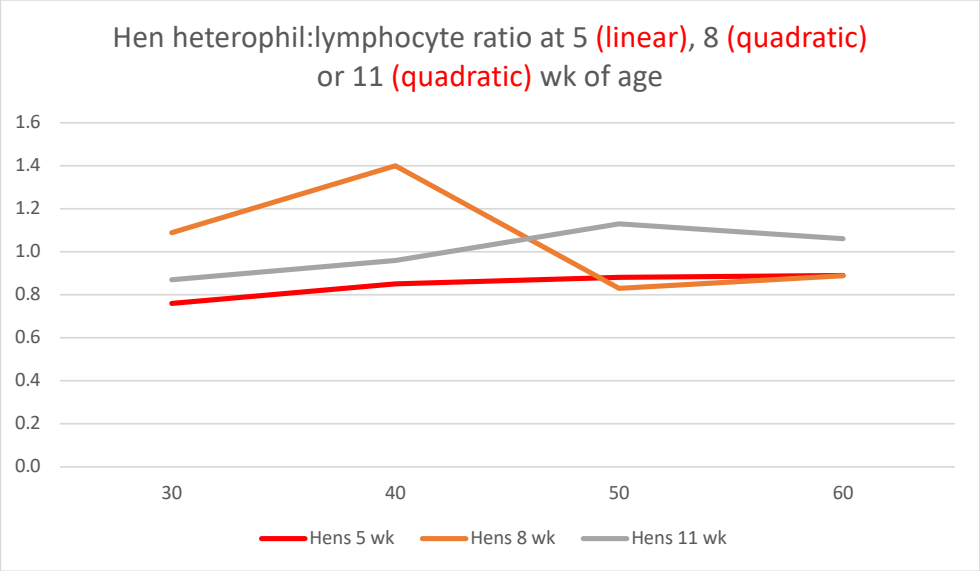

Figure 7. Heterophil:lymphocyte ratio (measure of chronic stress) in hens at 5, 8 or 11 wk of age

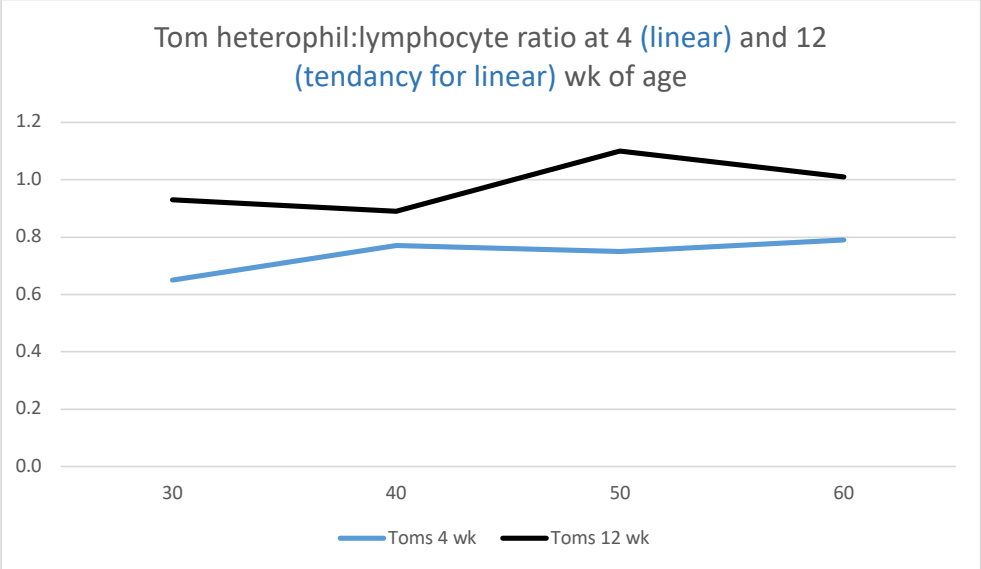

Figure 8. Heterophil:lymphocyte ratio (measure of chronic stress) in toms at 4 and 12 wk of age

Conclusions

To conclude, increasing stocking density has some effects on productivity traits of turkeys, including reduced body weight for 11 wk old hens and 16 wk old toms, and reduced feed efficiency in toms. However, increasing SD reduced turkey well-being in numerous categories, include footpad health, mobility in toms and other data not shown here (feather cleanliness and condition, behavioural expression). Care should be taken to ensure low stocking densities do not result in increased aggression as occurred in these works.

Acknowledgements

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Breast abnormalities in turkeys and implications on meat quality

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Introduction

The consumption of poultry meat has significantly increased over the past few decades and turkey meat currently ranks as the second most popular poultry meat-type around the World (Yalcin et al., 2019; Baéza et al., 2022). To achieve this result and meet the consumers' demand for high protein and low-fat fresh meat, breeding companies have implemented selection practices aimed at developing high growth-rate and breast yield genotypes. As a consequence of the selection practices, the growth-performances and cut-up yields of the modern turkeys have been remarkably improved. In fact, if compared to the productive performances of the genotypes reared in 1966, a 4-fold increase in the body weight has been achieved in the modern turkey lines (Havenstein et al., 2007; Aviagen, 2022). These impressive results have been achieved by inducing a hypertrophic growth of the fibers composing the muscle tissue itself since their number is basically determined at the time of hatch (Velleman, 2023). In detail, as for the pectoral muscles, the most valuable portion of the turkey carcass, these selection practices resulted in a significantly higher breast meat proportion (Hocking, 2014; Velleman, 2015; Yialcin et al., 2019). Besides the unquestionable improvements in the birds' productive performances, the selection for increased growth-rate and meat yield significantly affected the skeletal muscle architecture as well as the functionality of the fibers composing the tissue itself. That resulted in the occurrence of muscle fiber defects being strongly associated with the development of meat abnormalities (Velleman et al., 2003; Hocking, 2014; Velleman, 2015).

Historical evolution of growth-related abnormalities in turkeys: from past to present

The first scientific reports referring to the development of growth-related muscle abnormalities in turkeys dates back to the 1960s and 1970s when the first studies concerning the deep pectoral myopathy were published (Dickinson et al., 1968).

Later, during the 1980s, some quality defects were observed in turkey breast meat. They included an impaired cohesiveness and juiciness of processed turkey breast meat and an overall toughening of the *Pectoralis major* muscle which were associated with the presence of histological features deviating from the normal muscle architecture (Dutson and Carter, 1985; Grey et al., 1986; Grey, 1989; Sosnicki and Wilson, 1991). From that time, several studies were performed to investigate the pathology of skeletal muscles in fast-growing turkey lines (Sosnicki et al., 1988a,b; 1989; 1991). The outcomes of these investigations evidencing a compromised skeletal muscle architecture (i.e., hypercontraction and necrosis of the fibers, inflammatory cells infiltrations) along with a strong proliferation of connective tissues and increased fat deposition at endomysial and perimysial level (Sosnicki et al., 1991). Sosnicki and Wilson (1991) defined this condition with the term of "focal myopathy". This myopathy was thought to be related with a vascular defect which, in its turn, could induce the development of a localized micro-ischemia. This hypothesis is further corroborated by the impressive increase in the cross-sectional area of the fibers composing the pectoral muscles of the modern turkey lines which becomes 35-fold times larger during the first 15 weeks of life (Wilson et al., 1990). Overall, these findings seem to indicate that the selection for growth-rate and muscle development has likely resulted in muscles that have "outgrown their life-support systems" (Sosnicki and Wilson, 1991). In fact, the massive hypertrophic-based growth of muscle fibers was not accompanied by a concomitant growth of the connective layers, thus resulting in a poor vascularization of the tissue that could be associated with alterations in the musculoskeletal structure (Sosnicki and Wilson, 1991; Velleman et al., 2003; Hocking, 2014; Velleman, 2015) and a greater occurrence of growth-related abnormalities (Sosnicki and Wilson, 1991) which negatively affect the quality traits and technological properties of the forthcoming meat (Updike et al., 2005).

Intriguingly, the histological alterations observed 30 years ago in the pectoral muscle of fast-growing turkeys are similar to those observed in broiler chickens affected by the emerging growth-related abnormalities including the white striping (WS), the wooden breast (WB), and the spaghetti meat (SM) defects (Kuttappan et al., 2009; Sihvo et al., 2014; Sirri et al., 2016). These abnormalities manifest with distinctive phenotypes including the presence of white striations of variable thickness running parallel to the muscle fibers' direction (WS; Kuttappan et al., 2009), outbulging and pale areas having increased toughness (WB; Sihvo et al., 2014), and an overall detachment of the fiber bundles composing the *Pectoralis major* muscle (SM; Sirri et al., 2016). Besides their distinctive phenotypes, the abovementioned abnormalities share some common microscopic features including a compromised connective tissue composing both the perimysial and the endomysial septa (Petracci et al., 2019). In addition, each defect also exhibits distinctive histological traits such as an abnormal deposition of adipose tissue in WS, a proliferation and thickening of the perimysial network (up to fibrosis) in WB, and a progressive rarefaction of perimysial connective tissue layer in SM (Soglia et al., 2019). Within this context, it's worth mentioning that the outcomes of the histological evaluations performed on turkey *Pectoralis major* muscles evidenced similar features (Zampiga et al., 2019).

In turkeys, the presence of white striations in the superficial layer of *Pectoralis major* muscle, as well as the tendency of muscle fiber bundles to separate, are quite diffused and, although have been observed from a long time especially in heavy male turkey hybrids, are not considered a main quality issue by producers or consumers (Soglia et al., 2018; Zampiga et al., 2020). Past investigations (Grey et al., 1986; Grey, 1989) also described a turkey meat quality issue characterized by an increased toughness of the *Pectoralis major* muscle which at microscopic level revealed the presence of giant hypercontracted and necrotic fibers, having larger cross-section (Sosnicki and Wilson, 1991).

Incidence level and implications on meat quality

The genetic potential for growth-rate and breast yield is considered one of the main factors affecting the occurrence of breast meat abnormalities in broiler chickens (Petracci et al., 2019). Although even in turkeys the incidence of meat abnormalities and muscle fiber defects seems to be greater in fast-growing and high breast yield hybrids if compared with their unselected counterpart (Wilson et al., 1990; Sosnicki and Wilson, 1991; Velleman et al., 2003), the involvement of these factors in the onset of breast meat abnormalities is uncertain (Zampiga et al., 2020). In fact, previous studies assessing the incidence of breast abnormalities in turkeys belonging to different fast-growing genotypes, observed a remarkably high incidence of WS (as only a minority of the breasts were classified as unaffected cases) (Zampiga et al., 2019; Mudalal et al., 2019). On the other hand, the overall occurrence of WB and SM was negligible (Zampiga et al., 2019).

The occurrence of the emerging growth-related abnormalities (i.e., WS and SM) only marginally affects turkey meat quality. In fact, unlike the impact observed in broiler chickens, the detrimental effect of WS on turkey meat proximate composition was found to be limited: mainly the fat content significantly differs between unaffected and severely affected muscles (Soglia et al., 2018; Mudalal et al., 2019). Despite the limited effect exerted on meat composition, a visible-near infrared spectroscopy-based approach was effectively used to distinguish normal from severe WS turkey breasts (Zaid et al., 2020; Mudalal et al., 2020) that could be used for further processing. In detail, implementing the use of this tool will allow to limit the economic issues associated to the occurrence of WS for the poultry industry. In addition, consistent with the findings obtained for proximate composition, also the technological properties (i.e., water-holding and -binding capacities) of turkey breast meat were not affected by the occurrence of WS (Soglia et al., 2018; Carvalho et al., 2019). These findings evidence that, unlike the implications of WS in broiler chickens, the occurrence of this abnormality in turkey only marginally affects the quality traits of the forthcoming meat. According to the available literature, this difference could be ascribed to a species-specific dissimilarity in breast muscle morphology and development existing among turkeys and broilers (Soglia et al., 2018). In fact, as for broilers, the increase in breast meat yield obtained by genetic selection was mainly associated to a greater development of the pectoral muscle especially in terms of its thickness (Petracci et al., 2019). However, it was reported that the greater the Pectoralis major depth and yield, the higher the likelihood to find severe myopathic alternations within the muscle tissue (Griffin et al., 2018). Therefore, the limited impact of WS on turkey meat quality may be ascribed to a more harmonious development of the pectoral muscles that, being not merely restricted to the depth, as observed in broilers, could result in less severe changes within the muscle tissue (Soglia et al., 2018).

In conclusion, although the phenotypic features associated with the occurrence of WS in turkey breast meat seem to perfectly match with those observed for broiler chickens, the implication of its manifestation in terms of meat quality profoundly differ among the two specie. Based on that, even the implementation of processing strategies to manage the affected meat should be optimized.

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Ornithobacterium rhinotracheale in turkeys in Poland

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The avian respiratory tract is the main route of infection, and therefore its bacterial burden significantly impacts the overall health of birds and the performance of farmed bird species. One of the bacteria that may be present subclinically in the respiratory system of birds is *Ornithobacterium rhinotracheale* (ORT). This Gram-negative bacterium spread across the world in the 1990s, causing chronic diseases ornithobacteriosis and economic losses in the poultry industry. Transmission of this highly infectious pathogen can be horizontal *via* direct contact between birds through aerosols or drinking water. The possibility also exists of vertical transfer of ORT transovarially or through the oviduct. *Ornithobacterium rhinotracheale* can be a primary or secondary etiological agent, depending on the strain's virulence and the immune status of the host. Factors increasing the severity of ORT infections are poor flock management, high stocking density, high levels of ammonia and inadequate ventilation, and the occurrence of other respiratory coinfections such as *Escherichia coli*, *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, *Bordetella avium* or *Chlamydophila psittaci*. Viral respiratory pathogens such as turkey rhinotracheitis, infectious bronchitis, Newcastle disease and avian influenza viruses also predispose infected birds to develop more extensive clinical signs. Respiratory distress, airsacculitis, sinusitis, nasal discharge, sneezing, and facial edema are typical indicia of avian bacterial respiratory disease and other signs may also include depression, lower food intake, reduced weight gains and egg production, poorer growth and higher mortality. The lesions that are observed when turkeys are infected with ORT are often flattened tracheal mucosa, with reddish or hemorrhagic spots and accumulation of mucus. Blood may be excreted through the oral cavity from hemorrhagic lesions in the lungs.

Serological studies have identified the presence of at least 18 different ORT serotypes, and these have the designations A–R. The 97% of identified serotypes belong to four main types – A, B, D, and E – and they could be isolated from both chickens and turkeys. However, serotype A has the highest prevalence in chickens and far exceeds other types (95%), while turkey isolate serotypes varied more broadly. Unfortunately, serotyping is complicated by the limited availability of antisera, cross-reactivity between strains, and inconsistent results. Serotyping problems have led to the development of more effective molecular biology methods.

The main objective of the study was to evaluate the epidemiological situation in the ORT circulating in turkey flocks in Poland. The study also identified pathogens that could influence the clinical form of ornithobacteriosis in turkeys.

Material and Methods

Tracheal swabs or tracheal tissue samples were collected from 42 turkey flocks in the period 2019–2022. Tracheal tissue was aseptically obtained from birds sent for diagnostic purposes. Blood samples totaling 940 were also taken from 37 breeding turkey flocks and 57 broiler flocks during 2021 and 2022. Swab and blood samples were brought to the Department of Poultry Diseases at the National Veterinary Research Institute in Poland as part of routine diagnostic tests or monitoring programs. Clinical disease signs had been observed in some sampled birds.

Several molecular biology methods were employed for identification of bacterial infection. Use was made of real-time PCR, conventional PCR, and the traditional culture method with matrix-assisted laser desorption/ionization–time-of-flight (MALDI-TOF) analysis.

Tracheal samples were suspended in BWP buffer, then transferred to sheep blood agar and incubated at 37°C overnight. Bacterial colonies were cultured and purified and subsequently identified by MALDI-TOF. A small amount of cell colony was transferred from an agar plate directly onto the MALDI target plate and mixed with matrix solution. The mass spectra were acquired with a MALDI-TOF mass spectrometer (Bruker Daltonics, Billerica, MA, USA).

From swab and tissue samples DNA was extracted using a Maxwell RSC PureFood Pathogen Kit (Promega,

Madison, WI, USA) and a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturers' recommendations. The negative control in the DNA extraction was the Tris buffer used for sample preparation.

For the detection of the 16S rRNA gene of ORT, a real-time PCR was performed as described by Abdelwhab *et al*. with minor modifications. A conventional PCR assay was carried out to detect the same gene and it followed the protocol of van Empel *et al.*. This assay was performed on positive samples obtained in the real-time PCR.

Serological testing was carried out using a commercial ELISA, following the manufacturer's instructions (IDEXX Laboratories, Köniz, Switzerland).

Identification of other pathogens coinfecting birds with ORT was made using the same methods as for detecting ORT. In addition, to evaluate the composition of the bacterial community in the respiratory tracts of turkeys, a taxa identification based on the amplicon sequence of the V3–V4 region of the 16S rRNA gene was used.

Results and Discussion

Many pathogens of the respiratory tract are major risk factors in poultry health. In many cases, the clinical form of ornithobacteriosis is not observed in a large part of the flock, and infected birds without any disease manifestation remain carriers of the pathogen. Many infections most frequently occur as chronic and subclinical infections, or cause a mild form of the disease like a decrease in egg laying or eggshell deformation. In this study, the presence of ORT was not associated with a health problem in most of the tested flocks, which confirms that when it is the primary pathogen, ORT infects birds subclinically.

Of the 42 turkey flocks sampled, 21 provided tracheal samples harboring genetic material of ORT (50%). The years when flocks' samples were most frequently positive were 2019 with 65% of samples and 2020 with 80%.

In blood samples from breeding turkeys, ORT antibodies were found in an average of 86% of flocks with 87.5% in 2021 and 84.6% in 2022. In broiler turkey flocks, the prevalence of ORT was 66.6% in 2021 and 53.3% in 2022.

Identification of coinfecting pathogens by molecular methods and MALDI-TOF showed the presence of typical respiratory tract bacteria most likely to present as chronic and subclinical infections also causing respiratory disorders, sinusitis or airway inflammation, such as *Mycoplasma gallisepticum*, *M. synoviae*, *Enterococcus faecium*, or *Gallibacterium anatis*.

Analyzing the taxonomic composition of the microbial community of the respiratory system a phyla Firmicutes (43.2%) and Proteobacteria (42.6%) were the most dominated bacteria in this samples. The 10 most abundant families and the corresponding percentages of bacteria were *Enterococcaceae*, 19.66%; *Enterobacteriaceae*, 11.28%; *Lactobacillaceae*, 8.55%; *Morganellaceae*, 8.36%; *Moraxellaceae*, 7.16%; *Micrococcaceae*, 4.27%; *Carnobacteriaceae*, 4.07%; *Pasteurellaceae*, 4.05%; *Streptococcaceae*, 3.5% and *Weeksellaceae*, 3.18%. The most dominant genus in the respiratory tracts of turkey were *Enterococcus*, *Escherichia–Shigella* and *Lactobacillus*. Next on the genus level with abundance higher than 1% were *Proteus*, *Psychrobacter*, *Carnobacterium*, *Streptococcus*, *Rothia*, *Ornithobacterium*, *Stenotrophomonas*, *Morganella*, *Pseudomonas*, *Avibacterium*, *Neisseria*, *Acinetobacter*, *Staphylococcus*, *Massilia*, *Corynebacterium*, *Serratia* and unclassified *Pasteurellaceae.* Interestingly, in samples from turkeys where clinical signs were present, bacteria other than ORT of the genus *Ornithobacterium* were also found.

The occurrence of ornithobacteriosis in turkey flocks is of high significance to the poultry industry. Monoinfections with ORT are more aggressive in turkeys than in chickens and therefore represent a very important problem in broiler turkey flocks, and one which does not spare Polish turkey farmers. The prevalence of ORT in turkeys (50%) appears to be much higher than in chickens. This difference is particularly salient for the Polish poultry industry, considering that Poland is one of the largest producers of turkey meat in the EU.

Economic losses in the poultry industry caused by respiratory diseases including ornithobacteriosis are a serious problem, which in many cases may be exacerbated by the presence of other environmental factors or pathogens . The vertical transfer of ORT infection and the copathogenic aspect of the bacterium, which escalates morbidity and mortality in mixed bacterial infections demand that great attention should be paid to its control and prevention.

Turkey incubation using nature as a reference

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With the sophistication of single stage incubation came the question of how best to use this tool to achieve the best hatch results.

How do we cater to the developing embryos needs in regard to its incubation environment as it transitions through its fundamentally different stages of incubation?

To find this answer the best place to begin our search is nature.

There are of course intrinsic differences between natural and artificial incubation. Natural incubation takes place in a still air environment. Artificial incubation involves air movement. Natural incubation involves half the egg in contact with the floor of the nest. Artificial incubation has the full surface of the egg in contact with conditioned air. Natural incubation involves a nest of eggs from a single mother. Artificial incubation involves a machine containing eggs from a multitude of parent birds. This is not an exhaustive list of differences. The list is long.

But it is unwise to use these differences to dismiss the lessons to be learned from nature.

Problems that have always haunted the turkey hatchery are why, when we use artificial insemination on the breeder farms, is our hatch of fertile not higher, and why do we struggle to hatch all of our viable poults, losing so many at the pipped alive stage?

Nature holds the answer.

In nature the turkey hen commences laying her clutch of eggs when an increase in daylight and an increase of her own body weight tell her that the season is favourable. If her first clutch of eggs is destroyed in some manner, then she will more than likely begin a new clutch. If that second clutch is destroyed it is highly unlikely that she will commence a third on the basis that she had run out of time to fledge her offspring before the inevitable change in the season.

In the natural setting the mother bird completes her nest of a dozen or so eggs laying an egg per day. Only at the completion of her clutch does she commence incubation in earnest. Therefore, her nest contains a one2-day old egg, a twelve-day old egg and every age range in between.

In our commercial situation we know that when we set twelve-day old eggs we can expect a reduction in hatchability, a longer incubation time and poorer quality poults compared to what we would expect when we set fresher eggs.

And yet in the natural setting the mother bird hatches all of her poults in a very tight hatch window, and it is impossible to differentiate the poult that emerged from the one-day old egg and the poult that emerged from the twelve day old egg. They are of equal quality.

In the natural setting this is essential. The mother bird must possess the ability to move her clutch of poults away from the nest as quickly as possible to avoid detection by predators attracted by the smell emitted by the hatching process. The mother bird will then commence the task of teaching her poults how to feed themselves. Initially this is on benign materials such as sand and other nutritionally devoid matter to plump out the gut and equip the gizzard with abrasives while the poults are still able to subsist on the residual yolk sack.

This scenario is reflected in our commercial situation where the importance of a narrow hatch window affords the hatchery manager the luxury of processing the poults and delivering them to the farm in a timely manner for access to feed and water.

So, what can we learn from nature in this instance.

To understand how the mother bird can hatch her twelve eggs within a tight hatch window we must have an appreciation of the biology concerned.

A fertile egg, upon lay, contains a level of pre-incubation cellular development that is not consistent between eggs. Whilst not scientifically proven it would appear that the exact pre-incubation cellular development is determined by where the egg falls numerically within the clutch. Following this idea, if a clutch contains twelve eggs, the pre-incubation cellular development of the first egg in the clutch is markedly less developed than the last egg laid. When the mother bird visits her nest each day to lay that day's egg, she settles down and provides a short period of incubation. This incubation facilitates cellular development. Therefore, by virtue of progressive small periods of incubation, all the eggs in the clutch are brought to the same stage of pre-incubation cellular development by the time the mother bird goes broody and commences incubation in earnest.

This phenomenon still exists in our commercial breeder houses. The turkey hens are laying their eggs in clutches. Unlike the natural setting, every day in the commercial breeder house is a balmy day in paradise. Because the eggs are removed daily, and the hen sees no accumulation of eggs in a nest she does not go broody. Instead, when her clutch is complete, she goes off the lay for a few days and then starts laying again. This creates a situation of perpetual laying that is not reflected in the natural setting. As the bird advances in age the number of eggs that she lays in a clutch gets less and the number of days in between finishing one clutch and starting the next extends. This means that at the beginning of production from a farm and then again at the end of a farms production cycle, the number of first eggs in the clutch as a percentage of the total egg bank is greater. In nature these eggs required periods of incubation to make them as viable as the latter eggs in the clutch.

Therefore, in our commercial situation we should look for means to emulate these short periods of incubation to promote the viability of all of our fertile eggs especially those from very young and older flocks. At Petersime we have done this by designing a machine for this specific purpose. We call this our Restore machine. The Restore machine's function is reflective of the incubation created by the mother hen returning to her nest each day. By this means eggs are stimulated to advance in pre-incubation cellular development in the way intended by nature.

One of the great miracles of incubation is how an egg containing yolk and albumen can hatch a fully formed poult twenty-eight days later.

To understand this the incubation process can be roughly broken into two sections. The first ten days of incubation is the endothermic phase. This is when the egg progresses from a cluster of cells, through the blood-ring and black eye stages to the formation of a recognisable, all be it tiny, turkey embryo. From day eleven to day twenty-eight is the exothermic phase. The embryo gradually increases in size until it fills the egg and must hatch. During the endothermic phase the egg effectively isn't generating any heat and needs to be artificially warmed. During the exothermic phase the embryo is generating heat and effectively needs to be prevented from over-heating.

It is logical therefore that these two phases require a different incubation environment. But how do we determine how to do this?

In the natural setting the turkey hen did not commence lay until her body weight is such that she knows that she is physically capable of the arduous task of going broody and incubating her nest.

When she commences incubation in earnest, her body weight allows her to be extremely committed to the task. She sits tightly upon her nest and is reluctant to leave unless her own survival is dependent upon her doing so. She assiduously turns her eggs to always change the area of shell surface making contact with her skin, however apart from that she sits still and tight.

This of course corresponds with the endothermic phase of incubation. Her eggs are essentially liquid in nature. She is promoting the development of vascular growth. This vascular growth is effectively the lungs of her embryo.

This environment can be emulated in artificial incubation by minimising ventilation. An environment in which oxygen is marginally decreased is the perfect way to artificially stimulate vascular development in the developing, endothermic embryo. At Petersime we use the measurement of CO_2 – a naturally created waste product of the developing embryo – to control ventilation and emulate the nest environment for the first week of incubation.

Once the broody hen reaches the second week of incubation, she is more prepared to leave her nest for short periods each day to attend to her own needs. Her eggs are by now exothermic and can easily cope with periods of cooling. When she returns to the nest she sits higher on her clutch, thus facilitating gas exchange and moisture loss. The mother hen understands that air cell development is now of greater importance whereas it wasn't as critical during the endothermic phase. At Petersime we again use CO_2 set points to control an increased in ventilation. In this manner the

developing embryo can shed water as a waste product at the stage of development that is appropriate to this process. Temperature control is imperative. The embryo is now shedding heat into its environment. It's ability to overheat is a very real danger. At Petersime we address this by measuring eggshell temperature and allowing this measurement to control air temperature set points. Cooling water absorbs and removes excess heat from the environment.

At day twenty-five of incubation, embryos are preparing themselves to turn to the correct hatching position. They are capable of vocalisation and effectively tell their mother when they are ready to commence internal pipping. The mother knows that she has reached a critical point in her ability to serve her unhatched offspring in the best possible manner. She has already equalled pre-incubation cellular development. She has promoted strong vascular development for healthy dissipation of waste product and for harvesting oxygen. She facilitated a nest environment where her growing embryos could see the formation of a healthy air cell.

Her task at hatching time is to promote the narrow hatch window that she has thus far done everything in her power to create.

She therefore sits tightly upon her nest, almost suffocating the embryos to stimulate them to begin the process of cutting their way out of their shells.

At Petersime we again use naturally produced CO_2 as a means of stimulating the poults to progress from internal pipping through to hatch.

Every step that has been described above can be and indeed should be emulated in the artificial incubator. No one step in isolation can achieve the result of a narrow hatch window. They are interrelated.

Freshly laid fertile eggs must be stimulated to advance in cellular development. This will afford the hatchery the ability to commence incubating eggs that are already pre-conditioned for a narrow hatch window.

Eggs in the endothermic phase of incubation must experience an incubation environment that stimulates vascular development.

Eggs in the exothermic phase of development must experience an environment suitable for weight loss and temperature regulation.

By using nature as our reference, we can create the ideal environment for our fertile eggs and by this means increase the likelihood of a healthy hatch of viable, marketable poults.

Immunological Impact of Both Turkey Rhinotracheitis (Trt) And Newcastle Disease (Nd) Live Vaccines Application In Turkey Farms At 42 Days Old

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Background

TRT and ND are widespread viruses in French turkeys area productions: close to one out of three farms for TRT, and close to one out of five farms for ND, according to a seroprevalent study [1].

Despite these important viral circulations, vaccination program in turkeys is sometimes not fully complete. As a reminder, a classical vaccination program consists in three TRT (D0-D18-D42), three ND (D7-D25-D49) and one Hemorrhagic Enteritis (HE) (D25). Most of the time, the missing live vaccination applications are the oldest; certainly because of the repetition of live vaccine applications before D49, and the reduction of workforce dedicated to disease treatments and prevention [2].

But demedication has been in the hearth of sanitary actions for at least ten years [3], and avoiding viral diseases is one of the keys to prevent secondary bacterial infections.

An experimental trial [4], performed in Italy, showed the equivalence of results when TRT and ND live vaccines in combination are applied to one day-old turkeys.

In that way, the combination of TRT-ND live vaccines may be interesting for farm applications, in order to reduce the number of applications, to improve their quality, and to strengthen vaccine protection in turkeys.

Objective

The aim of the study is to compare serological profiles in farms using TRT and ND separated vaccinations respectively at D42 and D49, to farms using TRT-ND combined vaccinations at D42, without modifying the other vaccinations.

Material and method

Twelve turkey farms were randomly selected in the West part of France, the main turkey production area, between 2019 and 2022. In eight farms, vaccines were applied in combination; and in four farms, vaccines were applied separately.

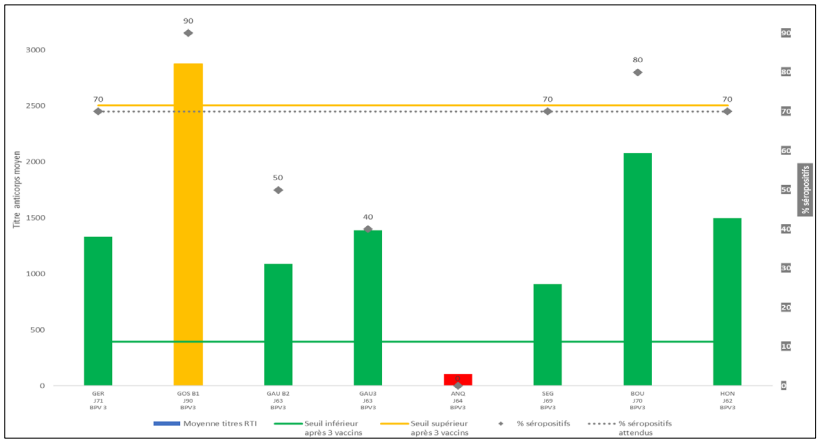
In all farms, the quality of vaccination was described. A default of live vaccine application was considered as play-off.

Serological analysis were performed around D42 and around three weeks later. Ten blood samples per age were collected, sent to the laboratory, then analyzed with IDVet kits : TRT kit for TRT valence and NDVS-CV kit for ND valence.

Serological results were treated according to thresholds defined by IDVet. For TRT valence, a mean titer between 396 and 2502 is expected after three live applications and more than 70% of serums up to 396. For ND valence, a mean titer between 993 and 4359 is expected after three live applications and more than 70% of serums up to 993. The cut off at 396 and 993 are the positive base lines defined in the IDVet kit.

Results

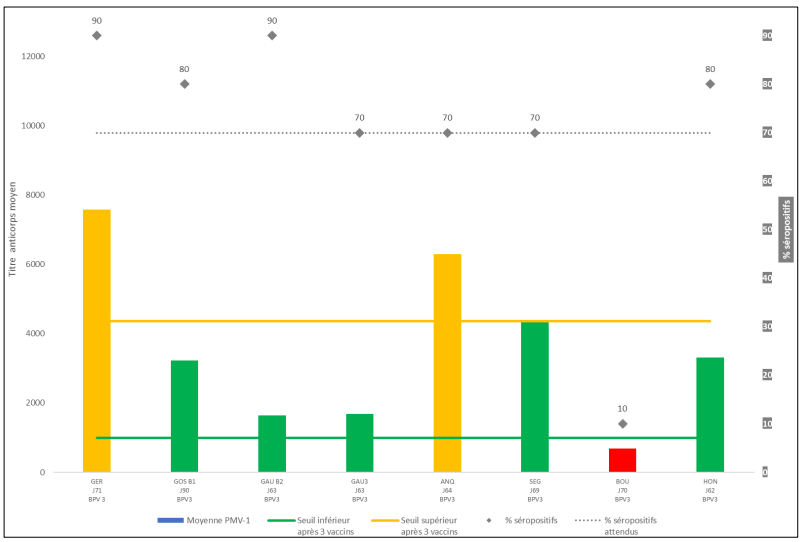
Figure 1 : TRT serological profiles after three live vaccinations (combined vaccinations)



Among the eight farms selected :

- six showed expected vaccinal values (mean titers between 396 and 2502, in green columns).
- one showed a higher mean titer (>2502, in yellow column).
- one showed a lower mean titer (<396, in red column).

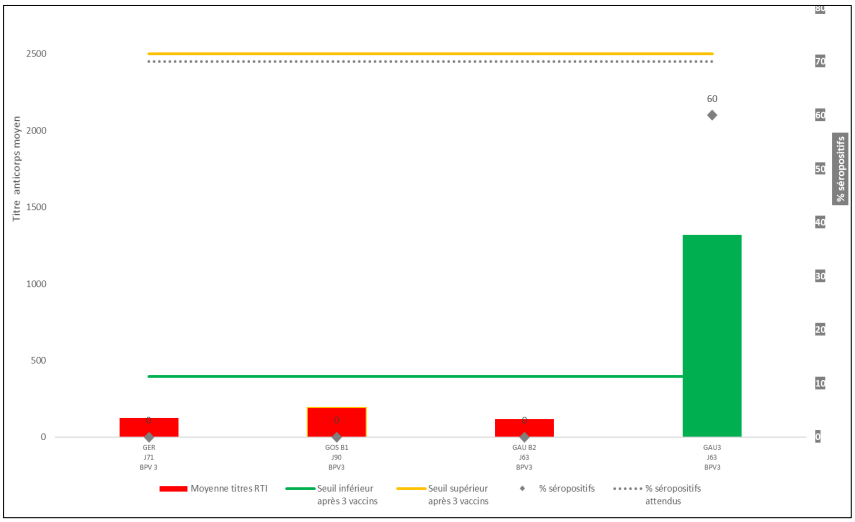
Figure 2 : ND serological profiles after three live vaccinations (combined vaccinations)



Among the eight farms selected :

- five showed expected vaccinal values (mean titers between 993 and 4359, in green columns).
- two showed higher mean titers (>4359, in yellow columns).
- one showed a lower mean titer (<993, in red column).

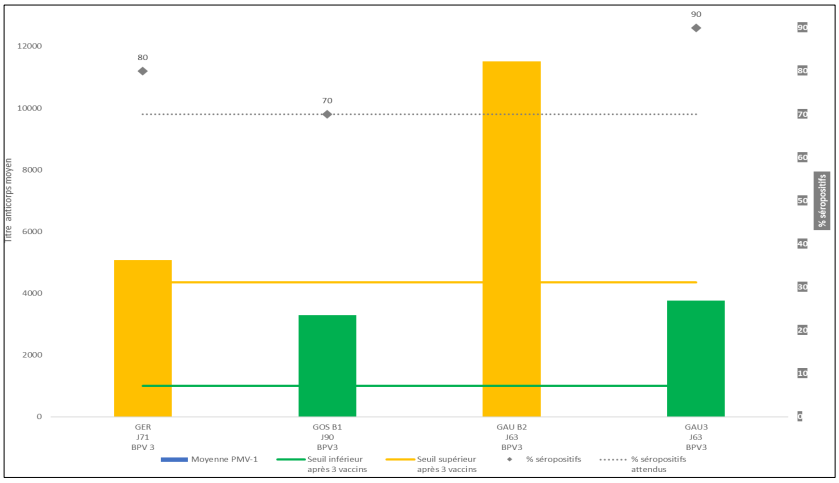
Figure 3 : TRT serological profiles after three live vaccinations (separated vaccinations)



Among the 4 farms selected :

- one showed an expected vaccinal value (mean titer between 396 and 2502, in green column).
- three showed lower mean titers (<396, in red columns).

Figure 4 : ND serological profiles after three live vaccinations (separated vaccinations)



Among the four farms selected :

- two showed expected vaccinal values (between 993 and 4359, in green columns).
- two showed higher mean titers (>4359, in yellow columns).

Discussion

In both combined and separated TRT-ND vaccine program, the majority of the twelve selected farms showed expected vaccinal values. Higher seroconversions were observed more often in the combined group than the separated group, but the difference of the sample size may be insufficient to conclude. In fact, the number of selected farms was difficult to balance between the 2 groups (combined and separated) due to economical context (decline of turkey production in France), to social context (participants' availability), and to health context (COVID-19 pandemic).

Concerning the particular cases :

ANQ farm (combined vaccinations) showed a low TRT mean titer and a high ND mean titer, unexpected because of its high quality of vaccination. An older serological analysis at D100 (unpublished data) showed expected vaccinal values that proved its high vaccine method. It might suggest a delay in the seroconversion due to an antigenic competition between TRT and ND, which are both respiratory viruses.

GOS farm (combined vaccinations) showed a high TRT mean titer and an expected ND mean titer. It revealed a recent TRT infection, with no clinical sign observed in turkeys.

GER and GAU farms (separated vaccination) showed low TRT mean titers and high ND mean titers, suggesting a recent ND infection without clinical sign in turkeys and probably an insufficient quality of vaccination. So, their vaccine methods were reviewed. Then, in GER farm, combined vaccinations were led. It showed an expected TRT mean titer and a high ND mean titer.

In all cases, the full TRT-ND vaccine program, was justified.

Conclusion

This study showed the equivalence of results in TRT and ND live vaccines combination when applied to D42 turkeys in farms.

Whatever, serological tool is an objective criterion for evaluating vaccination quality. By combining both TRT and ND valences, there is a way to qualify vaccination method, even if a field viral circulation occurs.

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Improving Hatchability and Quality via Communication between the Breeders and Hatchery

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"Collaboration is a key part of the success of any organization, executed through a clearly defined vision and mission and based on transparency and constant communication." – Dinesh Paliwal. One could not more accurately summarize what is required of the breeder farms and hatcheries to maximize hatchability and poult quality. Information should flow unbiasedly and freely between both groups, regardless if it is within the same company or between companies. Biased or poor communication only hinders the potential of everyone involved. Therefore, objective assessments and feedback must be developed and exchanged. One of these examples is the use of "Quality Control Egg Audits" at the hatchery.

"Quality Control Egg Audits" can be a very powerful tool when utilized correctly. They can bridge the communication gap that is often present between the hatcheries and the breeder farms. The intent of the egg audit is not to cast blame on either party, it is simply a means for the hatchery and the farms to work in collaboration with each other to resolve/identify issues, modify existing incubation parameters, and improve and maximize egg and subsequent hatchability and poult quality. Since processes differ between companies and geographical regions, each organization will need to customize its specific egg audit to make it relevant to its operation. Regardless of any operational differences, the basis of the audit is the same.

The Quality Control Egg Audit should be composed of four main categories:

1 Egg Sanitation

2 Egg Handling

3 Gross Appearance

4 Bacterial Presence/Load on the Eggshell

Egg Sanitation:

Eggs may be washed with various levels of chlorine and or quaternary ammonium, alkaline-based detergents and quaternary ammonium with a flat washer or brush washer. They may also be fumigated with formaldehyde, peroxide, glutaraldehyde/quaternary ammonium products in the egg houses. Egg sanitation may impact cuticle thickness and integrity. The egg cuticle directly impacts moisture loss, hatchability, poult quality, and bacterial contamination. Its consistency is important with regard to optimizing incubator and hatcher profiles as well as creating a narrow hatch window. Cuticle thickness and integrity can be examined using a 395nm LED ultraviolet black light flashlight that contains more than 50 LEDs. The cuticle color that fluoresces back will change and depends upon the types and concentrations of egg sanitizer used, fumigation used, and the age of the breeder hen. When auditing this parameter, it is very important to wait at least 24 hours after the egg has been sanitized, as it takes a while for the color change to occur.

Due to operational differences in egg sanitation, each company will need to establish a target cuticle color and use that as a benchmark. The consistency of the established target can be then evaluated using UV light. When auditing, the points should be based on overall general appearance as opposed to individual eggs. The goal is to observe the consistency in the targeted cuticle color. There will be some natural variation in the cuticle color between eggs, but it should not be stark. You do not want to see a lot of variation (light-dark pink) between the eggs or on the eggs themselves. The variable cuticle color may indicate that sanitation was suboptimal. Point deductions should be made for improper cuticle color and consistency. The level of points deducted should reflect the severity of the deviation.

Egg Handling:

Eggs should be evaluated for air cell size, location, and any fine line cracks in the shell. The air cell forms as the freshly laid egg cools. The air cell is very small at first but increases in size with egg age and moisture loss. The purpose of the air cell is to give the embryo orientation, which is required for proper development and hatching. The air cell should be firm and fixed to the center of the large end of the egg. Air cells are considered misplaced if the air cell does not touch the midline of the large end of the egg at all. If the air cell is misplaced, it may indicate improper cooling of the eggs or transporting the eggs too fresh, and the air cell has not "fixed" yet. It is advised the eggs are not shipped long distances until at least 24hrs post-lay. Eggs with misplaced air cells have an increased chance of having malpositioned embryos at the time of hatch.

The size of the air cell indicates how much water/moisture is contained within the egg and gives insight into cooler humidity levels. If the air cell is too small, the egg may not be able to lose enough moisture during incubation. If this occurs, the embryo may drown in its own fluid when pipping through the inner membrane into the air cell. If the air cell is large, the egg contains less water. If the air cell is very large, the poult may end up with too little moisture at hatch, dehydrate, and get stuck in the shell. Air cell size will increase with egg age, which is why it is important to audit the eggs shortly upon arrival at the hatchery. The goal is to receive flocks with approximately the same size air cell so the hatchery can design proper incubation profiles. Air cell target size is typically 2 cm in diameter. Smaller air cells will be seen if the eggs are audited within the first two days of being laid. (Fresh eggs). They may also indicate that the egg is gaining moisture (or not losing enough) Dehumidification should be used in on-farm egg coolers to maintain optimum humidity requirements. Larger air cells can be an indicator of excessive moisture loss. Seasonal changes and subsequent outside humidity levels can greatly impact cooler humidity and therefore air cell size. This can be assessed using a small high-intensity flashlight. Point deductions should be made on a per-egg basis.

Fine line cracks can be easily seen using a candling light as well, whether it be from rough egg handling or a body check from the AI tube during insemination. The older the crack the brighter the crack will be when candled. The more specific you can be with the type or size of crack observed the easier it will be to address the issue, whether it be a farm or transportation issue. Cracks can lead to premature embryo dehydration and increase the chance of contamination. These can be assessed using the same process as the air cells and point deductions should be made on a per-egg basis.

Gross Appearance:

In this category, one should simply be looking for physical cleanliness, cull eggs that may have slipped through farm grading, as well as eggshell characteristics. This category can be tough to audit. One must keep in mind that these eggs were laid in a barn, and there are going to naturally be minor imperfections. The greater the incidence of organic material the greater the risk of contamination. Litter and or straw that is small and easily flecked off is less risky than anything stuck on the egg. The greatest risk is fecal material.

Smears and scratches create breaches in the cuticle. Smearing typically happens when debris is wiped off the egg when the cuticle is wet. A light feathery appearance that is created by the hen when the egg is freshly laid or accidental smearing during egg collection is normal. This happens if the egg is picked up/laid very close to the collection time and the cuticle has not had time to fully dry or if the hen moves when the cuticle is still wet.

Debris on the shell should be easily flicked away, and evidence of doing so should be minimal. It is never acceptable to sand, scrub, or use a metal knife to scrape/clean eggs. Sanding, scrubbing, and scraping compromise the integrity of the cuticle and allow bacteria to easily enter the egg. In fact, the simple act of sanding, scrubbing, and or scraping can sometimes physically push bacteria into the pores and the egg itself thus increasing the chances of contamination. These eggs tend to have higher amounts of contamination and reduced hatch rates.

Shell quality is an indicator of egg quality and correlates with hatchability and poult quality. Shell quality can also be indicative of breeder flock health. This doesn't mean that eggs with suboptimal shell quality should be culled out, but it is a way of tracking trends and making necessary changes. For example, if one sees a trend in the number of eggs with calcium deposits, thinner shells, etc., it may be time to reevaluate the diet. Pimply eggs are often due to excessive air pressure in AI guns. These eggs very seldom hatch because they dry out too quickly. They easily facilitate the growth of mold and bacteria and should be removed.

Physical appearance should be evaluated by looking at individual eggs. They should be examined for cleanliness, debris, smears, scratches, and the presence of cull eggs. Eggs outside the accepted parameters should receive individual deductions. Shell quality should be assessed visually as well as by physically picking up the eggs and

touching the eggshell. The poor texture is not always visually apparent. Deductions in this category should be based on individual eggs.

Bacterial Presence/Load on the Eggshell

Eggs will not be sterile, but the microbial load on the eggshell should be minimal and void of specific harmful types such as E. coli, Pseudomonas, and aspergillus. Harmful bacteria or fungi on the surface of the eggs can lead to an increased number of rots and contamination of the hatchery. Eggs may become infected prior to, during, or post-hatch. Bacteria or fungi that make their way through the shell may simply cause a rotten egg or more seriously hide out in between the inner shell membranes and infect the poult when they pip and begin to hatch. Bacteria on the outside of the shell can lead to excessive poult mortality. When the poult hatches, the fresh navels may pick up any bacteria present on the eggshell as they move across the eggshells. This may subsequently lead to omphalitis and higher poult mortality. Microbial presence, type, and load can be evaluated by rolling eggs on various bacterial agars and incubated for a period of time. This evaluation does not capture any bacteria already drawn inside prior to being sanitized.

Conclusion

Using the four areas described, the audit can be used to quickly pick up on trends and address issues, it is recommended that each flock should be audited at a minimum of every other week. For the sake of consistency, the audits should be conducted by a designated person and done within 24-48 hours of the eggs arriving at the hatchery. Straying away from this structure can lead to inconsistent feedback due to color changes and moisture loss associated with holding eggs.

Each audit should start out with 100 points and then any suboptimal observations/characteristics found should be deducted from there. To make the audit more meaningful, each of the large categories should be weighted based on the potential impact on hatchability and quality. There should be maximum deductions for each category. Each observation should be tallied or tracked for informative reasons, but the maximum deduction should only be used for scoring purposes. For example, the auditor may tally 11 misplaced air cells but may only deduct 5 points in that category. Individual observations may be weighted in each category. Some observations may not be deducted and may simply be tracked. Some observations may carry more weight than others, for example, “Fecal Material” found on an egg may result in a 2-point deduction per egg. Even when deductions are made, that doesn’t mean that the eggs should not be set, it simply helps to fine-tune and direct the process. In addition, a score of 100 is not a realistic expectation. If farms are scoring the full 100 points, they are likely throwing away good eggs! A score of 90+ points is amazing, 80-90 points are good, 75-80 points are ok, and anything less than 70 points should elicit a phone call or conversation.

A very purposefully detailed audit is intended to give very detailed feedback to resolve issues easily and quickly. For example, one can say very subjectively that I do not like the quality of eggs coming into the hatchery and the grower doesn’t have a clue as to what to investigate. Or one can say very objectively that the eggs had 20 misplaced air cells. Based on that information, the grower should then look at how long the eggs are sitting before they are collected and cooled. The observation indicates that the eggs were cooled improperly. Again, this audit tool is to help improve communication between the farms and the hatchery to ultimately improve hatchability and poult quality. The continual feedback, good or bad, helps the breeder farm fine-tune its processes and provide the hatchery with the best quality egg possible.

Natural technologies to improve performance and prevent histomoniasis

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A healthy digestive system is crucial for high productivity, as it ensures a proper utilization of the diet and, therefore, a good productive performance. There are **many challenges**, though, that **can affect gut health** in intensive turkey farming. For example, **protozoan infestations**, such as histomoniasis, coccidiosis, and cochlosomiasis.

Histomoniasis, also known as **blackhead disease or infectious enterohepatitis**, is a disease of great relevance in turkeys because of their high susceptibility. It is caused by the extracellular protozoon *Histomonas meleagridis* that infests the ceca and, later, the liver of turkeys. *H. meleagridis* uses *Heterakis gallinarum* (roundworm) as its mechanical carrier and may cause severe cecal and hepatic necrotic lesions.

This disease has a rapid course in young birds, which usually die within few days after signs appear. Mortality of an outbreak may reach up to 80-100% of the flock. The chronic form of the disease appears in older turkeys, which can survive and become carriers, presenting chronic enteritis and alternating constipation and diarrhea.

Currently, the are no animal drugs approved to prevent, treat, or control this disease in the US and many other countries worldwide. In Europe, there is one antibiotic (aminoglycoside) registered for treatment in Italy.

Therefore, conventional solutions are obsolete for one of the huge problems of the turkey production, and alternative solutions may be researched to comply with the needs of the turkey industry.

Natural Technologies To Prevent Histomoniasis

Pronutrients are proposed as a natural innovative tool to reduce the severity of the disease and its impact on the performance of the flock.

Pronutrients (botanical molecules) to prevent protozoan infestations

Pronutrients, which are active molecules from specific plants, have a metagenetic mechanism of action: they are capable of optimizing the functioning of their target organ.

Some genes of the DNA need a stimulus to be active or to increase their activity. Pronutrients act as one of those stimuli, and help to activate specific genes that, otherwise, would not be expressed.

Genes activated by pronutrients are related to specific cell functions, which means that the stimulation of the DNA leads to the synthesis of functional proteins responsible for a better physiology of the target organs.

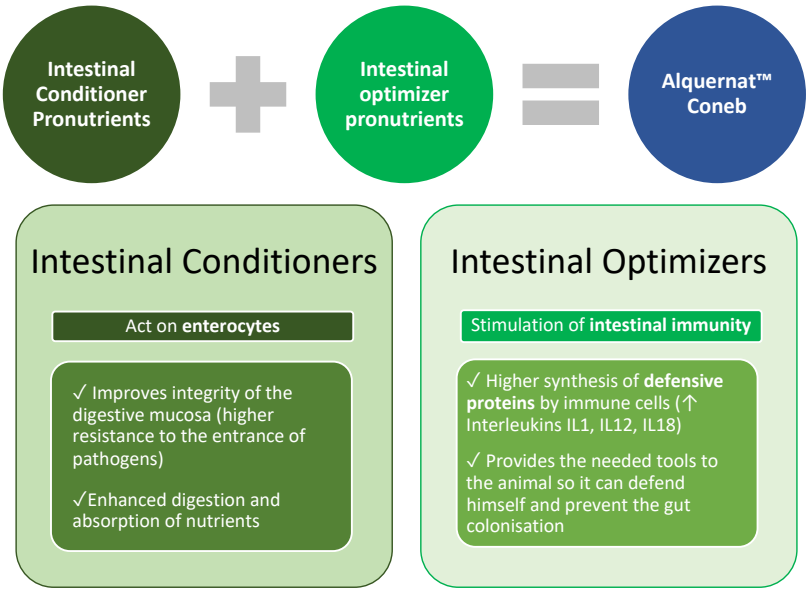
In conclusion, **pronutrients can activate target cells to optimize the organs functioning**. They can be classified into ten groups depending on their target organ. The two groups of pronutrients related to gut health are discussed below.

Intestinal optimizer and intestinal conditioner pronutrients

Intestinal conditioner and intestinal optimizer pronutrients **act synergistically to improve the resistance of animals against protozoan enteric diseases**. The combination of pronutrients targets two types of intestinal cells: enterocytes and intestinal immune effectors.

On one hand, intestinal conditioner pronutrients promote the regeneration and improve the integrity of the intestinal epithelium, strengthening the cytostructure and the tight junctions of enterocytes. In this way, the intestinal mucosa is better-organized, and the proper renewal rate of enterocytes avoids the presence of undigested feed and limits bacterial and protozoan colonization, while more nutrients are absorbed.

On the other hand, intestinal optimizer pronutrients stimulate the development and activation of immunity at intestinal level, promoting especially cellular immunity, key in the response of birds for the elimination of protozoa. This way, the local immune cells are ready to fight protozoa such as coccidia, *Histomonas*, *Cochlosoma* and other pathogens during their stages in the gut.



The combination of these type of pronutrients allow the animal's digestive mucosa to maintain its correct nutritional and defensive function. **A better protection and status of the intestine will have a positive impact on performance and will prevent the consequences of a protozoan challenge**: better feed utilization, higher final weight, and reduced mortality.

Because of their mechanism of action, these pronutrients have a safe nature, and they do not have risk of residual accumulation (nor need of withdrawal period), nor risk of resistance development (as they do not directly affect the parasite).

The use of pronutrients has been extensively researched and there are multiple trials that demonstrate that adding pronutrients to turkey diets, or even to the drinking water, can naturally prevent and/or treat protozoan infestations, just by optimizing gut cells functioning. Thus, its effectiveness and beneficial effects are proved.

Trial: Use of Pronutrients in Experimentally Challenged Turkeys

Objective: A trial was conducted at the University of Arkansas to evaluate the efficacy of a product that combines the mentioned types of pronutrients, marketed as Alquernat™ Coneb, as preventive natural tool against histomoniasis in experimentally challenged turkey poults.

Animals: 368 one-day-old commercial turkeys were placed in groups of 23 turkeys per floor pen for 42 days. Animals were distributed into 4 treatments, with 4 replicate pens per group.

Treatment groups:

(T1) Negative control: no challenge, no treatment.

(T2) Positive control: challenged animals, no treatment.

(T3) Coneb – No Challenge: animals were NOT challenged and received pronutrients at 1 kg/t in the feed from day 1 until the end of the trial.

(T4) Coneb – Challenge: animals were challenged and received pronutrients at 1 kg/t in the feed from day 1 until the end of the trial.

Challenge: 1000000 *Histomonas meleagridis* per bird were intracloacally administered to groups T2 and T4 on day 14 of age.

Parameters evaluated: Weight, feed intake and feed conversion rate were assessed at the start of the trial, and at days 14 and 42. Mortality was evaluated daily and gross lesion scoring on days 28 and 42.

Results:

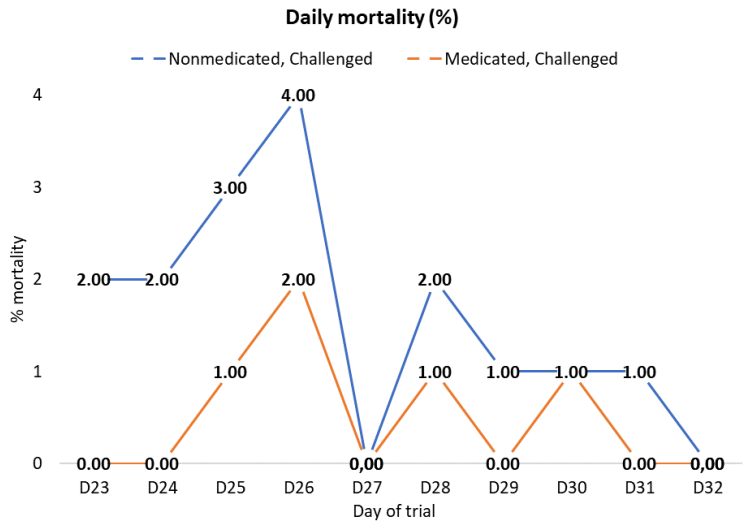
Productive parameters of the groups

Letters indicate statistically significant differences (P<0.05)

	T1 Negative Control	T2 Positive Control	T3 Coneb – No challenge	T4 Coneb - Challenge
Weight (g) D42	1269.90 ± 33.913[bc]	1233.50 ± 31.337[c]	1357.40 ± 26.172[a]	1325.20 ± 30.483[ab]
Feed intake (kg) D0-42	40.199 ± 4.496[ab]	36.825 ± 0.797[b]	48.358 ± 3.037[a]	44.829 ± 3.092[ab]
Adjusted FCR D0-42	1.86 ± 0.02	1.91 ± 0.04	1.88 ± 0.02	1.88 ± 0.03
Mortality linked to histomoniasis (% of the total)	0[a] (0/89)	17.2[b] (16/93)	0[a] (0/91)	5.6[a] (5/89)

Pronutrients significantly improved weight in non-challenged birds (+87.5g T3, versus T1) and in challenged birds (+91.7 g T4, versus T2). T4 group achieved higher weight than T1 (+55.3 g), despite T4 was challenged and T1 was not.

Comparing challenged groups, T4 reduced mortality by 67.4% compared to the non-treated group (T2). In addition, the mortality outbreak was milder and was controlled faster in the treated group (T4) than in the non-treated group (T2).



Conclusions: Pronutrients are an effective tool to improve turkey performance in the absence of a parasitic challenge and have proved to be an effective tool to control the outbreak of histomoniasis and its negative effects on performance.

Summary

The turkey industry is in demand of a proved treatment against histomoniasis, a disease which is often fatal in turkeys. In the absence of approved drugs for prevention nor treatment, pronutrients are positioned as a natural, unique, and effective tool to control histomoniasis.

Intestinal conditioner and intestinal optimizer pronutrients are active compounds of plant origin capable of reinforcing gut structure and immunity, so the animal can defend himself from parasitic invasions.

The use of pronutrients in turkeys has demonstrated their ability to reduce the number of affected animals and the decrease in the severity of the hepatic and cecal lesions, which is translated into a better final weight (+91 g) and a lower histomoniasis mortality (-67%), compared to a challenged and non-treated group.

Furthermore, in the absence of a challenge, pronutrients can enhance turkey production by increasing final weight (+87.5 g) with a similar feed conversion, ensuring the profitability of the investment.

Exploring the Frontier of Immediate Feed/Water Access for Poults

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New innovations in hatcher design now permit poults to hatch with immediate access to feed, fresh water and light, expanding into the turkey industry.

This innovation will challenge everything the industry has accepted about the best practices of maximizing the value of every fertile egg by positively impacting hatchery, farm and processing results while enhancing animal welfare. Much more research is required to optimize this technology for turkey production committed to investigating the overall impact early nutrition will have on the entire turkey life cycle.

Incubation & hatching:

Traditionally, early hatched birds are at the greatest risk of early mortality and dehydration. The industry goes through great pains to tighten and push back the hatch window as close to servicing as possible without reducing the hatch percentage.

Using innovative approaches such as HatchCare, an incubationist can plan the hatch window earlier to provide plenty of extra time for late poults to emerge, which improves overall hatch. Early hatched birds may actually get stronger and larger compared to their standard-hatched peers because they are immediately on nutrition. This allows huge improvements in the overall development of internal organs and prepares birds for the stressful moves of servicing and transport to farms.

[Pictured: Comparison of two standard hatched intestine [left] vs Next Nest intestine [right] on service day]

Servicing:

The size difference in conventional vs. HatchCare birds on service day is variable depending on how early the hatch window occurs but fed/watered birds are larger 100% of the time coming out of the hatcher.

While there is certainly additional manure and meconium generation, sexors have reported no negative impact on their jobs. In fact, the general feedback provided has been an improvement in the sex organ growth to a point that the job becomes easier to perform and speed/accuracy has increased. This bears further examination and quantification, but early indications have been positive.

Pre-shipping:

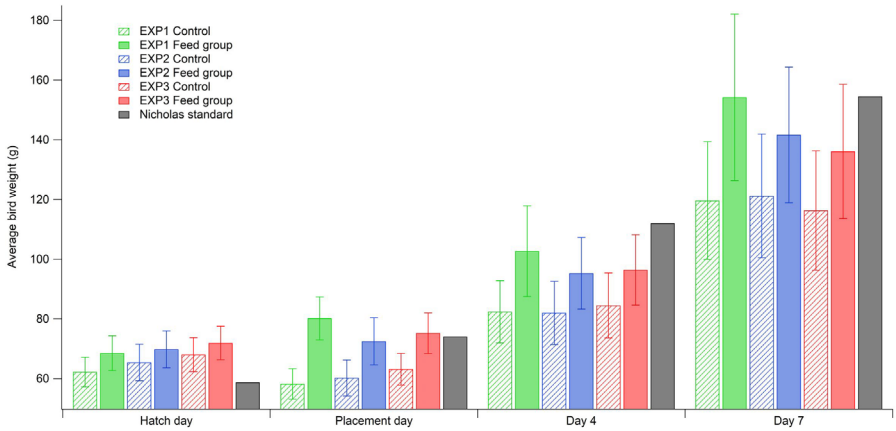
In modern hatching, placement is wise when logistically possible. The average bird may lose 10% of their weight between service day and next-day placement. However, with HatchCare, the urgency is drastically reduced – as serviced poults are returned to their hatch baskets and back into the ideal environment of HatchCare to continue to eat/drink while awaiting the truck to the farm. In fact, our overall experience has been that Next Nest poults are 37% larger upon departure from the hatchery.

Brooding:

We are looking forward to further investigating the impact of HatchCare on the brooding process. Our farmers have loved the concept of getting their poult deliveries at a more convenient time rather than the first possible moment they can be delivered. This flexible scheduling in a world short on workers willing to show up at 1 am and work all night is a boon for the industry.

We believe barn temperatures at placement will need to be reduced for Next Nest birds, as they are already producing a large amount of heat at placement. In early trials we attempted to split barns between control and test sides with fences and it rapidly became obvious that these are two quite different birds coming out of flocks incubated together until just 3 days prior.

Much work still needs to be done to optimize the barn arrival. There will likely be methods to improve this transition even more once we gain more commercial use. Regardless, we know we are not yet optimized and we still see excellent early results.



[Pictured: Three control vs test trials and the Nicolas Standard growth curve at 7 days]

Enhanced Results that Span the Test of Time:

While we scale-up our own hatchery operation in Willmar for full production, we have had a few external opportunities to observe the impact of early feed/water all the way to processing. Three 48-pen control tests have been achieved with a third-party partner which indicate a retained advantage for with feed and water access in the hatchery in the processing plant.

Preliminary results show performance improvement at processing averaged ~2-3% additional weight through market, in the same timeframe as contemporary control birds. This growth advantage was accomplished with the same amount of feed as standard-hatched birds; providing a significant cost savings.

Toms								
	Control weight (lbs)	Headstart weight (lbs)	Weight difference (lbs)	FCR adjust (FCR/lb)	Less feed (lbs)	Feed ($/ton)		Savings per processed tom
Less feed	45.00	45.9	0.9	0.05	2.03	$ 300		$ 0.304

[Positive impact of Next Nest birds at processing, at historical average feed cost, per tom]

Improved mortality for Next Nest birds was also identified, especially when all trial birds were challenged with an all-flock issue:

Trial #1: 0.3% disadvantage to HeadStart = $-0.009/tom [minimal mortality trial for the full flock]

Trial #2: 1.9% advantage to HeadStart = $0.057/tom

Trial #3: 2.6% advantage to HeadStart = $0.078/tom

Care from Egg to Farm

We have only just begun to learn and optimize around this innovative technology. However, potentially increased weight and less feed may offer real bottom-line savings that reveals an opportunity that cannot be ignored. Along with the potential cost savings, the technology offers further improvement to the welfare of every poult during the first critical days of life.

Next Nest Hatching, LLC has been created by a 75-year turkey producer to further explore and open the door for all turkey producers to access the incredible advantages of technology to provide access to feed and water in the hatcher. In partnership with HatchTech, the team at Next Nest is excited to invite other interested operations to embrace this opportunity early and join our efforts to define the impact of this exciting new tool for the industry!

"Reduction of the environmental impact and the consequence on the competitiveness of the turkey sector by the specific use of feed raw materials"

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Introduction

As feed is a major contributor to the environmental impact for poultry products, the aim of the trial was to evaluate the effect of including raw materials with lower environmental impact on performance of male turkeys. Bases on the results, the impact on environmental criteria was estimated and economic simulations were carried out to linked with the competitiveness for the turkey sector.

Material and methods

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

288 Aviagen Premium males turkeys were raised in 24 floor pens (2.6 m²) from 1 day to 119 days old and split in 4 groups.

The feeding program contained 6 phases. Feed presentation was a crumble from d0 to D35, then pellets of 3.25 in diameter. The 4 groups were fed with the same nutritional levels. The nutritional levels were adjusted according to Techna France Nutrition specifications, matrix and energy system for commercial turkeys (Table 1) and contained xylanase and phytase. Feed and water were provided *ad libitum*.

Table 1 : Nutritional value of diets

	Age	0-21	21-35	35-56	56-77	77-91	91-119
*Nutritional value of the feed**							
METABOLISABLE ENERGY	Kcal/kg	2750,0	2850,0	2900,0	3050,0	3150,0	3200,0
CRUDE FAT	%	3,5 - 4	4 - 6,7	4,2 - 5,7	4,7 - 6,9	5 - 5,9	5,2 - 6,7
CRUDE PROTEIN	%	25,9 - 26,8	24,9 - 25,3	21,9 - 23,2	20,5 - 21	19 - 19,5	18,5 - 18,9
DIGESTIBILE LYS	%	1,55	1,43	1,27	1,13	1,04	1,01
CALCIUM	%	1,40	1,25	1,10	0,95	0,85	0,80
PHOSPHORUS Av	%	0,75	0,63	0,57	0,46	0,41	0,38

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

The raw materials in the feed which differed for each group was the source of protein (table 2). In group 1, which will be considered as the control group, the raw materials used were those conventionally used for poultry feed in France, with imported soyabean meal as the main source of protein.

Group 1 diet contained soyabean meal. To replace non-EU soyabean meal the other diets were created through the selection of high protein sources of alternative raw materials. Specifics ones have been selected for their high protein level:

Group 2 : Hipro Sunflower meal with 46% of crude protein content and low fiber value

Group 3: An extruded commercial mix with 33% of crude protein containing faba bean and French OGM-free soyabean seed

Group 4 contained dehulled faba bean (31% of crude protein) and same Hipro sunflower meal as in group 2. This was the only group to use, only in started feed, imported soyabean meal at a level of 10% in order to meet CP specifications.

For all groups, synthetic amino acids were incorporated in feed to achieve TECHNA's specification for the ideal amino acid profile of turkey requirements. The variation of protein and fat level between the different groups was due to raw materials characteristics. Table 2 presents the rate of incorporation of raw materials for all diets.

Table 2 : Diets composition

		Diets Raw materials											
Phase		**0-21**				**21-35**				**35-56**			
Composition of the feed		**G1**	**G2**	**G3**	**G4**	**G1**	**G2**	**G3**	**G4**	**G1**	**G2**	**G3**	**G4**
CORN	%	10	10		10	10	10		10	10	10	10	10
WHEAT	%	30,0	30,0	18,0	23,0	37,1	32,4	30,4	28,2	42,3	44,5	22,6	32,7
WHEAT BRAN	%			12,5				6,0	6,0				
SOYABEAN MEAL	%	**41,80**			**10,00**	**37,00**				**31,80**			
HIPRO SUNFLOWER MEAL	%		**35,00**		**15,00**		**36,10**		**15,00**		**30,70**		**12,00**
SUNFLOWER MEAL	%	2,00		4,00				7,00				6,00	3,10
EXTRUDED MIX	%			**35,00**				**35,20**				**33,00**	
DEHULLED FABA BEAN	%				**15,00**				**15,00**				**20,00**
RAPESEED	%	2,80				3,80	3,40		2,00	3,70			
RAPESEED MEAL	%			4,00	4,00			6,80				6,00	2,00
PEA	%		5,00	5,00	5,00			6,00	6,00			8,50	
POTATO PROTEIN			2,60	3,80	2,00			2,00	4,20				
DDGS		4,00	2,60	4,00	4,00	4,00	4,00	4,00	4,00	6,00	2,00	6,00	6,00
RAPESEED OIL	%	0,40	2,50		1,45	0,40	3,50		1,35	0,40	3,30		2,05
AMINO ACIDS, PREMIX AND MINERALS	%	9,00	12,30	13,70	10,55	7,70	10,60	2,60	8,25	5,80	9,50	7,90	12,15

Phase		**56-77**				**77-91**				**91-119**			
Composition of the feed		**G1**	**G2**	**G3**	**G4**	**G1**	**G2**	**G3**	**G4**	**G1**	**G2**	**G3**	**G4**
CORN	%	10	10	10	10	25	16	10	10	12	10	10	10
WHEAT	%	45,5	48,0	33,9	40,5	42,1	50,0	45,1	44,4	55,0	54,7	49,6	45,6
WHEAT BRAN	%												
SOYABEAN MEAL	%	**28,40**				**24,90**				**21,60**			
HIPRO SUNFLOWER MEAL	%		**24,80**		**10,00**		**19,80**		**7,50**		**18,90**		**6,60**
SUNFLOWER MEAL	%												
EXTRUDED MIX	%			**28,50**				**25,00**				**25,00**	
DEHULLED FABA BEAN	%				**20,00**				**20,00**				**20,00**
RAPESEED	%		2,90										
RAPESEED MEAL	%			6,00			2,00					2,00	
PEA	%			8,00			7,00					2,00	
POTATO PROTEIN													
DDGS		2,90	2,10	6,00	6,00			2,40	5,50	2,00	3,00	2,60	5,30
RAPESEED OIL	%	2,65	4,00	0,40	2,50	3,10	4,30	0,40	3,55	3,40	5,00	0,55	3,85
AMINO ACIDS, PREMIX AND MINERALS	%	10,55	8,20	7,20	11,00	4,90	0,80	17,10	9,05	5,60	8,40	8,25	8,65

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

The value of environmental criteria was calculated according to the proportion of the different raw materials in the formula (Table 3). The environmental assessment of raw materials was calculated for 6 indicators: greenhouse gases (GHG), non-renewable energies (NRE), phytosanitary treatments index (PT), water consumption (WC), phosphorus consumption (PC) and land use (LU). These values were determined according to TECHNA's calculations based on life cycle analysis from production to factory gate for GHG and NRE. PT, WC and PC were drawn from published databases (Wilfard, 2016). Land use corresponds to 1/average yield of the crop. The environmental index considers these 6 criteria for which a coefficient was assigned.

Birds and feeds were weighted at 21, 35, 56, 77, 91 and 119 days per pen to determine the average daily weight gain (ADG), daily feed intake (DFI) and feed conversion ratio (FCR). At the end of the study, the calculation of feed environmental impact was done proportionally to feed intake.

Litter quality was evaluated at 35, 69, 77, 91 and 105 days by measuring litter dry matter.

At 119d, 18 turkeys from each group were selected and slaughtered. The day after, the carcasses were processed with weights taken of the total carcass, breast, thigh, and abdominal fat, to determine yields.

Statistical analysis of data was completed using R, version 1.2.1335. Analysis of variance (ANOVA and Tukey test $\alpha = 0,05$) was used for weight, ADG, FCR, litter dry matter, carcass weight, carcass yields, litter dry matter and environmental criteria ; Chi-2 test for mortality.

Results

At day 119, the weight and ADG of the male turkeys was significantly lower for group 3 compared to others (p= 0.001). In the first phase, 0-21d, group 3 had the lowest weight and ADG; highest growth was seen in group 2. Thereafter, and throughout the rest of the trial, only the weight of group 3 differed significantly from the other groups.

From 0d to 91d, feed intake of group 3 was significantly lower than other groups, and after 91d there was no difference between groups. The result, therefore, was the total feed intake was lowest for group 3 for the total period (p=0,001). There was no difference between the groups on FCR for the total period, even if group 4 had a better feed efficiency (- 0,05pt of FCR compared to group 1).

Table 4 : Weight, average daily weight gain (ADG), daily feed intake (DFI) and feed conversion ratio (FCR)

	Item	Treatments				SEM	p-value
		G1	G2	G3	G4		
	Initial BW,g	59,9	59,9	59,9	59,8		
0-21 days							
	BW, g	659 ab	710 a	614 b	698 a	34	**0,001**
	ADFI, g/j	41,3 a	41,4 a	37,0 b	42,1 a	1,7	**0,000**
21-35 days							
	BW, g	1776 a	1844 a	1632 b	1815 a	74	**0,000**
	ADFI, g/j	119,1 a	119,0 a	109,6 b	117,0 ab	5,3	**0,016**
35-56 days							
	BW, g	4869 a	4969 a	4333 b	4936 a	180	**0,000**
	ADFI, g/j	241,2 a	246,9 a	221,2 b	244,2 a	10,7	**0,002**
56-77 days							
	BW, g	9164 a	9136 a	8288 b	9295 a	272	**0,000**
	ADFI, g/j	406,8 a	410,4 a	379,8 b	420,4 a	16,6	**0,003**
77-91 days							
	BW, g	11876 a	11799 a	11064 b	12044 a	334	**0,000**
	ADFI, g/j	508,9 a	492,0 a	480,0 a	506,3 a	18,4	**0,045**
91-119 days							
	BW, g	17870 a	17679 a	16895 b	17882 a	363	**0,001**
	ADFI, g/j	623,3 a	604,9 a	590,3 a	583,4 a	31,4	0,073
0-119 days							
	ADG, g/j	149,7 a	148,1 a	141,5 b	149,8 a	3,1	**0,001**
	FCR	2,287	2,279	2,267	2,239	0,046	0,172

Litter quality differed at 69d, 77d and 91d, with driest litters for group G4, and wettest litters for group 1 (Data not shown). No impact of raw materials profiles was observed on mortality (data not shown).

No significant difference between groups on dressing weight and yields was found (Table 5).

Table 5 : Carcass weight and yields at 119d

	Item	Treatments				SEM	p-value
		G1	G2	G3	G4		
	Number	18	18	18	18		
Weights 120 days							
	Life, g	17872	17880	17033	17739	1168	0,102
	Carcass, g	14550	14544	13790	14475	1013	0,078
	Breast, g	4211	4161	3908	4143	437	0,171
	Full leg, g	4077	4095	3944	4122	244	0,137
	Abdominal fat, g	149	158	149	175	45	0,257
Yields 120 days							
	Carcass, %	81,4	81,3	80,9	81,6	0,9	0,138
	Breast, %	23,5	23,2	22,9	23,3	1,5	0,585
	Full leg, %	22,8	23,0	23,2	23,3	0,9	0,394
	Abdominal fat, %	0,83	0,88	0,88	0,99	0,24	0,266

The substitution of soyabean meal with vegetable alternative protein sources was significant for the environmental impact. The most important decrease through feed formulation obtained was for greenhouse gases: it was reduced by 39 % for group 2, by 46 % for group 3 and by 41 % for group 4. Many other criteria showed a significant decrease by using alternatives to imported soyabean meal, but in lower proportions (table 6) : for all groups a reduction was seen between 5% to 8,5%, 23% to 27% and from 8,5% to 16% respectively for NRE, WC and PC.

However, in Group 3 the impact was higher than diets with imported soyabean meal for phytosanitary treatments index (+ 293% - P=0,000) and land use (+16% - P=0,000). Land use was also unfavorable for group 4 (+ 11% - P=0,000).

Overall, Global Environmental Index was significantly improved (P=0,000) for the 3 formula profiles compared to a formula with imported soyabean meal.

Table 6 : Environmental criteria

	Item	Treatments				SEM	p-value
		G1	G2	G3	G4		
Global period							
	Green house gases, Kg Eq. CO_2/t	735 a	450 b	399 d	434 c	0,84	**0,000**
	Non- renewable energies, MJ/t	4097 a	3759 d	3905 b	3783 c	1,29	**0,000**
	Phytosanitary treatments	13,0 b	12,0 d	53,5 a	12,6 c	0,05	**0,000**
	Water consumption, L/t	11,6 a	8,9 b	8,5 d	8,9 c	0,03	**0,000**
	Phosphorus consumption, Kg P/t	4,6 a	4,1 c	3,9 d	4,1 b	0,00	**0,000**
	Land use, ha/t	0,19 c	0,17 d	0,21 a	0,21 b	0,00	**0,000**
	Environmental Index, %	**3,37** a	**1,96** b	**1,58** c	**1,60** c	**0,04**	**0,000**

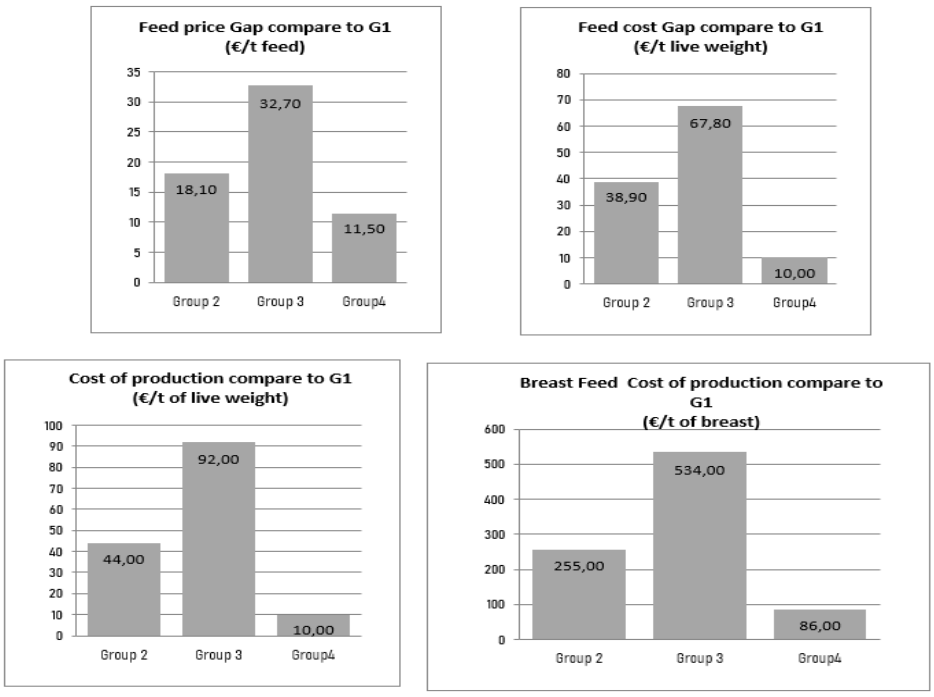
Discussion

The result of this trial confirms most of the theorical scenarios used by authors for the estimation of poultry production and the environmental impact. As mentioned by Leinonen (2013), to maintain growth and feed efficiency, it's necessary to formulate the feed with well-balanced amino acid by using synthetic ones. However, it's also important to consider raw materials characteristics and their potential antinutritional effect. Thus, we supposed that group 3 lowest performance was surely due to too high levels of wheat bran in the starter diet. The weight difference at the end of the starter period for group 3 was then maintained throughout the test.

Best performance was obtained with group 4, including dehulled faba bean; this group was also the most effective when reducing the environmental impact. Litter dry matter was also better than other groups, which can benefit litter quality and animal welfare.

From these results, an economic simulation based on the '3 point-contract", the most common French model (Magdelaine, et al 2015), has been created considering the French context of May 2022. 4 economic criteria have been evaluated: feed price, feed cost, cost of production, breast feed cost (Messaoud, 2020). All these criteria were increased with the use of more sustainable ingredients; compared to soybean meal, the smallest difference was for diets with dehulled faba bean.

Figure 4 : Economic gap between each group and the group 1



Another feeding strategy to reduce the environmental impact of diets is to improve feed efficiency by reducing feed consumption (Leinonen, 2016). Reducing FCR also have an impact on production cost (Messaoud, 2020). So, in addition to using alternative raw materials, the nutritional specifications of turkey diets should be adapted in order to compensate as much as possible for the additional cost for lower environmental impact feeds and to maintain competitiveness of turkey production.

Conclusion

Producing turkeys' diets with alternative protein source in substitution to imported soyabean meal is feasible while maintaining performance. To optimize competitiveness of the turkey industry and to improve their environmental impact, a global strategy should be set: the use of specific raw material with low environmental impact and modification of nutritional specifications to improve feed efficiency. Moreover, the availability of such raw materials is a real issue to be addressed if formulation with low environmental impact raw materials is to be developed.

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Updates on Welfare and Sustainability for the European Turkey Industry

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Summary

Welfare and sustainability have an increasing influence on the political agenda in Europe. To meet this there are increasing cross sector initiatives to become more robust in transparency and communication whilst staying with the facts. Primary breeders make a valuable contribution to improving the welfare and environmental sustainability of turkey production through a balanced breeding approach. The welfare of birds can be assessed through the measurement of Key Welfare Indicators and there is publicly available information showing the welfare improvements in the field. Although the environmental impact of turkey production relative to other animal protein sources is favourable, there is still a need to improve resource use and reduce environmental outputs. Improvements in feed utilisation efficiency represents the greatest opportunity to reduce the environmental impact of turkey production. The historic and projected improvements in this area mean an expected reduction of 9% reduction in the Carbon Footprint of Turkey production by 2030.

Global Trends

There are two global trends that are having an influence on the supply and purchase of turkey meat in the last few years.

The first is the movement from a producer to a consumer market where the buying power of the consumer has a greater influence on what food is produced. As the population is moving away from countryside and into the towns and cities then the links with agriculture are reduced; the understanding of how food is produced is lost as it no longer forms a part of everyday life for the majority of the population. Increasingly the consumer wants to have choice over what to buy and is becoming concerned about how their food is produced. This is due to a combination of the influence of the retail and food service sectors along with welfare organisations.

The second trend is that we need more from our planet as the population increases. The United Nations have estimated the global population will reach 8.5 billion in 2030, increasing by 1/2 billion people from 2022 (UN 2022). At the same time the agricultural land per capita will decrease. It was 0.63 hectares in 2018 when the global population was 7.7 billion. Additionally developing economies want to add animal products into their diets as their wealth increases. To be able to achieve these needs we must look to sustainable intensification where we try to get more animal production output from the resources available i.e. less water, feed and land per kg of product.

Non-Governmental Organisations are shifting the food system paradigm. They are becoming involved and having influence on what is considered good farming practices and good food. For example the Eat Lancet group were leading UN Food Systems Summit ‘sustainable diets’ and Eurogroup for Animals is secretariat for Intergroup on Welfare and Conservation of Animals at European Parliament. The concern with such involvements is that they are feeding on the disconnect between society and agriculture. We are finding that conversations about the goodness of food do not take into account the fact that animal based foods are wholesome and nutrient dense. There is a focus on some metrics such as calories or protein whilst others such as vitamins, minerals and bioactive compounds are neglected. With such a focus it is important to realise that the most “sustainable” options per kg or per kcal are often poor nutrient sources and diets low in animal source foods are usually less nutritionally robust.

In the area of communication the industry is playing catch-up. For many years the approach was taken that it was better to say nothing rather anything at all. In recent years it has been realised that the best approach is to tell your own story rather than respond to others. With that approach the associations are starting to have their own

communication people and organisations such as the Animal Agricultural Alliance and European Livestock Voice have been established. These are multi-stakeholder groups of American and European partners in the livestock food chain who understand the importance of communicating rather than just talking. This communication is based on the need to focus on bridging the disconnect with society and bringing back an understanding of agriculture by the citizen. This is particularly important to provide an alternative narrative to the one which puts forward that modern agriculture is bad.

Animal Welfare and Politics in Europe

As noted earlier consumers are concerned about how there food is produced and across Europe there are several pieces of legislation specifically aimed at farm animal welfare. Currently the European Commission is reviewing its welfare strategy. This includes a fitness check to assess the relevance of its current legislative framework. The check confirmed that the current legislation was beneficial, but that some needed to be reviewed to take into account of where science and farming methods are currently placed. Additionally the check found that welfare legislation enforcement could be more uniform across all the member states.

The Commission has also asked European Food Safety Authority (EFSA) to supply five scientific opinions with three relating to poultry: Meat chickens – including breeders and hatchery; Egg layers – including breeders and hatchery; Transport –in containers for poultry

The Commission has stated that there will be no new legislation without an EFSA Opinion being sought therefore we can assume that these will be the scientific input to new EU legislation to be proposed by the end of this year.

Currently there is no turkey equivalent of the broiler and laying hen directives, but there will be an EFSA scientific opinion on turkeys, however this is not expected to be requested until 2024 at the earliest.

The EFSA scientific opinions on transport was published in September 2022 and poultry was included as in the section focusing on transport in containers (EFSA 2022). This covered both the transport of poults and livehaul. There were a number of recommendations made which are different from current practice and if made law would have a significant effect on how the industry operates (Table 1).

Topic	Day old transport	Livehaul
Key Recommendation	To avoid welfare issues with the transport of day old birds on-farm hatching is re-commended	
Journey start	When first poult emerges	When feed withdrawn
Journey end	When poult received feed and water on farm	When last bird unloaded
Time requirement	Poult must receive food and water within 48 hours	Journey must be completed in 12 hours

Table 1. Summary of major recommendations relating to poultry from the 2022 EFSA Scientific Opinion on transport in containers.

It is interesting to note that there is no maximum journey time for day old bird, just that they must have food and water within 48 hours. Recently a large scale holding trial resulted in liveability on arrival and 7 Day liveability results at 60 and 72 hours after pulling the birds from the hatcher (**Özlü** *et al*., 2022). These results are in line with the experience of the breeding companies transporting day old birds. The concept of the journey starting when the first bird emerges is an assumption from research and current legislation. However, European day old bird transport is based on one study, Warriss *et al.* (1992), which concerns birds obtained from a commercial hatchery at pull. In addition, the abundance of research in day old bird holding, is based on 'pull', not when the first day old bird emerges. These examples show the importance of ongoing research and open dialogue and due care should be taken before legal measures are put in place. The EFSA opinions cover welfare aspects only. The European Commission, European Parliament and Member States aim to involve all stakeholders, inviting for extra information and proof, in a follow up process that is currently taking place in the area of animal transport.

The Germany government has decided that there is a need for specific turkey legislation and they have put forward a number of proposals which will bring similar controls as the broiler directive. These include:

- A maximum permissible stocking density in the fattening phase of 35kg/m² (3.1 birds/m²) for hens and 40 kg/m² (1.9 birds/m²) for males. The 2023 version of the QS standard is 52 kg/m² for hens and 58kg/m² for males (QS 2023).

- Activity material must be available in sufficient quantity at all times. In principle, all animals must have the opportunity to use the activity material.
- Sufficient structuring of the barn to create opportunities for retreat
- Feed withdrawal maximum of 12 hours before slaughter, taking into account the duration of transport and standing times at the slaughterhouse, which is the same as recommended in the EFSA opinion on Transport.

The UK government started to consider the legislation on animal transport in 2020 as part of the opportunity post Brexit to ban the movement of livestock into the European Union for slaughter. The current proposals (DEFRA 2021) cover transport of all species, but in terms of poultry they still permit the export of live poultry. However they have introduced maximum journey times (Table 2)

Category	Time	Comments
Day old birds	24 hours	There will be more time if additional criteria fulfilled. The specification of additional criteria has not yet been agreed.
Broilers	4 hours	This refers to broiler chickens only going to the processor. The journey starts when the last bird is loaded.
All other birds	12 hours	This covers all poultry that is not a broiler. The journey starts when the last bird is loaded.

Table 2. Summary of maximum journey times from the UK government response to the consultation on improving welfare in transport.

Additionally there is a proposal that journeys over 65 km are only permitted within a temperature range of 5 - 25°C unless a temperature controlled vehicle is used.

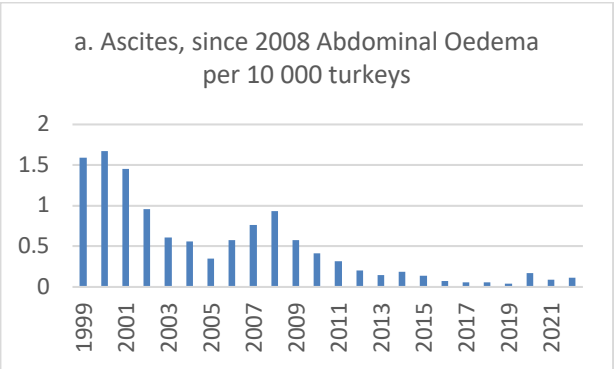
At this time the changes in Germany and the UK are still proposals and the industry is in discussion with the responsible ministry about the actual legislative proposals to be tabled before parliament in the second half of this year.

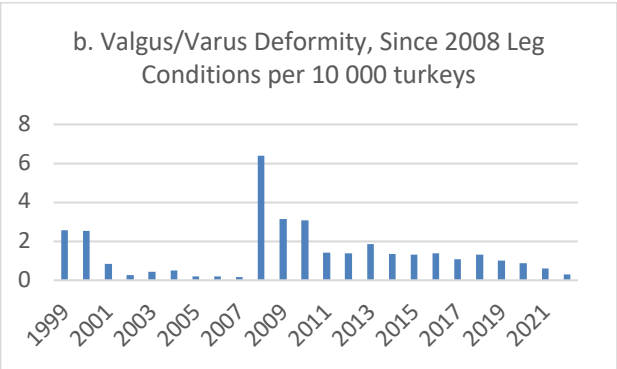
Welfare Indicators

When considering welfare we should think about both the physical and mental state of the animal and avoiding the tendency to anthropomorphize the bird's needs, which can lead to inaccurate understanding of biological processes.

An important aspect of assessing welfare is to look at key welfare outcomes which give the ability to track how birds are performing. Currently, the easiest place to make some of these measurements is at the end of life in the processing plant.

The role of the primary breeder is to consider the welfare of the both in the birds in their care and of future generations by selecting to improve the welfare traits at the same time as other traits of interest. Public information from the Canadian Food Inspection Agency (CFIA,2023; Figure 1) shows the improvements from breeding are reflected in practice – ascites, reflective of heart and lung health, leg health and the number of dead on arrival have demonstrably improved between 1999 and 2022.





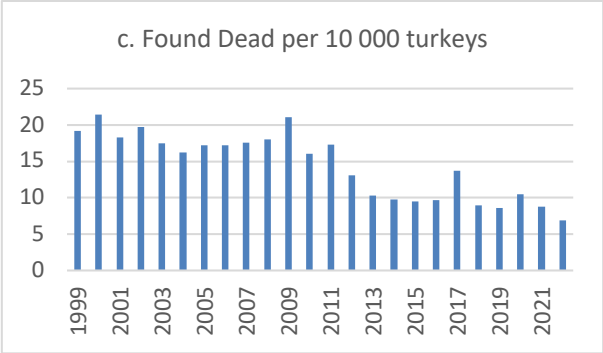


Figure 1. Ascites (as from 2008 abdominal oedema) (a), Valgus/varus (as from 2008 leg health) (b), Dead on arrival (c) related condemnation rates in turkeys. Per 10,000 (1999-2022). Canadian Food Inspection Agency (CFIA), 2023). Note the assessment criteria were altered in 2008

Animal based measures assess something specific to the bird. Resource based measures consider the inputs, for instance breed or environmental enrichment. The Dutch Greenwell project looked at the benefits of the four farming "concepts" currently being used to grow broilers from an economic, environmental and welfare point of view. The four concepts were

- Conventional – the standard broiler;
- Dutch retail – which is a slightly slower growing broiler (50g/day) with environmental enrichment;
- Beter Leven 1* - even slower growing broiler (46g/day) with environmental enrichment, lower stocking density and on-farm hatching;
- Organic.

In the welfare aspect of the study they initially looked to establish repeatable and reliable indicators and drew up a list of 5 animal based and three resource based criteria to evaluate welfare (De Jong, I.C., & te Beest, D., 2020). These criteria were used to calculate welfare scores for farms operating the different concepts (De Jong, et al., 2022). Due to the low number of farms using the organic concept these were not included in the final results. When all the welfare indicators were counted there are three distinct, but overlapping distributions with the Conventional (no resource based scores) being lower than the Dutch Retail (one resource based score); than the Beter Leven 1*(three resource based scores). However, when the resource based indicators are removed and only the animal based measures are considered, the variation between the concepts is vastly reduced. With far greater overlap between the three concepts. When looking purely at the animal based measures the importance of management is very clearly seen. It is possible to farm conventional birds with as good if not better welfare score than a Beter Leven 1* (Figure 2).

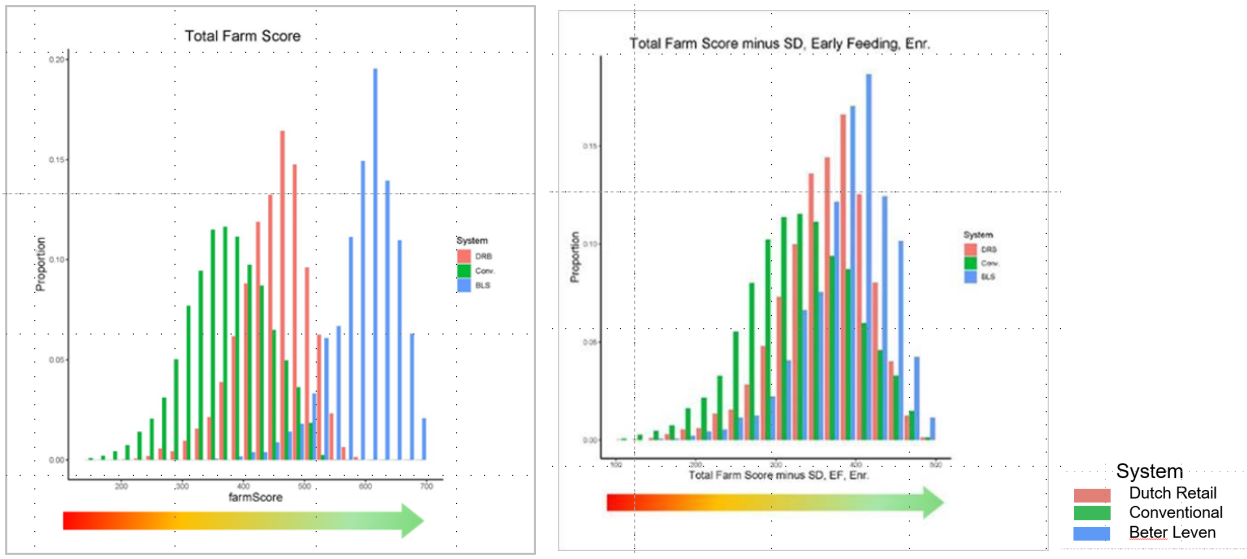


Figure 2 Welfare Scores with and without resource based measures for three different farming systems in the Netherlands (De Jong et al., 2022).

One of the difficulties of using Animal based welfare indicators is that there needs to be a standardisation of approach to ensure the repeatability between the people using them in different locations. The International Poultry Welfare Alliance (a multistakeholder initiative including industry and scientists) has published a reference guide on animal based Key Welfare Indicators to provide a reference to help those managing and caring for poultry understand key welfare indicators and how they can be used to improve welfare outcomes (IPWA, 2023). The document provides a number of animal based key welfare indicators, explaining why they can be used and how to use them.

As we can see from the Greenwell project, management has a significant impact on the welfare of the birds. To help the farmer with this essential part of the jigsaw, the health and wellbeing of the birds in their care, Avec have published a Turkey Management Guide (Avec, 2023). The aim of the guide is to promote harmonized best practices for European turkey production and to ensure the quickest possible implementation of advances into turkey farming. The document can be used at the turkey house for guidance and communication and may at the same time be used as a checklist to make sure the important issues that influence the health and welfare of the turkeys are considered.

Increasing importance of sustainable turkey production

Another area we have seen grow in importance over the years is environmental sustainability as consumers and producers become more aware of the increasing global population along with the increased need to make better use of available resources.

In terms of animal products poultry meat is in a good position as we can see it has the smallest carbon cost per kilogram across the supply chain compared to the major meats (Figure 3).

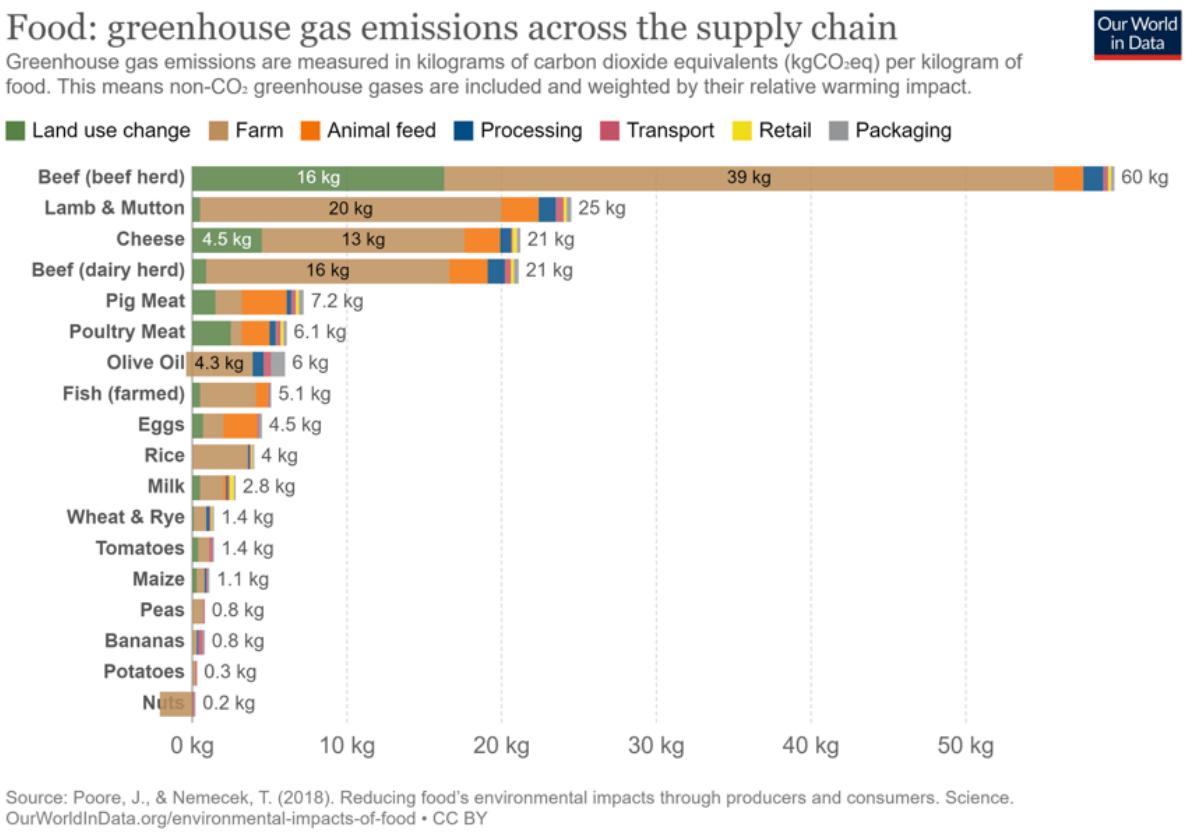


Figure 3. Greenhouse gas emissions per kilogram (kilograms of carbon dioxide equivalents (kgCO_2eq) per kilogram). (Poore, J., and Nemecek, T. 2018; Ritchie and Rosser, 2020).

Breeding companies are able to help improve environmental sustainability as part of the balanced breeding approach as this brings improved performance and improvements in environmental impact. Looking at the biological performance of the BUT6 relative to 2020 (Figure4), since 1977 we can see that for males raised to 140 days the liveweight has increased 42% and is expected to rise by a further 11% by 2030 while at the same time the feed conversion rate (FCR) has decreased and is expected to be at 86% of 2020 level by 2030

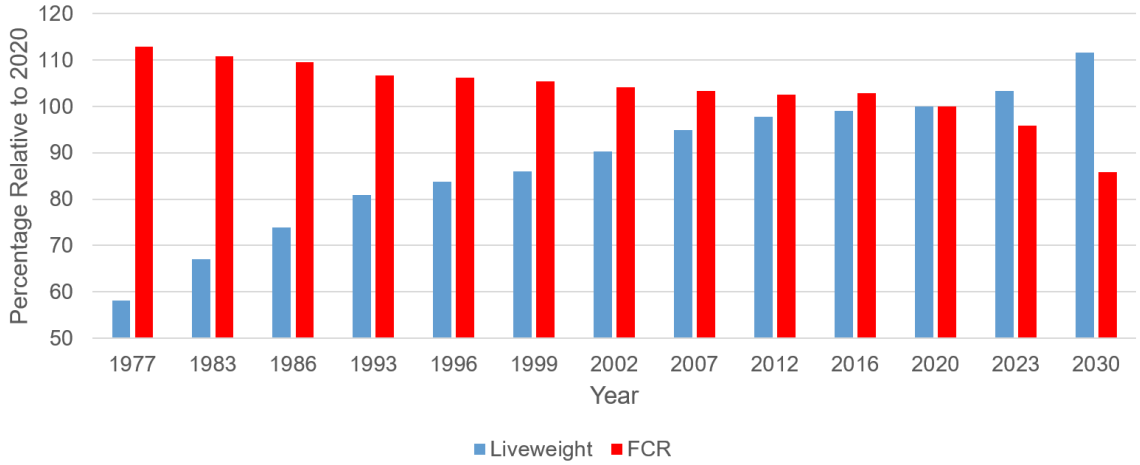


Figure 4. Published BUT6 performance objectives since 1977 and expected future performance as a percentage relative to 2020.

Assessing sustainability improvements arising from genetic progress in turkeys

Calculations of benefits arising from current rates of genetic progress for FCR, weight, water consumption, liveability and processing yields show substantial annual improvements on practical resource usage, contributing significantly to sustainability of the turkey sector (Table 3). These effects are cumulative over time.

Resource	Quantity Saved	Units
Feed	99,000	T
Land use	22,600	Ha
Haulage	7,630	Trips
Water	168,300	m^3
Birds slaughtered	1.24	M

*Table 3. Potential annual impact of genetic progress of the BUT6 on practical resource use by the European turkey industry. Assumptions: Rate of genetic progress applied to the European turkey industry. 2.1MT Carcass wt 2020 (Avec2022). Estimated average liveweight 14.5kg. Arable production of feed at 4.38t/Ha. Haulage capacity 26t from farm to mill, mill to barn. Water use at 1.7*feed.*

Assessment of the environmental impact of genetic progress in turkeys was made using the turkey specific Life Cycle Assessment (LCA) model developed by an industry–academia collaborative research project supported by the UK Technology Strategy Board. This LCA model calculates farm level environmental impacts. The model utilises information from typical UK productions systems and raw materials to calculate the main environmental impacts (including carbon footprint) per kg live weight and carcass weight using input data given by the user (e.g. bird performance, feed composition, energy use) (Leinonen *et al.*, 2016).

The turkey LCA model was used to estimate the environmental impact of historical and projected changes in BUT6 performance. The results show that from 1977 to 2030, there is a reduction of around 0.45% per year in the carbon footprint (Figure 5).

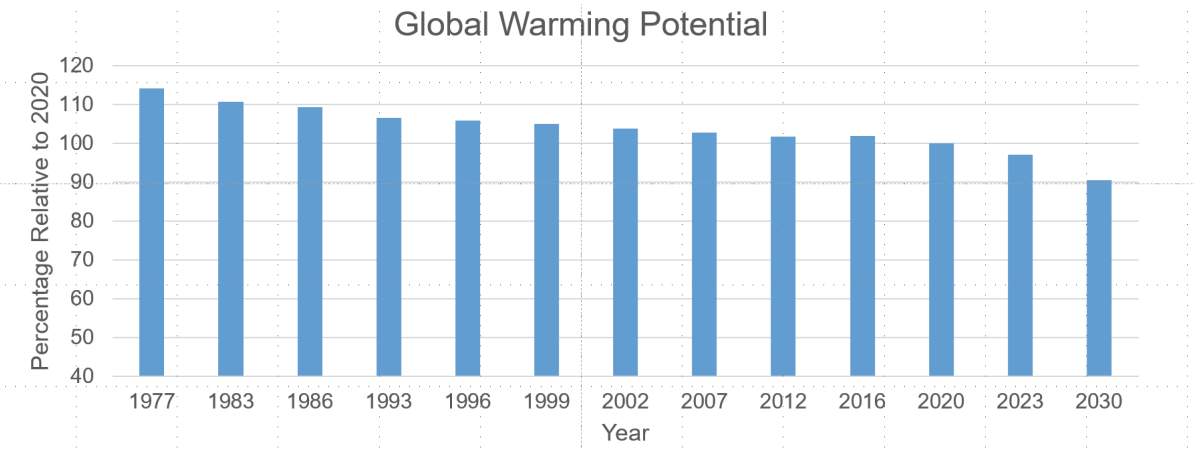


Figure 5. LCA model outputs for greenhouse gases (kg CO_2 equivalent) as a percentage of 2020 performance. The scenario considered was for a 20 week commercial male BUT6 grown to the maximum stocking density under the FAWC recommendations for turkeys, resource inputs per bird (gas, electricity) were estimated from UK field information (Leinonen et al., 2016). Rations and raw materials are typical of that used within the UK industry. For simplicity, an all-in, all-out production system was modelled.

Feed has the greatest impact on the Greenhouse gas emissions accounting for around 60 - 70% of the total carbon. Actions that result in a reduction of feed consumption, while maintaining performance, will result in the lowering emissions. Since FCR is all about feed efficiency, genetic progress of this trait accounts for the majority of the reduction observed in our calculations. With the continued progress on FCR we are expecting the greenhouse gas production in 2030 to be 9% lower than 2020.

Final Considerations

With the ongoing changes that we are seeing in the poultry industry it is important to remain informed. We engage with relevant stakeholders as welfare and sustainability considerations, including legislation, evolve. Our breeding efforts focus on using a broad and balanced breeding approach that contributes to the long-term sustainability of turkey production and improved welfare outcomes.

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Gut-barrier integrity in turkeys in response to different dietary ratios of limiting amino acids and induced inflammation

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Introduction

The concentrations and different proportions of essential amino acids (EAA) in turkeys diets of are established according to the recommendations of either the British United Turkeys (BUT) or the National Research Council (NRC, 1994), which, however, differ with regard to the dietary levels and ratios of important EAA, including lysine (Lys), arginine (Arg) and methionine (Met). For example, the dietary Arg to Lys ratio in turkey diets recommended by the BUT is approximately 2–5% higher than that recommended by the NRC. Similarly, the lowest and the highest values of the Met to Lys ratio recommended by the BUT exceed those recommended by the NRC by around six and three percentage points, respectively. The above differences may appear to be relatively small, but they raise concerns in the turkey production sector due to their potential impact on both feed cost and bird physiology. In practice implications, the most important goal of proper balancing of the dietary concentrations and proportions of EAA is usually the economic aspect, assuming the highest possible weight gain of birds at the same time low feed consumption. However, an increasing number of studies indicates that limiting amino acids play a key role in supporting the function of the gastrointestinal tract and the immune system related to the digestive tract in poultry (Subramaniyan et al., 2019). Although most of these studies concern broiler chickens, it can be assumed however, that due to the high conservation of the processes related to the immune system response in poultry, limiting amino acids also have a similar effect on the immune system of turkeys. In line of the above, this report summarizes the results of experiments investigated the effect of different dietary Arg, Lys and Met levels and their different proportions on performance, gut and immunological parameters in turkeys reared under optimal conditions or exposed to various stressors. We hypothesized that different EAA levels than that recommended by NRC, would be effective in maintaining or improve bird performance by supporting gut integrity and immune function of turkeys, particularly in the challenge conditions.

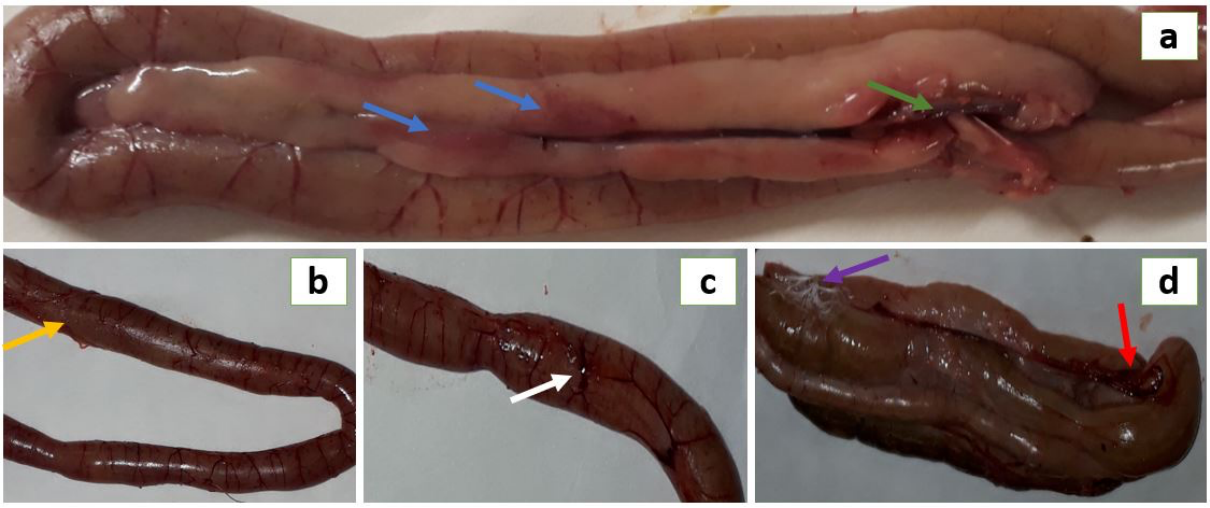
The immune status, oxidative and epigenetic changes in gut tissues as a response to different ratios of Arg and Lys in optimal conditions

Considering different levels of Arg and Lys, our previous reports indicated that in turkey diets with Lys concentration consistent with NRC recommendations, the optimal inclusion rate of Arg was 100% Lys, but Met concentration should be higher than that recommended by the NRC to improve the growth performance and the immune and antioxidant status of turkeys. It was found that in diets with Lys content consistent with NRC recommendations, the inclusion rate of Arg set at 90% Lys compromised the growth performance and the immune and antioxidant status of young turkeys during the entire rearing period (Jankowski et al., 2020; Ognik et al., 2021a). More to that, diets with high Arg content (110% Lys) contributed to an increase in the proportion of breast muscles in the final BW of turkeys and did not induce the oxidation of lipids, proteins or DNA, but promoted undesirable protein nitration and an increase in thyroxine levels (Ognik et al., 2021b; Jankowski et al., 2021; Konieczka et al., 2021). The obtained results are mostly in line with other reports, in which was indicated that the growth performance of turkeys is affected by the dietary inclusion levels and ratios of Arg and Lys whereas, the Arg:Lys ratio higher than 1:1 may improve performance results (Veldkamp et al., 2000; Oso et al., 2017).

Different dietary ratios of Lys and Arg in diets with high or low Met levels on the gene expression of tight junction proteins in *Clostridium perfringens* - challenged turkeys

In turkeys, necrotic enteritis (NE) is caused by *Clostridium perfringens* (*C. perfringens*) anaerobic bacteria and their toxins, and it causes considerable economic losses in poultry farming (Wade and Keyburn, 2015). NE manifests by

strong inflammation and necrosis of the intestinal mucosa, which impairs nutrient absorption, weakens immune and antioxidant systems and consequently, compromises growth performance and increases bird mortality (Hardy et al., 2020). Although intestinal barrier and intestinal permeability are important for health and disease, the mucosal barrier and its role in enteric disease are still poorly understood in turkeys. Therefore, the molecular basis of differential responses to infections caused by certain microorganisms in the turkey gut should be elucidated because they are critical for the birds' health. To the best of our knowledge, the dietary inclusion levels of limiting amino acids (Lys, Arg and Met) and their optimal ratios in the diets of young turkeys challenged with *C. perfringens* have not been reported in the literature to date. Therefore, we hypothesized that the optimal concentrations and ratios of Lys, Arg and Met in turkey diets can limit oxidation and methylation of biologically important molecules and improve gut barrier function (Ognik et al., 2020). To investigate birds' response to EAA and infection, our laboratory developed a model for studying mild NE (a sub-clinical form of infection that does not cause lethal effects) in turkeys using *C. perfringens* bacteria. This is of particular importance because it is the sub-clinical form of *C. perfringens* infection that causes the most economic losses and, more importantly, it is difficult to detect, and therefore may be a potential vector for transferring pathogens and/or their toxins into the food chain - hence poses a serious threat to consumers (Olkowski et al., 2006). The induction of sub-clinical symptoms of NE depends on many factors (including the content of protein and non-starch polysaccharides in the diet, zoohygienic conditions in the room), but above all on the occurrence of coccidiosis. Since there are no registered vaccines on the European market (based on attenuated coccidia found in turkeys - mainly *E. gallopavonis*), using field strains in combination with *C. perfringens* bacteria can lead to lethal effects.



Phot. 1. Representative photographs illustrating changes in different intestinal segments of turkeys challenged orally with *C. perfringens*. The typical responses of the host included: A - multifocal, minimal to mild hyperemia in the duodenum (blue arrow), and advanced necrosis in connective tissue (green arrow) in the duodenum; B - lumpy coating reminiscent of a pseudomembrane (yellow arrow) in the jejunum; C - manifested swollen vessels on the jejunum surface (white arrow); D - multifocal hyperemia and hemorrhages (red arrow) and fibrinous coating on the surface of the caeca (purple arrow) (Ognik et al., 2020).

In this experiment, we observed moderate necrotic lesions in different segments of the gut after *C. perfringens* administration (Phot. 1). This finding confirmed that necrotic model consisting in *C. perfringens* administration in three periods preceded by coccidia challenge was successful at inducing gut inflammation, without causing bird death. Thus, it provides a good model for studying the specific interactions between diet and host response under challenge conditions. In the present study, the expression of occludin, ZO-1, GLP2, OGG1 and TFF2 genes increased in the wall of the small intestine, which indicates that *C. perfringens* infection in turkeys compromised intestinal barrier integrity. The expression of the above genes increased in birds fed diets with both low and high Met content, equivalent to 30% and 45% of Lys content, respectively. A review report by Awad et al. (2017) indicates that *C. perfringens* endotoxin increases intercellular permeability and disrupts intestinal mucosal barrier function in infected chickens, which is in line with our results. We concluded from this experiment that in turkeys infected with *C. perfringens*, fed diets with high Lys content, Arg content should be decreased to 90% Lys and Met content should be increased to 45% Lys. The above dietary amino acid ratios contribute to suppressing oxidative processes and epigenetic alterations in important molecules in the wall of the ileum and in the blood, and maintaining intestinal barrier integrity. Even though the analyzed amino acid ratios interacted with the systems responsible for the maintenance of gut integrity in the host organism, this dietary intervention probably enabled birds to cope with NE (Ognik et al., 2020).

Response in different challenge conditions

Intestinal health is critical for maximizing growth performance and production efficiency in turkeys. When gut homeostasis is disrupted by pathogens, nutrient digestion and absorption are altered since priorities are shifted from maintaining regular physiological processes to fighting off the pathogens (Korver, 2006). EAA, Arg, Lys and Met, play a key role in supporting gastrointestinal function and the gut-associated immune system. Experiments performed on chickens demonstrated that increased dietary levels of EAAs stimulated local immunity (Zulkifli et al., 2016), and contributed to reducing intestinal mucosa atrophy and maintaining intestinal microbiota diversity under both optimal and stress conditions (Dong et al., 2017). However, the majority of studies investigating the regulatory role of EAAs involved animal models other than turkeys. Due to considerable differences in metabolism and EAA requirements resulting from different growth rates, data cannot be directly extrapolated from other poultry species to turkeys. Therefore, in the next experiment we investigated effect of two levels of dietary Arg, Lys and Met (high or low) and challenge with *Clostridium perfringens*, *Escherichia coli* lipopolysaccharide (LPS) or no challenge (placebo). We evidenced that increased dietary levels of Arg, Lys and Met had a beneficial effect on turkey performance and immunological parameters, and it improved selected indicators responsible for maintaining gut integrity in different challenge conditions. Under optimal conditions (with no challenge), high ArgLysMet diets maintained bird performance and they improved selected performance parameters in challenged birds. The immune system of turkeys was not excessively stimulated by high ArgLysMet diets, which did not disrupt the redox balance and had no negative effect on gut integrity. High ArgLysMet diets increased the expression levels of selected genes encoding nutrient transporters and tight junction proteins. We found also that neither high nor low ArgLysMet diets significantly affected serum FITC-d (marker of gut permeability) concentrations in turkeys. Serum FITC-d concentrations were significantly higher in birds infected with *C. perfringens* than in uninfected birds, whereas the administration of LPS did not induce significant differences relative to the control group (Figure 1). However, the influence exerted by different dietary inclusion levels of Arg, Lys and Met on gut integrity was largely interactive and determined by the stressor (*C. perfringens* vs. LPS) (Konieczka et al., 2022a). Further studies are required to investigate the role of Arg, Lys and Met levels in the diet on the immune response, gut function and performance of turkeys in different challenge conditions.

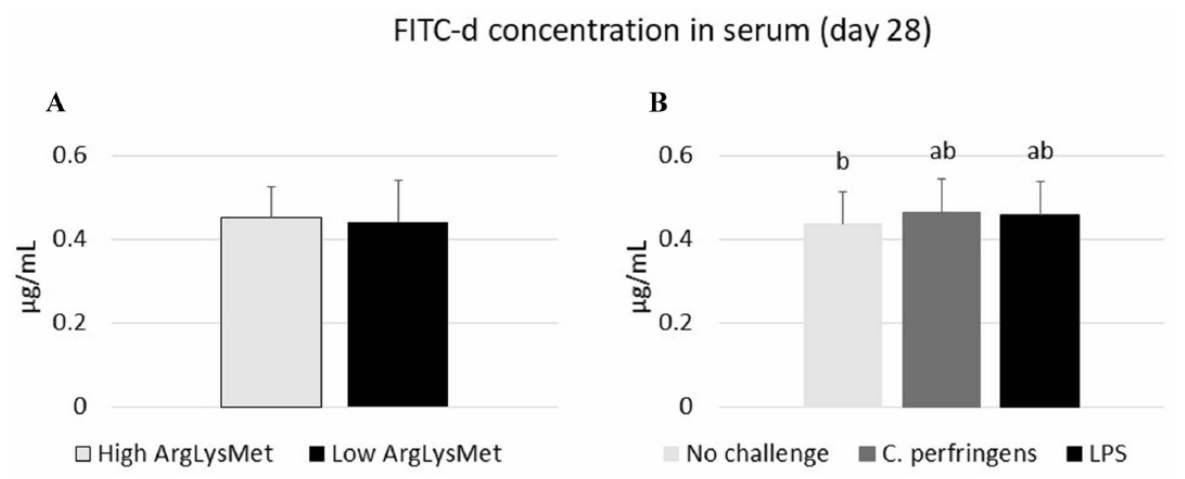


Figure 1 Serum FITC-d concentrations in turkeys fed high or low dietary arginine, lysine and methionine levels (A) and as a result of either *C. perfringens* infection, *E. coli* LPS challenge or no challenge (B). [a,b]Means within a row with different superscripts differ significantly ($P < 0.05$).

Konieczka et al. (2022a)

Gut barrier condition vs. meat quality indices

In other experiment (Konieczka et al., 2021), we showed that the increased level of Arg and Lys in the diet of birds significantly reduced the concentration of putrefactive short-chain fatty acids, ammonia and lowered the pH while increasing the concentration of short-chain fatty acids, including butyric acid (beneficial effect on the intestinal mucosa Figure 2; Konieczka et al., 2020) in the contents of the cecum. The above results indicate a beneficial effect of increased levels of Arg and Lys in the diet on the activity of bacterial microflora in the turkeys' digestive tract.

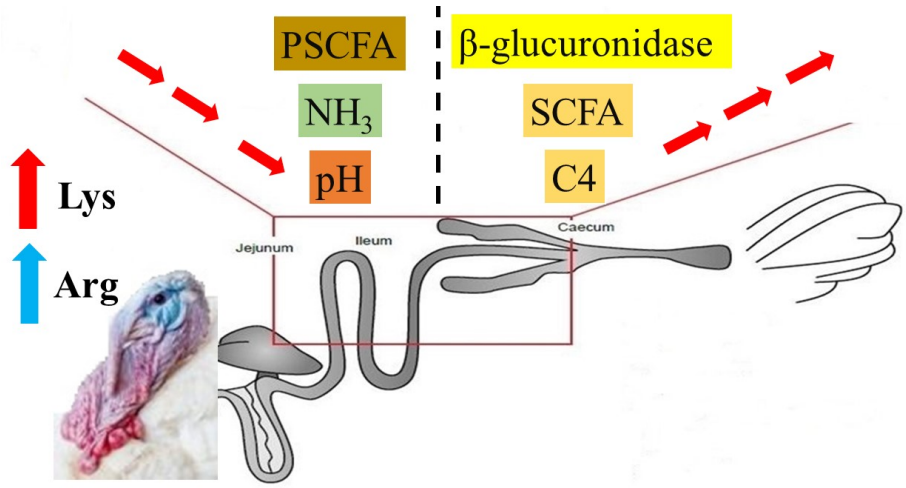


Figure 2. Beneficial effect of increased (compared to NRC, 1994) levels of Arg and Lys in the diet on the functional state of the digestive tract (Konieczka et al., 2021).

In another experiment (Konieczka et al., 2022b), we investigated if the varied levels and proportions of Arg to Lys in low- and high-Met can influence the sarcoplasmic protein profile of breast muscles from turkeys reared under optimal or challenge (*Clostridium perfringens* infection). Because, we previously evidenced that feeding different levels and ratios of EAA may affect significantly the transcripts levels not only of tight junction proteins genes but also selected genes encoding nutrient transporters are engaged in processes related to the maintenance of homeostasis in whole biological system (Castro and Kim, 2020), we speculated that by targeting them, it may be an effective intervention to manage the initial stage of inflammation in the host, which could in turn affect metabolic processes contributing to meat features associated with quality in turkeys. Because the metabolism of key elements, including peptides, fatty acids, and amino acids in the host depends on gut barrier functions to a high extend (Bortoluzzi et al., 2018), we assumed that different ratio of essential amino acids, would be effective in supporting gut integrity and thereby shall affect meat features in challenge conditions (Konieczka et al., 2022b). The results of this experiment showed a significant effect of all studied factors (infection by *C. perfringens* as well as dietary Arg and Met levels) on the sarcoplasmic protein profile. However, the lack of abovementioned effects of the tested factors on pH or color brightness indicates that despite the different enzymatic profiles of individual groups, no significant differences in the extent of postslaughter glycolysis or postmortem changes were found in muscle tissue. However, the results of many other studies have shown evidence that some glycolytic enzymes are complex multifunctional proteins with unexpected functional roles, such as transcription regulation (HK, LDH, GAPDH, and ENO), apoptosis (HK and GAPDH) and cell motility (GPI). However, the most pronounced effect of both infection and EAA manipulation was manifested in the principal components analysis, in which was shown that the most distinguished groups were the one infected and fed different than recommended EAA levels.

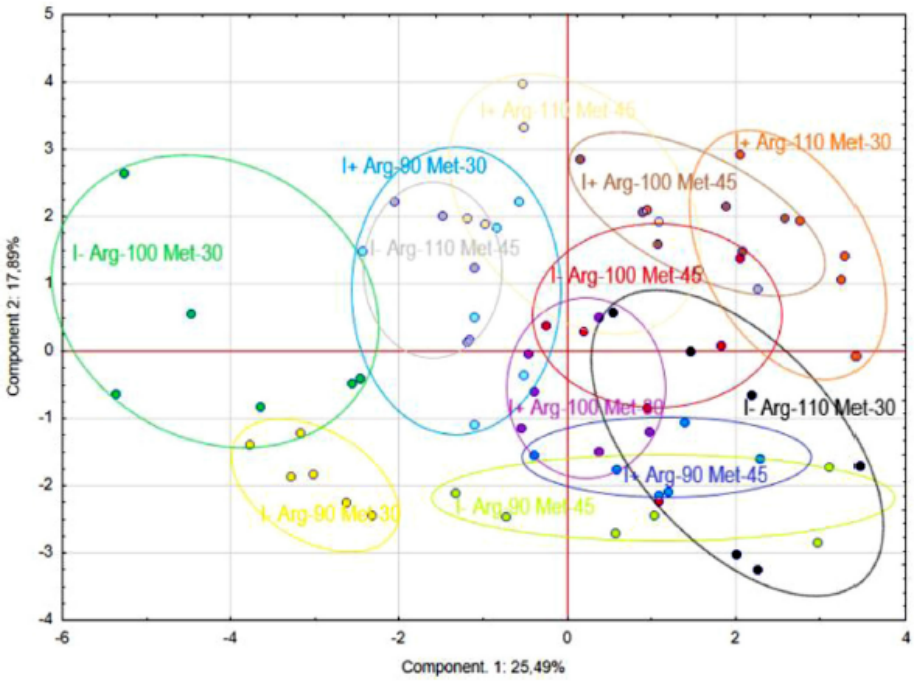


Figure 3. Results of principal component analysis (PCA) and projection of the cases on the PC plane. Explanation: I+ C. perfringens infection; I- no C. perfringens infection, three levels of Arg (90%, 100%, and 110%) relative to the content of dietary Met (30% or 45%) (Konieczka et al., 2022b).

In conclusion, experiments run in our laboratory have shown the existence of many relationships (including interactions) between the level and ratio of Arg, Lys and Met in the diet and the processes determining intestinal integrity in turkeys. The obtained results indicate a beneficial effect of elevated levels of these amino acids on physiological processes in growing turkeys. The particular potential of using an increased level of limiting amino acids in the diet of turkeys resulted from the fact that they did not cause overactivity of the immune system in birds, which usually results in the compromised performance. However, it can be concluded that the beneficial effect of the increased content of Arg, Lys and Met in the diet on the functional state of the gastrointestinal tract and the general health status of birds justifies the development of detailed nutritional recommendations for turkeys, taking into account not only performance results, but also health benefits. Since the turkey sector has grown rapidly in the past decade, a further understanding of the EAA needs of birds is essential to achieving high growth rates and maintaining a profitable and sustainable production.

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Feeding strategies for commercial turkeys: responding to marketplace volatility

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Introduction

Choosing the most effective feeding programme has never been more important given the volatility of the raw material market and lack of consistent supply of materials. The current raw material market appears to be more settled however, given recent events, the future is far from predictable.

In such situations, it is critical that nutritionists can make informed decisions in order to choose the most efficient feeding programme for different circumstances. This must also be considered within the context of an ever changing growing environment where the turkey's response to a given feeding regime is influenced by enteric challenges, increased removal of antibiotics and, in some cases, removal of coccidiostats.

Biological response to nutrient density

Identifying the most efficient feeding programme starts with an understanding of the birds response to nutrient density. Energy and protein are the two main contributors to diet cost and therefore are the main nutrients to consider from an economic point of view.

Aviagen Turkeys Ltd, in collaboration with their research partners, Moorgut Kartzfehn GmbH, conducted a series of trials examining the impact of various energy and amino acid densities on heavy strain male turkey performance. Each of these trials assessed different nutrient levels, Table 1 shows the amino acid and energy levels assessed, nutrient levels are expressed as a percentage of a commercial standard.

Table 1: A summary of energy and amino acid response trials.

Trial Number	Age	Treatments (% of standard)		
	Weeks	Days	Amino Acids	Energy
1	21	147	90, 120	95, 105
2	20	140	90, 100, 110, 120	100
3	21	144	90, 105	97.5, 102.5
4	20	140	90, 100, 100, 120	100

A summary of the 'commercial standard' feeding programme and nutrient levels are shown in table 2.

Table 2: Summary commercial standard (100%) nutrient levels.

		Period						
	Unit	P1	P2	P3	P4	P5	P6	P7
Age Fed	weeks	0 - 3	3 - 6	7 - 10	11 - 13	14 - 15	16 - 18	18 - 20
Digestible Lysine	%	1.59	1.46	1.32	1.14	1.09	1.00	0.91
Energy	MJ/kg	11.5	11.8	12.2	12.5	12.6	13.0	13.2
Energy	Kcals/kg	2749	2820	2916	2988	3011	3107	3155

Performance data from these trials were collated to assess the response of turkeys to a range of nutrient densities. The surface plot graph (Fig.1a,b) shows liveweight and feed conversion ratio (FCR) responses to altering nutrient density.

Both liveweight and FCR are responsive (expressed as percentage relative to the 100% control) to both amino acid and energy density, both liveweight and FCR responses are optimised at the highest nutrient densities. The surface plot graphs also shows that energy density appears to impact positively on the response to increasing amino acid concentration suggesting that diet energy density should be considered when feeding higher amino acid densities.

Figure 1a,b: 20.5 week liveweight and FCR responses to a range of amino acid and energy densities.

Fig 1a: Liveweight (kg) at 20.5 weeks

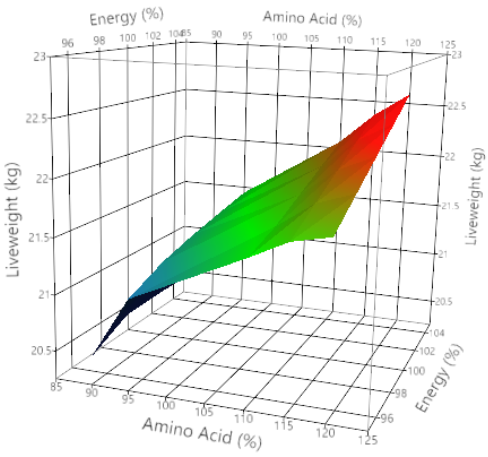
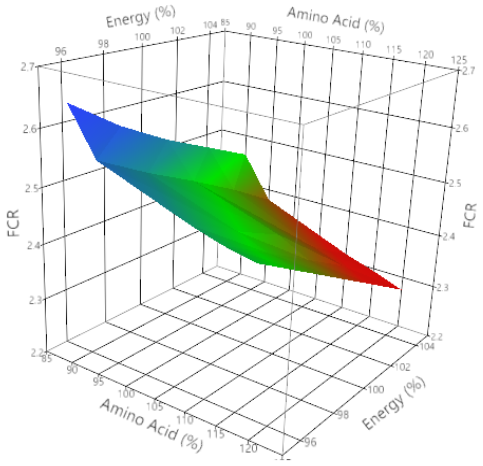
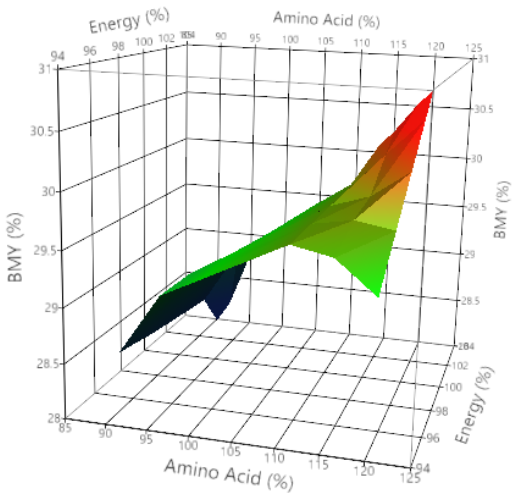


Fig 1b: FCR at 20.5 weeks



Processing traits were also assessed, as with liveweight and FCR responses, increasing nutrient density had a positive impact on breast meat yield (see figure 2). At lower energy densities breast meat yield (BMY) showed a curvilinear response to increasing amino acid density. However, BMY continued to respond to increasing amino acid concentration at higher energy densities, again, this reinforces the importance of considering both nutrients particularly when optimising processing yield.

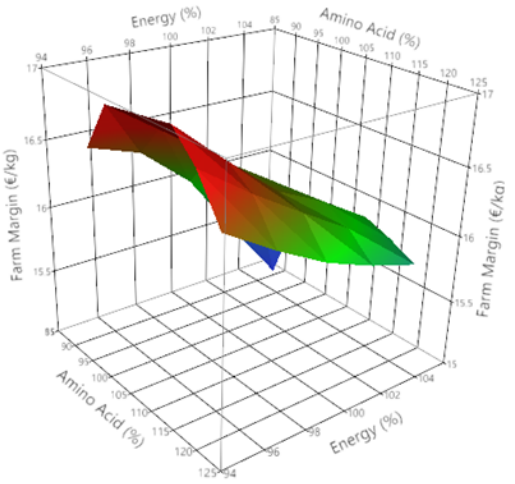
Figure 2: BUT6 20.5 week breast meat yield response to a range of amino acid and energy densities (% relative to standard).



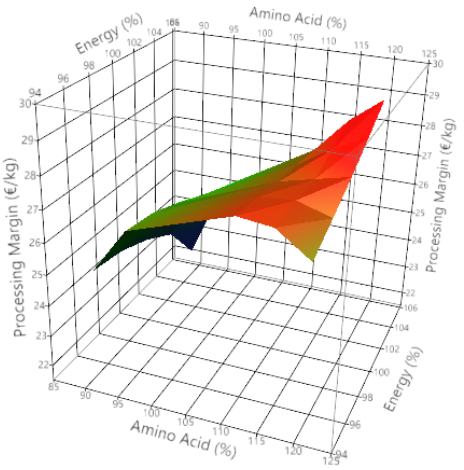
Having established performance responses to different nutrient densities, economic responses can also be derived. Margin (after feed cost) is calculated by estimating the difference between revenue (value of live bird or meat) and feed cost at each nutrient density. By examining the profile of the surface plot graph (figure 3a) the effect of nutrient density on farm margin can be established. The same approach can be taken to derive an estimate of margin for processed products, margin was estimated at each nutrient density (figure 3b) based on revenue (the value of breast meat yield) minus feed cost per bird.

Figure 3a,b: Farm and processing margin (revenue minus feed cost Euro/bird) at differing amino acid and energy densities (% relative to the commercial standard).

Farm Margin[1] (Euro/bird) after feed cost



Processing Margin[2] (Euro/bird) after feed cost



[1]revenue based on Euro 1.71/kg liveweight

[2]revenue based on Euro 7.45/kg breast meat

Optimal farm margin is achieved at an amino acid density above the standard (105% of standard) and at the lowest energy density (95% of standard). Energy density has a much greater influence on margin than amino acid density and reflects the impact of the recent increase in the cost of dietary energy. The lowest farm margin was at the highest energy (105%) and lowest amino acid concentration (90%). Based on existing raw material costs, higher margin is achieved at higher amino acid density and lower energy density than the existing standard. Higher energy densities may achieve higher biological performance however this is outweighed by increased feed cost per bird.

Contrary to farm margin optimal processing margin was achieved at both the highest amino acid and energy density. This reflects the response of breast meat yield to both nutrients and the higher revenue associated with processed products relative to liveweight. Increasing amino acid density without a concomitant increase in energy results in lower margin, lowest margin was at the lower amino acid densities.

In summary, based on current feed prices, optimal farm margin is achieved at lower energy levels and higher amino acid densities relative to the commercial standard. Optimal processing is achieved at higher energy and amino acid levels relative to the commercial standard.

These assessments were conducted at current day raw material prices, as discussed previously raw material costs have fluctuated considerably, the change in raw material prices since 2020 to date is considerable (see figure 4).

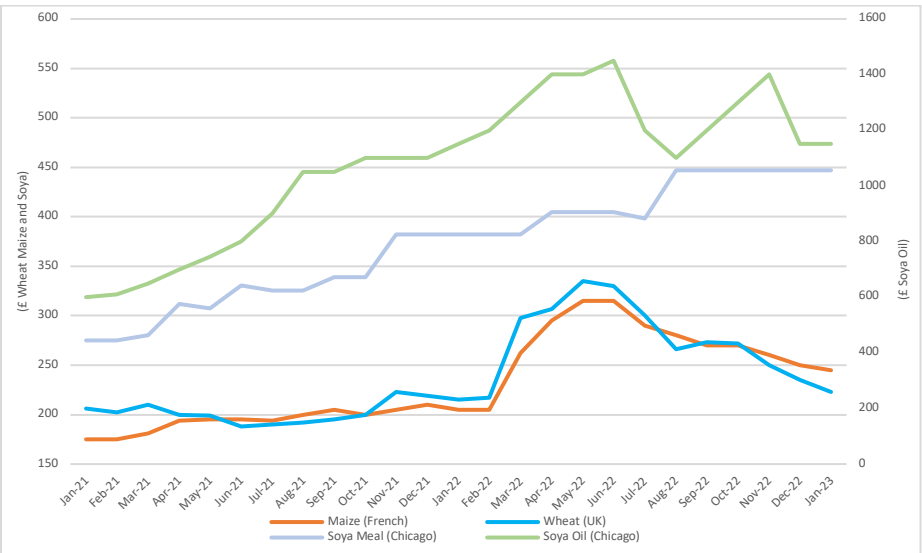
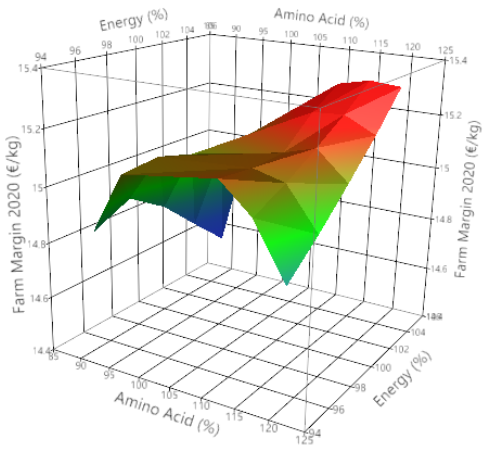


Figure 4: Raw material prices (£/MT): January ’21 to January ‘23

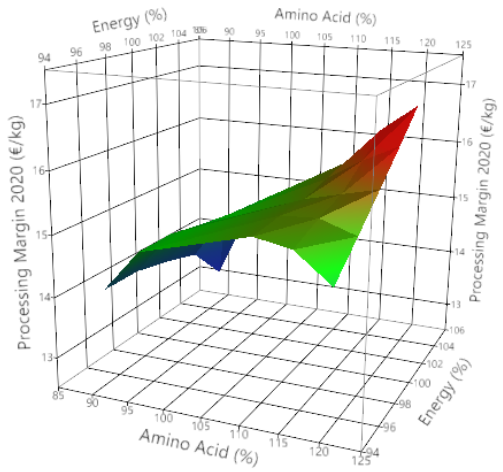
Feed prices were reduced to reflect Jan 2021 raw material costs, farm and processing margin were estimated based on these lower costs (fig.5a,b). Liveweight and processing revenues were adjusted to reflect 2021 levels.

Figure 5a,b: The impact of reduced raw material cost on farm and processing margin at differing amino acid and energy densities.

Farm Margin[3] (Euro/bird) after feed cost



Processing Margin[4] (Euro/bird) after feed cost



[1]revenue based on Euro 1.25/kg liveweight

[2]revenue based on Euro 4.25/kg breast meat

Based on the 2020 raw material cost base highest margin was achieved at the highest energy levels (see figures 5a,b), this is the opposite outcome to the assessment based on the 2023 raw material cost base. Feeding a higher amino acid density relative to the commercial standard achieved higher margin, regardless of energy density. Processing margin based on the 2020 raw material cost base resulted in a very similar trend to margin based on the 2023 raw material cost base which suggests that processing margin is very resilient to raw material price change.

In summary, the data demonstrates the ability of the modern bird to respond to a wide range of nutrient levels. Liveweight, FCR and breast meat yield are responsive to nutrient density and show optimal responses on or above the commercial standard. Optimal economic performance is realised at different nutrient densities and are dependent on the objectives of the business, for example farm or processing margin. Feed prices have a significant impact on the optimal diet nutrient density and highlights the importance of nutritionists reviewing feeding programmes especially during periods of volatile raw material prices.

Methionine Sources in Turkeys – an Update

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Abstract

In this review, scientific data on the relative bioefficacy value (RBV) of methionine sources in turkeys will be presented and discussed. A compilation of scientific information on RBV of methionine sources in turkeys was done and based on 15 available data sets from studies conducted since 1981. An average RBV of 76 % (weight gain), and 68 % (feed conversion) for liquid methionine hydroxy analogue (MHA-FA) compared to DL-methionine (DL-Met) in turkey was found. Despite these values being slightly higher, a RBV of 65% on a weight-to-weight basis is recommended for liquid MHA-FA in turkey nutrition which is confirmed by a recent opinion of the European Food Safety Authority for monogastric animals. Applicability of this recommendation is justified and validated by several challenge-feeding trials with turkeys where DL-Met and liquid MHA-FA were supplemented in a respective 65:100 ratio in corresponding treatments. While this recommendation was always confirmed, it is evident that especially at marginal dietary Met+Cys levels, which represent more sensitive test conditions, liquid MHA-FA could be replaced by DL-Met in turkey diets without compromising performance. Even the contrary, results indicated extra benefits regarding improved breast meat yield, antioxidant capacity of liver and foot pad health with DL-Met.

Introduction

Commercial poultry diets are routinely supplemented with methionine or its precursors to precisely meet their sulphur amino acid (**SAA**) specifications. Methionine (**Met**) is an essential amino acid for poultry and serves as a building block for protein synthesis, being also a precursor for cysteine (**Cys**) and important methyl donor (Selle *et al*., 2020). Most of the supplemental Met commercially available is supplied as crystalline DL-methionine (**DL-Met**, 99% content) or as liquid DL-2-hydroxy-4-methylthio butanoic acid (methionine hydroxy analogue-free acid, **MHA-FA**; 88% content) (Willke, 2014). Both products provide Met activity to poultry, but chemically MHA-FA is not an amino acid due to the replacement of the characteristic amino group by a hydroxy group (Yang *et al.*, 2020). In addition to the chemical differences, there are studies indicating slower and less efficient absorption of liquid MHA-FA due to differences in transport systems in intestinal brush border membrane and potential catabolism by enteric bacteria (Maenz and Engele-Schaan 1996, Drew *et al.*, 2003). Therefore, understanding the nutritional value indicated by the relative bioefficacy value (**RBV**) of liquid MHA-FA compared to DL-Met is an important precondition to cost-effective purchasing, feed formulation, and optimum animal production (Sauer *et al*., 2008).

A relative bioefficacy of 65% for liquid MHA-FA is recommended for turkeys

A proper evaluation of potential differences in the bioefficacy of two products is strongly dependent on an adequate trial design. In such experiments, the treatments comprise a basal diet deficient in Met+Cys and a set of diets with incremental Met+Cys levels. The effect of the incremental Met supplementation on performance criteria describes the dose-response relationship which is not linear but follows the law of diminishing returns. The first unit of supplemental Met results in a relatively strong effect, i. e. significantly increases the performance. This effect diminishes progressively with each additional amount of Met supplementation. Eventually an optimum supply status is reached where no further increase in performance can be achieved. This curve can accurately be described by an exponential regression equation (Rodehutscord and Pack, 1999). The more data points describe the non-linear first section of the response curve, the more robust is the conclusion. The methodology of simultaneous exponential regression as proposed by Littell *et al.* (1997) assumes that both nutrient sources under test allow for the same asymptote of the response curves. Although the experimental setup to determine the RBV has been a matter of discussion and different asymptotes have been proposed by Gonzales-Esquerra *et al.* (2007), the meta-analysis performed by Sauer *et al.*

(2008) including 46 dose-response experiments with broilers, as well as a more recent broiler trial (Lemme *et al.*, 2020), provided evidence that the asymptotes are the same for liquid MHA-FA and DL-Met.

The European Food Safety Authority (EFSA), which examines and assesses dossiers for product registration in the European Union, released a scientific opinion on liquid MHA-FA and its calcium salt in 2018 (Rychen et al., 2018). This opinion concludes a lower RBV for MHA-products for non-ruminant animals and fish and suggests an RBV of 75% for MHA-products compared to DL-Met on equimolar basis which is equivalent to 66% on product-to-product basis. This is in line with a recent meta-analysis by Lemme et al. (2020) concluding that RBV of MHA-FA was 62%. In contrast to broilers, less scientific dose-response data on turkeys is available. While a couple of studies were published back in the 1980's, Hoehler et al. (2005), Gonzales-Esquerra et al. (2007) and Batonon-Alavo et al. (2022) conducted trials to determine the RBV of MHA-FA relative to DL-Met in turkeys using the simultaneous dose response approach more recently **(Table 1).** If only these studies would be considered, average RBV for liquid MHA-FA would be 75% and 66% for body weight gain and feed conversion ratio, respectively, and are therefore slightly lower than the overall average. Indeed, particularly the RBV for feed conversion ratio would be in line with the conclusion by EFSA (2018) indicating a RBV of 66% for monogastric animals on product basis. Therefore, like recommendations for broilers, laying hens, swine and aqua species (Lemme *et al*., 2012; Htoo and Rademacher, 2012; Lemme, 2010) an RBV of 65% for liquid MHA-FA compared to DL-Met is recommended for turkey nutrition.

Table 1. Relative bioefficacy of liquid MHA-FA compared to DL-met (%) in turkeys from fifteen available data sets[1]

			Relative Bioefficacy	
Author	Institute	published	Weight gain	Feed conversion ratio
Schmidt	Virginia Polytech. Ins. & Univ., USA	Master Thesis, 1981	75	63
Schmidt	Virginia Polytech. Ins. & Univ. , USA	Master Thesis, 1981	64	67
Blair	Virginia Polytech. Ins. & Univ. , USA	Master Thesis, 1983	80	72
Blair	Virginia Polytech. Ins. & Univ. , USA	Master Thesis, 1983	105	84
Noll et al.	Univ. Minnesota, USA	Poultry Science 63:2458-2470, 1984	98	84
Noll et al.	Univ. Minnesota, USA	Poultry Science 63:2458-2470, 1984	76	48
Noll et al.	Univ. Minnesota, USA	Poultry Science 63:2458-2470, 1984	89	81
Harms	Univ. Florida, USA	Carolina Nutr. Conference, 1987	63	64
Potter et al.	Virginia Polytech. Ins. & Univ. , USA	Trial report, 1987	59	80
Potter et al.	Virginia Polytech. Ins. & Univ. , USA	Trial report, 1987	56	55
Hoehler et al.	Akey Inc., Lewisburg, USA	Journal of Appl. Poultr. Res 14:296-305, 2005	66	44
Hoehler et al.	Michigan State Univ. , USA	Journal of Appl. Poultr. Res 14:296-305, 2005	68	56
Gonzales-Esquerra et al.	Grupo Techn. Agroindustrial, Mex	Poultry Science 86:517-527, 2007	81	66
Gonzales-Esquerra et al.	Grupo Techn. Agroindustrial, Mex	Poultry Science 86:517-527, 2007	87	95
Batonon-Alavo et. al	Missouri Contract Poultry Research, USA	26th World's Poultry Congress, ID:2191, 2022	76	66

[1] updated table from Lemme et al. (2012)

The following example is given to demonstrate an appropriate experimental design, as well as the proper mathematical model which should be used for data analysis and proper interpretation. In a feeding trial from day 7 to 50 with female Hybrid Converter turkeys, the effects of DL-met (99 % pure), a diluted DL-Met (65%) which was made by blending DL-Met (99%) with glucose, and liquid MHA-FA (88%) on performance were compared (Hoehler *et al.*, 2005).

Corn-soy diets were supplemented with graded levels of the products (0.07, 0.14, 0.21, 0.28%). In this trial, a RBV of 65% was assumed *a priori* for diluted DL-Met (65%) relative to pure DL-Met (99%). Thus, these treatments could be regarded as an internal standard to check the validity of simultaneous regression analysis as suggested and validated by Lemme *et al.* (2020). With 3 out of 4 inclusion levels within the curve-linear part of the curve, the weight gain response of turkeys to graded levels of DL-Met, diluted DL-Met (65%), and liquid MHA-FA described a nonlinear trend and, therefore, data were suitable for simultaneous exponential regression according to procedure proposed by Littell *et al.* (1997) (**Figure 1)**. The determined RBV for diluted DL-Met (65%) confirmed expectations although it was slightly lower than expected 65% which is due to biological variation of data. RBV of both diluted DL-Met (65%) and liquid MHA-FA were significantly lower than 88%.

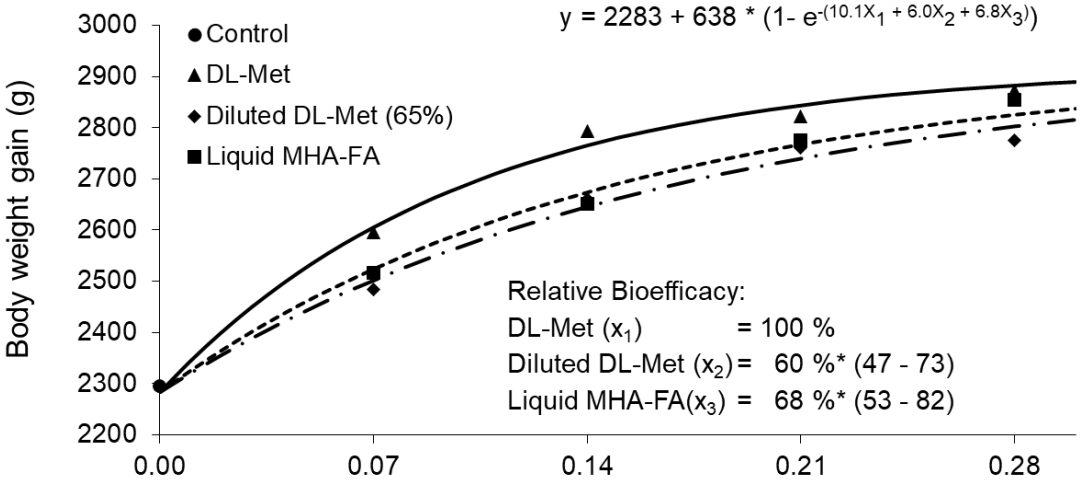
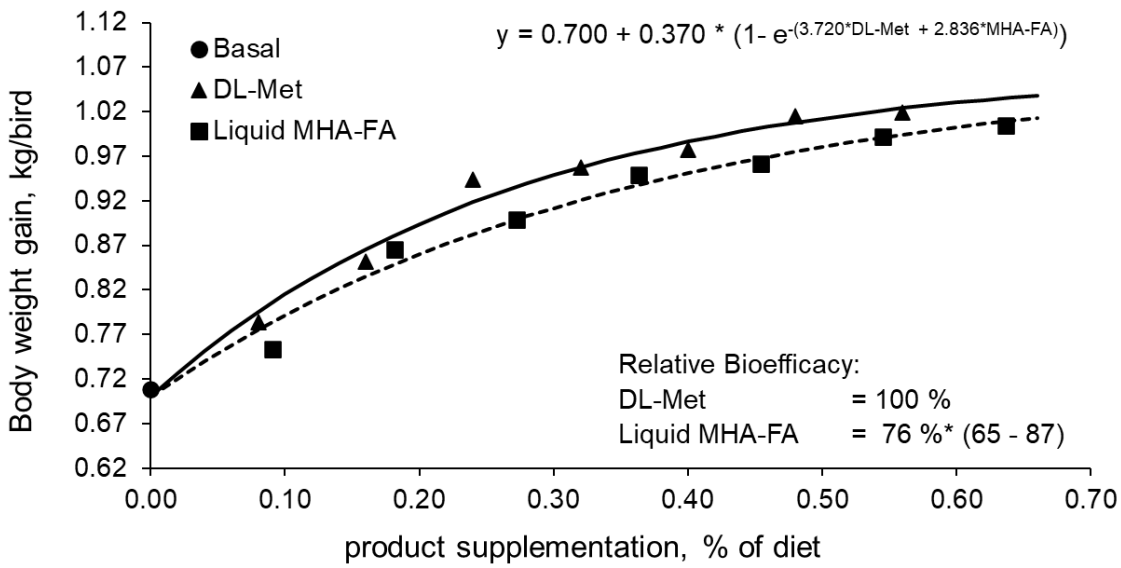


Figure 1. Weight gain of female Hybrid turkeys 7 to 50 days of age fed incremental levels of DL-Met, diluted DL-Met (65%), or the liquid hydroxy analogue of Met (MHA-FA; 88%). Values in brackets indicate the 95% confidence interval. *Significantly less than 88% ($P < 0.05$).

A recent publication by Batonon-Alavo *et al.* (2022) proposed Single and Two Slope broken line regression to determine Met+Cys requirements of turkeys with DL-Met and liquid MHA-FA from 0 to 28 days. The authors concluded there is no difference in requirement regarding Met sources. While data confirm that both products can be used to meet the Met+Cys requirement of turkeys, the required supplementation to achieve the maximum response is different between the sources. The data by Batonon-Alavo *et al.* (2022) were re-analyzed by simultaneous exponential regression. The supplemental levels of DL-Met were calculated as the difference between the dietary SAA in the supplemented feeds and the SAA in the basal diet (0.700 % Met+Cys). For liquid MHA-FA, equimolar inclusion levels were assumed as suggested by the authors (88%). The results of the re-evaluation can be obtained from **Figure 2**.



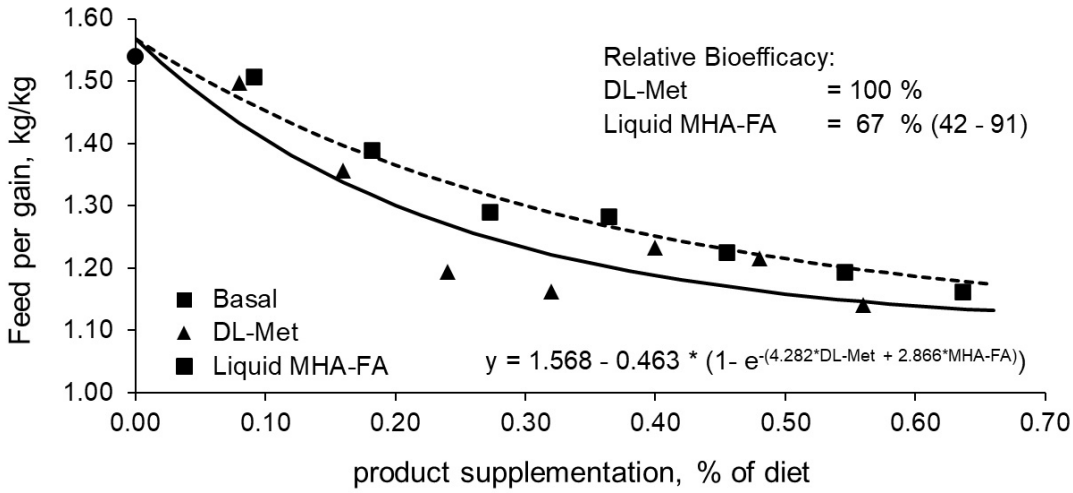


Figure 2. Re-evaluation of data reported by Batonon-Alavo *et al.* (2022) using simultaneous exponential regression on weight gain and feed conversion of Hybrid Converter poults fed increasing doses of either DL-Met (99%) or MHA-FA (88%) from 0 to 28 days of age. Values in brackets indicate the 95% confidence interval. *Significantly less than 88% (P < 0.05).

These results are in line with overall averages shown in **Table 1**. However, in contrast to the conclusion by Batonon-Alavo *et al.* (2022) that "requirements are similar for both MHA-FA and DL-Met", it is clear in the simultaneous exponential regression that the maximum gain or minimum FCR is achieved by lower levels of DL-Met compared to MHA-FA. A few years earlier Gonzales-Esquerra *et al.* (2007) reported two simultaneous dose-response trials with 1–3-week-old turkeys and particularly the first dose-response trial suggested responses similar to Batonon-Alavo *et al.* (2022).

The validation of the recommendation on relative bioefficacy

The recommended RBV of 65% for liquid MHA-FA as compared with DL-Met (on product basis) was challenged and validated in feeding trials. Male 1-d-old B.U.T. Big 6 turkeys were evaluated in a 21 day feeding trial where dietary treatments comprised a basal diet, which was deficient in Met+Cys, and six diets with three graded levels of liquid MHA-FA (0.154, 0.308, and 0.462%) or DL-Met (0.100, 0.200, and 0.300%; Hoehler *et al.*, 2005). The ratio between both products at each of the three corresponding inclusion levels was 65%, assuming that 100 units of liquid MHA-FA can be replaced by 65 units DL-Met without compromising performance. The summary of the results is presented in **Table 2**. Accordingly, performance in corresponding treatments (2 vs 5, 3 vs 6, and 4 vs 7) did not differ, thus, confirming and validating the recommendation.

Table 2. Effect of graded levels of DL-Met and the liquid hydroxy analogue of Met (MHA-FA; 88%) on weight gain and feed conversion in male B.U.T. Big 6 turkey poults from 1 to 21 d of age, trial 2 (Hoehler *et al.*, 2005)

Treatment	Met Source	Addition of Met source (% of product)	Weight gain ± SD	Feed per gain ± SD
1	Basal	---	481 ± 33[c]	1.547 ± 0.042[a]
2	DL-Met	0.100	520 ± 21[bc]	1.490 ± 0.057[ab]
3	DL-Met	0.200	550 ± 32[ab]	1.458 ± 0.033[b]
4	DL-Met	0.300	567 ± 29[a]	1.462 ± 0.075[ab]
5	Liquid MHA-FA	0.154	525 ± 31[abc]	1.496 ± 0.071[ab]
6	Liquid MHA-FA	0.308	550 ± 12[ab]	1.476 ± 0.034[ab]
7	Liquid MHA-FA	0.462	564 ± 21[ab]	1.469 ± 0.035[ab]

[a–c]Means with no common superscript within a column indicate significant differences (P < 0.05).

Lemme and Meyer (2009) conducted a trial with B.U.T. Big 6 turkey toms in a 6-phase feeding program. Also in this study, DL- Met or MHA-FA were supplied in a 65:100 ratio to either meet adequate Met+Cys levels in turkey diets or to be below requirement by supplementing half of the dose of both products. Dietary Met +Cys levels (normal vs

half) resulted in a significant final body weight difference indicating that at half dosage Met+Cys supply was limiting performance (**Table 3)** making the 65:100 test more sensitive at lower supply. Supplementation of the products in a 65 (DL-Met) : 100 (MHA-FA) ratio revealed similar body weight, mortality and carcass traits. However, there was a trend for better feed conversion ratio which may confirm a basically lower RBV observed in the literature review (**Table 1**).

Table 3. Growth and slaughter performance of male B.U.T. Big 6 turkeys toms fed with adequate and low Met+Cys levels and supplemented with either DL-Methionine (DL-Met) or liquid methionine hydroxy analogue free acid (MHA-FA) at a ratio of 65:100 after 21 weeks of life (Lemme and Meyer, 2009)

Met+Cys level	Normal[1]		Half (50%)			Statistics	
Met source	DL-Met	MHA-FA	DL-Met	MHA-FA	P-value	P-value	P-value
Treatment	1	2	3	4	(level)	(source)	L x S
Final body weight (kg)	21.42	21.68	20.86	20.94	**0.01**	0.430	0.679
FCR (g/g)	2.613	2.635	2.598	2.618	0.13	*0.054*	0.925
Mortality (%)	7.13	9.74	7.3	8.12	0.61	0.264	0.568
Carcass yield (% LW)	71.56	72.27	69.05	69.5	0.022	0.424	0.915
Breast meat (% CW)	42.85	42.56	41.2	42.43	0.334	0.381	0.184

LW = Live weight, CW = carcass weight, FCR= feed conversion ratio
[1]Met+Cys levels were 10.8, 9.9, 9.0, 8.0, 7.3, and 6.7 g/kg feed in phases 1 to 6.

Agostini *et al.* (2017) conducted a study with similar design. Male B.U.T. Big 6 turkeys were used. Four dietary treatments comprised two products (DL-Met, liquid MHA-FA) and two dietary Met+Cys levels. Sub-optimal digestible Met+Cys levels were around 87% of the optimal levels which were set at 9.9, 9.0, 8.5, 7.4, 6.6, and 5.8 g/kg feed in phases 1 to 6. The amount of DL-Met added in both Met dose groups was 65% of that of added MHA-FA (on product basis). Again, growth rate of turkeys fed the sub-optimal Met+Cys diets was significantly lower confirming that sub-optimal Met+Cys levels limited performance although no difference in feed conversion was observed (**Table 4**). In corresponding treatments neither a difference in body weight nor in feed conversion ratio was observed. In contrast, especially at low Met+Cys supply, breast meat yield was higher with DL-Met than with liquid MHA-FA.

Table 4. Growth and slaughter performance of male B.U.T., Big 6 turkeys fed diets with adequate and marginal Met+Cys levels and supplemented with either DL-Methionine (DL-Met) or liquid methionine hydroxy analogue free acid (MHA-FA) at a ratio of 65:100 after 21 weeks of life (Agostini *et al.*, 2017)

Table 4. Growth and slaughter performance of male B.U.T., Big 6 turkeys fed diets with adequate and marginal Met+Cys levels and supplemented with either DL-Methionine (DL-Met) or liquid methionine hydroxy analogue free acid (MHA-FA) at a ratio of 65:100 after 21 weeks of life (Agostini *et al.*, 2017)

Met+Cys level	Normal[1]		Low			statistics	
Met source	DL-Met	MHA-FA	DL-Met	MHA-FA	P-value	P-value	P-value
Treatment	1	2	3	4	(level)	(source)	L x S
Body weight (kg)	20.025	19.956	19.259	19.609	**0.03**	0.55	0.37
Feed intake(kg)	53.136	52.853	51.675	52.499	0.12	0.63	0.33
FCR (g/g)	2.665	2.659	2.658	2.69	0.63	0.68	0.52
Mortality (%)	5.9	8.9	7.1	8.5	0.84	0.26	0.67
Slaughter yield (% LW)	77.8	75	77.2	78.1	0.51	0.69	0.40
Breast meat (% CW)	37.2	37.1	36.5	35.7	**<0.001**	**0.02**	0.12
Litter score (wk8)	5.6	5.8	5.1	5.1	0.19	0.72	0.83

LW = live weight, CW = carcass weight, FCR = feed conversion ratio
[1]Met+Cys levels were 9.9, 9.0, 8.5, 7.4, 6.6, and 5.8 g/kg feed in phases 1 to 6.

More recently, Lingens *et al.* (2021) evaluated the effects of DL-Met and liquid MHA-FA in adequate and low protein diets on performance parameters, footpad health, liver health and oxidative stress. 63 day-old female turkeys (B.U.T. Big 6) were randomly assigned to four groups fed with diets differing in methionine source (DL-Met (65%) vs. liquid MHA-FA (100%)) and crude protein content (15% vs. 18%) for 35 days. The results showed no interactions between

the dietary crude protein and Met sources. Strong protein reduction significantly impaired water intake, feed intake, weight gain and feed conversion ratio, but improved footpad health. There was a trend for higher final body weight and weight gain, respectively with DL-Met, which was due to significantly higher feed consumption. However, feed conversion ratio was not affected by products at either dietary protein level. DL-Met resulted in a significant increase in the liver's antioxidative capacity compared to liquid MHA-FA. Although the protein reduction resulted in impaired performance, the study showed that MHA-FA can be replaced by DL-Met in a 100:65 weight ratio without compromising performance and additional benefit of DL-met to improve the antioxidative capacity of the liver.

Table 5. Performance of female turkeys fed experimental diets with different dietary protein content and using either DL-Methionine (DL-Met) or liquid methionine hydroxyl analogue-free acid (MHA-FA) from day 63 to 98 days (Lingens *et al.*, 2021)

Crude Protein (CP) level	18%CP		15%CP		Statistics		
Met source	DL-Met	MHA-FA	DL-Met	MHA-FA	P-value	P-value	P-value
Treatment	1	2	3	4	(protein)	(source)	P x S
Final body weight (kg)	9.06	8.85	8.56	8.27	**<0.05**	*0.09*	0.80
Body weight gain	4.991	4.857	4.505	4.255	**<0.05**	*0.08*	0.59
Feed intake(kg)	13.006	12.589	12.532	11.868	**<0.05**	**<0.05**	0.53
Water intake (kg)	26.464	25.428	23.862	22.623	**<0.05**	**<0.05**	0.83
Water : Feed intake	2.04	2.02	1.9	1.91	**<0.05**	0.81	0.71
FCR (g/g)	2.61	2.6	2.78	2.79	**<0.05**	0.97	0.72
TAC (umol UAE/g protein)	1.080	1.000	1.020	0.930	0.11	**<0.05**	0.86

TAC = total antioxidant capacity of liver, FCR = feed conversion ratio

Additionally, Lingens *et al.* (2021) observed a trend for lower footpad dermatitis (**FPD**) scores for DL-Met compared to MHA-FA fed turkeys at d 98. This result confirms earlier findings by Abd El-Wahab *et al.* (2014), who found that young turkeys fed higher levels of methionine (2 or 3g/kg diet) supplemented with DL-Met led to significantly lower FPD scores compared to those same levels in MHA-FA supplemented diets (4.54 vs. 5.04 and 4.12 vs. 5.19, $p<0.05$). The level of dietary Met plays an important role for health of skin of foot pad. Already in 1974 Chavez and Kratzer (cited in Kamphues *et al*., 2011) reported higher frequency and score of FPD in turkeys fed liquid MHA-FA compared to turkeys fed DL-Met. Thus, it seems that Met has a structural function regarding foot pad health via protein synthesis and continuous production of keratin. Consequently, low availability of Met could affect protein synthesis negatively and affect skin of foot pad.

Table 6. Comparison of responses to liquid MHA-FA and DL-Met[1] on body weight gain or feed conversion ratio at adequate or marginal dietary Met+Cys

Parameter	Body weight gain				Feed conversion ratio			
Met+Cys supply	adequate		marginal		adequate		marginal	
Met source	MHA-FA	DL-Met	MHA-FA	DL-Met	MHA-FA	DL-Met	MHA-FA	DL-Met
	%	%	%	%	%	%	%	%
Hoehler *et al.*, 2005 (1)[2]	100.0	99.8	100.0	98.7	100.0	103.2	100.0	99.2
Hoehler *et al.*, 2005 (2)[3]	100.0	100.5	100.0	99.0	100.0	99.5	100.0	99.6
Hoehler and Hooge, 2003 (1)[4]	100.0	98.5	100.0	100.0				
Hoehler and Hooge, 2003 (2)[4]	100.0	100.6	100.0	102.2				
Lemme and Meyer, 2009	100.0	98.8	100.0	99.6	100.0	99.2	100.0	99.2
Agostini *et al.*, 2017	100.0	100.3	100.0	98.2	100.0	100.2	100.0	98.8
Lingens *et al.*, 2021[5]	100.0	102.4	100.0	103.5	100.0	100.4	100.0	99.6
Average	**100.0**	**99.8**	**100.0**	**100.2**	**100.0**	**100.5**	**100.0**	**99.3**

[1]DL-Met responses relative to MHA-FA responses, MHA-FA = 100% [2] adequate and marginal represented by highest and lowest supplementation of liquid MHA-FA or diluted DL-Met [3] adequate and marginal are represented by highest and lowest supplementation level [4] FCR was not reported [5] no Met+Cys reduction but crude protein reduction.

Table 6 summarizes the results of seven turkey feeding trials where DL-Met and liquid MHA-FA were supplemented at a 65:100 ratio at either adequate or marginal Met+Cys supply. These trials comprise experiments conducted in well controlled pen facilities as well as under commercial production conditions (Hoehler and Hooge, 2003). These commercial trials used a total of 54,906 turkeys and it should be noted that MHA-FA fed birds at adequate Met+Cys supply were slaughtered one day later which would explain the differences in final body weight. This compilation in **Table 6** serves as strong validation for the recommended RBV of 65% for liquid MHA-FA relative to DL-Met. Accordingly, it is applicable at any dietary Met+Cys level. This appears to be in contrast with Jankowski et al. (2017) who supplemented equimolar levels of liquid MHA-FA or DL-Met at Met+Cys at NRC (1994) recommendations, which were considered low, and at about 40% higher dietary Met+Cys level. At both dietary Met+Cys levels, there were no performance differences between both products. There were indeed small but significant differences (10.8 vs 11.0 kg final body weight; 2.40 vs 2.37 kg/kg FCR) between dietary Met+Cys levels; however, respective recommendations by the breeder (Hendrix Genetic Company, 2014) suggested final body weights of 10.8 kg which indicates that Met+Cys supply was not strongly limiting performance. Indeed, such pairwise comparison is particularly sensitive at marginal Met+Cys supply and results in **Table 6** are therefore strong evidences.

Conclusions

A relative effectiveness of 65% of liquid MHA-FA relative to DL-Methionine on a weight basis (1kg liquid MHA-FA to 0.65 kg DL-Met) is recommended. A number of recently published validation studies not only provide the evidence that 100 units of MHA-FA can be substituted by 65 units of DL-Met at any dietary Met+Cys supply level or at different protein levels without affecting bird's performance, but that DL-Met also provides additional benefits regarding improved breast meat yield and foot pad health.

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The importance of a robust microbiome in Turkeys

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Abstract

The microbiome is a term describing the collection of trillions of microorganisms, including bacteria, inside humans and animals. It plays a key role in the operation of the human and animal body. Although humans have been using good bacteria in their diets for a very long time (e.g., sour cabbage, vegetables or yogurt), we've only just started to understand that good bacteria can have a positive impact on some diseases like Alzheimer's, autism, and depression.

What is easily overlooked is that bacteria are an integral part of an animal (including humans) and are often viewed as a separate organ, the forgotten organ. A human or animal body exists of more bacteria than specie specific cells. Making the microbiome very important, as we have observed that it has a significant impact on animal health. The relationship between microbiome composition robustness and the growth of animals and their resistance to pathogens is something we learn more and more about every day.

We are beginning to understand a lot more about the effects the microbiome has on its host, be it human, or animal. Diseases of the gut are an obvious one, whilst others are much less so, such as how Short Chain Fatty Acids produced by some bacteria can affect behavior via the gut-brain axis. These SCFAs (Short Chain Fatty Acids) have been shown to regulate appetite, the immune system and even lessen stress under harsh conditions. However, what is of most interest to the animal nutrition and feed industry is that we now have plenty of experimental data showing that good bacteria can increase the feed digestibility of protein and carbohydrates. And, therefore, have an impact on better feed efficiency.

One example of the importance of a robust microbiome, is a study we call the big birds, small birds' study. In this study, we followed a couple of hundred broiler chickens. We ended up with some birds only weighing 1.5 kg, while others were more than twice as heavy but from the same flock. We sequenced the microbiome of the 25 smallest and 25 heaviest birds. The microbiomes were markedly different between the two groups, with the big birds having significantly richer alpha diversity, meaning the diversity of bacteria within each gut. More importantly, we also found that the microbiome of the big birds was significantly more uniform; they looked alike. The smaller birds' microbiomes were all over the place. This is in line with the Anna Karenina principle for microbiomes stating that "All healthy microbiomes are similar; each dysbiotic microbiome is dysbiotic in its own way." It seems that healthy or robust microbiomes look the same under certain conditions, and having unstable microbiomes indicates a fragile and potentially lower-producing microbiome.

Gut health directly impacts a bird's overall health, welfare, and performance. So, all the above can be translated into production profitability. To summarize: the animal's performance and production economics are partially dependent on the microbiome of animals. That is why we want the microbiome to be robust and able to respond quickly to coming challenges. Challenges like chronic stress, heat stress, pathogens like salmonella or clostridia, antibiotic therapy, or nutritional factors. With nutritional factors, we can understand the diet change and the quality of the raw materials used.

A robust microbiome is critical in maintaining birds' resilience to these challenges.

Optimising the use of fats and oils in turkey diets

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Introduction

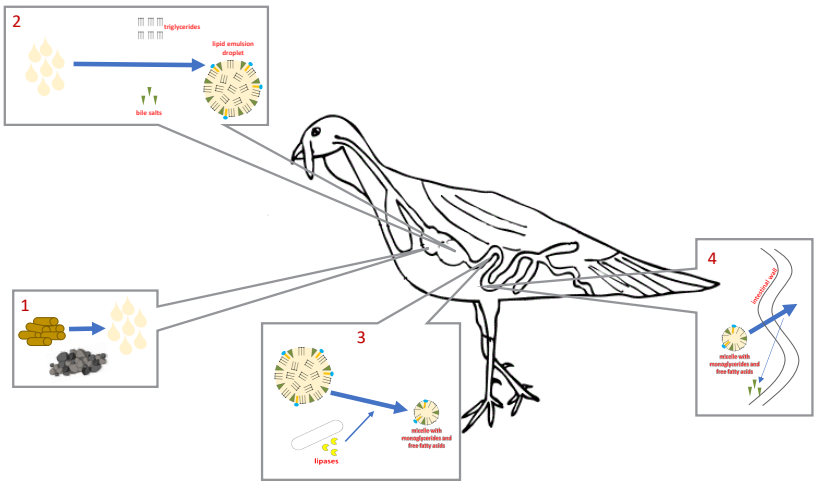
Fats and oils are the major energy source in a compound feed, with fibre digestion releasing only limited amounts of energy (Noblet and Shi, 1993). Increasing dietary fat content tends to increase growth rates and efficiency (Plavnik et al., 1997) and slows feed passage rate, allowing more time for digestion and absorption of other nutrients. However, there are practical limits to fat inclusion in commercial diets, as increasing fat levels are associated with reduced pellet hardness and durability (Thomas et al., 1998), as well opportunities for oxidation and rancidity, wet litter and footpad dermatitis (Youssef et al., 2011). Some authors have suggested that poor digestion of fats in young poults can also contribute to skeletal disorders through mineral imbalances resulting from soap formation in the feed and digesta, though this has not been conclusively proven (Leeson and Atteh, 1995). Economic considerations can also limit the inclusion of fats and oils to a ration, especially considering the volatile and high prices of feed grade oils seen in recent years.

Therefore, it is important to optimise the use of dietary lipids – obtain the best growth performance whilst maintaining economic sustainability. Whilst this topic has been well reviewed in broilers and pigs (Wealleans et al., 2021; Ravindran et al., 2016), there is comparatively limited information available about the factors influencing fat digestibility and absorption in turkeys. Therefore, this paper aims to present an examination of lipid digestion in the turkey and opportunities to optimise the utilization of fats and oils in turkey diets.

Fat Digestion And Absorption In Turkeys

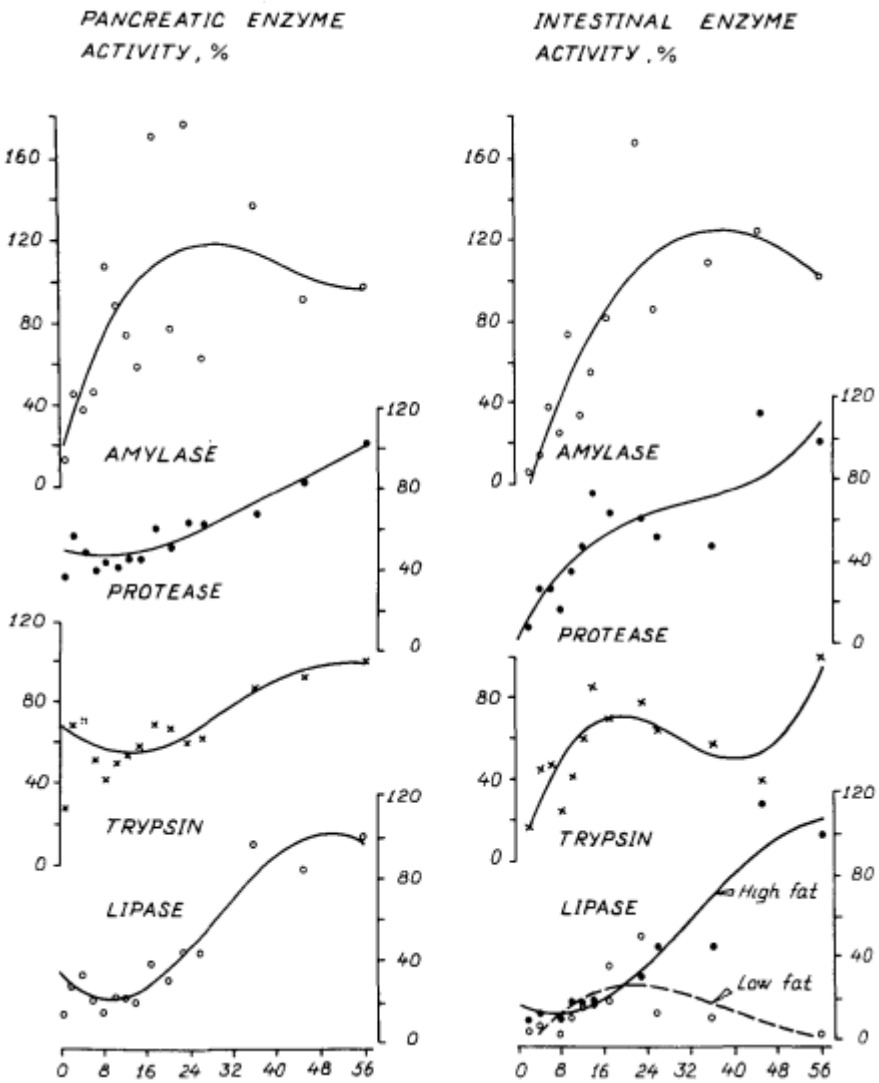
Fat digestion and absorption in animals and birds consists of three stages: emulsification, hydrolysis and absorption. The process of fat digestion and absorption in poultry was extensively reviewed by Ravindran et al. (2016), and is summarised in Figure 2.

Figure 2. Simplified schematic overview of the three crucial steps in lipid digestion in the turkey: emulsification, hydrolysis and absorption. 1) As feed enters the gizzard, primary grinding alongside other feed particles frees fat from the feed matrix. 2) On entry into the proventriculus and gizzard, fat mixes with free triglycerides, bile salts and fat-soluble nutrients, creating emulsion droplets. 3) In the duodenum, lipases hydrolyse triglycerides into monoglycerides, and mixed micelles form. These mixed micelles are composed of triglycerides, free fatty acids and fat-soluble nutrients. 4) Mixed micelles are absorbed across the intestinal wall, and bile salts are released back to the gall bladder.



In brief, after consumption, feed containing both added fats and those native to other feed materials enters the gizzard, where it is ground and vigorously mixed. The process of fat digestion begins later in birds than mammals, where the grinding action of teeth and the secretion of salivary lipase begin the digestive process directly upon consumption (Wealleans et al., 2021a). Anti-peristaltic movements ensure that digesta does not pass through the gizzard and the small intestine without sufficient mixing (Duke et al., 1972; Dziuk and Duke, 1972). As digesta passes into the duodenum it is mixed with bile, which contains pigments, salts, native phospholipids, cholesterol, electroltyes and proteins (Krogdahl, 1985). Hurwitz et al. (1979) reported that 60% of fat is hydrolysed and absorbed in the duodenum of turkeys, and that absorption rate was increased with the degree of unsaturation. Changing dietary fat content has limited effects on turkeys' intestinal and caecal pH (Zubair et al., 1996), but can affect the secretion of endogenous lipases: Krogdahl and Sell (1989) reported the concentration of digestive enzymes including lipase in the pancreas and digesta of turkeys, as shown in Figure 3. Excreted fat is present both in regular and caecal droppings, though is resent in greater quantities in caecal droppings (Zubair et al., 1996).

Figure 3. Development of digestive enzyme concentrations in the pancreas and digesta of turkey poults up to 56 days.



Nutritional Value Of Fats And Oils In Turkey Diets

The nutritional value of a fat or oil is species and age dependent, and varies with a number of chemical factors relating to the lipid itself. Developed for broilers and pigs, the Wiseman equations estimate apparent metabolizable energy (AME) based on the saturation level (U/S ratio) of the fatty acids with the lipid and the content of free fatty acids (FFA) (Wiseman et al., 1998). Average values for Wiseman values, energy-diluting factors such as moisture, impurities and unsaponifiables (MIU), and measurements of oxidative quality for common oils used in turkey diets are shown in Table 1.

Table 1. Average values for fatty acid profile, PV, TBA, MIU, FFA and U/S measurements for oils commonly used in turkey diets[1]

	Oxidative Quality			Energy Related Characteristics			Fatty Acid Characteristics		
Oil type	TBA ppm	PV meq/kg	OSI H	MIU %	FFA %	U*/S	SFA %	MUFA %	PUFA %
Corn	0.24	2.58	19.16	2.54	11.94	5.06	16.63	32.21	51.16
Linseed	0.14	8.65	15.55	1.03	1.02	7.77	13.04	23.48	63.48
Palm	0.29	5.81	29.18	0.51	3.52	0.95	51.56	39.75	8.69
Rapeseed	0.27	8.23	17.78	1.10	0.59	14.31	6.80	67.35	25.85
Soybean	0.19	6.10	12.79	0.81	0.60	4.78	17.42	25.86	56.72
Sunflower	0.13	15.45	8.96	0.90	0.69	8.24	10.87	30.73	58.40

U/S: Ratio of unsaturated and saturated fatty acids with consideration of FA with a short carbon chain length (i.e. ≤ C14:0), are regarded as unsaturated compounds irrespective of their degree of saturation*

[1]Adapted from Wealleans et al. (2020b)

When thinking in particular about the value of fats and oils for turkey production, it is important to remember that the Wiseman equations were not developed for turkeys, and it is likely that there will be differences in availability of energy by fat source between species. Mossab et al. (2000) and Lall and Slinger (1973) reported that turkeys were more able to utilise saturated fatty acids and less sensitive to lipid U/S ratio than chickens. Murugesan et al. (2019) compared the available energy content of ten oil sources in broilers, laying hens and turkeys; comparative results are shown in Table 2. This work suggests that, at the same chronological age, turkeys derive less nutritional benefit from dietary lipids than chickens, though the extent of the difference varies with lipid source.

Table 2. N corrected AME value of ten common lipid source (kcal/kg) for broilers and turkeys[1].

		AMEn		
	Gross energy	**Broilers, 21d**	**Turkeys, 21d**	**% difference between species**
Choice white grease	9401	6045	5998	0.78
Corn oil	9405	10226	7444	27.21
Poultry fat	9407	7829	5995	23.43
Soybean oil	9541	8123	7288	10.28
Distillers corn oil 1	9464	7803	7803	0.00
Distillers corn oil 2	9565	9871	5805	41.19
Methyl soyate esters	9512	7977	7333	8.07
Animal-vegetable blend 1	9392	8094	6007	25.78
Animal-vegetable blend 2	8985	7482	5547	25.86
Animal-vegetable blend 3	8997	9154	8421	8.01

[1]Adapted from Murugesan et al. (2019)

It is likely that lower nutritional values for lipids in turkeys versus chickens of the same age is related to relative physiological maturity and gastrointestinal development, though this is contrast to the findings of Mossab et al. (2000), who saw increased saturated fat digestibility in turkeys compared to broilers at 1 week old. Initial studies into the digestibility of fats and oils by poults saw significant increases between weeks 1 and 8 of age: for example, Whitehead and Fisher (1975) reported increased utilisation of tallow from 54% at 2 weeks to 74% at 8 weeks, though no such increase was seen for lard or corn oil. Other studies have also reported conflicting accounts on the effect of poult age and sex on fat digestibility (Schulte et al., 2012; Mossab et al., 2000; Sell et al., 1986; Halloran and Sibbald, 1979; Salmon, 1977).

The fat sources assessed by Murugesan et al. (2019) had relatively similar gross energy contents (8985-9565, CV=2.12%), while there was a large variation in observed AMEn (5547-8421, CV=14.81%). Schulte et al. (2012) also reported large differences in digestibility between fat sources, noting a detrimental effect of fat blends containing high levels of FFA, in line with the prediction of the Wiseman equation.

Further complicating the assessment of the nutritional value of fats and oils is their incorporation into compound feed. The production of compound feed often includes the application heat and pressure during the pelleting process, both of which can be a trigger for oxidation (Yang et al., 2020). As part of ongoing monitoring activities at Kemin Animal Nutrition and Health, turkey feed samples collected from commercial mills and integrations across Europe were analysed for indicators of oxidative quality, as shown in Table 3. These values are in line with the average oxidative parameters reported for commercially obtained oils (Table 1), suggesting oxidative status of turkey feed is largely driven by the oxidative status of the oils it contains.

Table 3. Assessment of lipid oxidative quality in commercial turkey feeds.

	Min	Average	Max
Extracted peroxide value (meq/kg)	3.00	8.64	16.8
TBA value (mg/kg)	0.80	1.28	2.68

Improving Performance With Lipid Absorption Enhancers

Compared to many species, there is a paucity of information on the use of products to improve fat digestibility in turkeys.

When fat digestibility is improved, the amount of fat in the diet required to maintain performance can be decreased. A trial to demonstrate this principle was conducted, with two treatments: a positive control, formulated to standard nutrient requirements; and a negative control (energy reduced in phases 3-6) supplemented with a LPL-based additive containing LPL, monoglycerides and a synthetic emulsifier (Lysoforte® Extend, LEX). Each treatment was replicated with four pens, and 71 male BUT 6 birds/pen. The bodyweight of 20 birds/pen was measured at the end of each feeding phase and of all birds on day 145. Feed consumption (in kg per pen) and feed conversion (FCR) was measured weekly. Table 4 reports the effect of treatment on performance parameters. Bodyweights in the LEX group at day 145 were numerically lower than those in the control group (-300 g/bird, 1.5%), though the difference was not statistically significant (P=0.1498). However, FCR was substantially lower in LEX treated birds (2.82 control vs 2.72 LEX; P=0.0269). At processing, 23 birds per treatment were randomly assessed for carcass characteristics. There were few differences between treatments, with LEX-supplemented birds recording 29.32% breast meat yield compared to 29.2% in control birds.

Table 4. Effect of LEX supplementation on growing male turkey performance

	Control	LEX	P-value
Weight P1 (random sample, kg)	0.378	0.364	-
Weight P3 (random sample, kg)	1.36	1.35	-
Weight P4 (random sample, kg)	4.63	4.41	-
Weight P5 (random sample, kg)	9.71	9.69	-
Live weight, kg, 145 days	19.76	19.46	0.1498
Live weight corr., kg, 145 days	19.93	19.62	0.1508
SD	1.24	1.34	-
CV %	1.53	1.78	-
Daily gain, kg, d0-145	0.136	0.134	0.1498
FCR, d0-145	2.82	2.72	0.0269
Feed conversion ratio corrected	2.66	2.65	0.9599
Mortality in pen, %	5.99	4.93	0.6871
Birds in sick pen	7.04	1.06	0.0335

Furthermore, to investigate the interaction between increasing fibre and increasing fat digestibility, a study was conducted, using a 2 x 2 factorial arrangement, to compare the effects of LEX and xylanase supplementation on growth performance in turkeys. BUT 6 mixed-sex turkeys (4 per pen and 12 replicates per treatment) were assigned to four treatments: (1) negative control diet (NC), (2) NC diet with LEX (500 g/T), (3) NC diet with xylanase at a concentration of 45,000 U/kg (15 g/t Xygest™ HT) and (4) NC diet supplemented with LEX (500 g/T) and xylanase (45,000 U/kg). Water and diets based on wheat, soybean meal, full-fat soybeans and corn were available ad libitum. Body weight and feed intake were measured weekly from 0 to 35 days, and body weight gain and feed conversion ratio were calculated. No interactions were observed for any of the performance parameters. LEX improved ($p<0.05$) the final average body weight of turkeys by almost 100 g compared to the control (1629g vs 1530g). Feed intake was not significantly affected by any of the treatments. During the total trial period, a trend ($p=0.08$) was observed for the effect of xylanase on feed conversion ratio (FCR) compared to the control (1.79 and 1.88, respectively). Additionally, the FCR was further improved in the treatment receiving the combination of xylanase and LEX, 12 points less than the control.

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Advances in Fermented Soybean Meal: Quality and Application with focus on Young Poultry

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Introduction.

Pre-starter and starter diets for poultry - notably turkey poults - are considered some of the most highly specialized products produced by the compound feed industry. These types of feeds have grown in importance over the past decennia with the recognition that, growth in the early life stages is critical to subsequent performance and overall diet utilization, i.e. production efficiency.

To realize the growth potential in young turkeys, high levels of dietary protein are used in poults up to 6-8 weeks of age and these can range between 24 and 28%. Under most conditions the supply of these proteins is largely assured through the inclusion of soy products - generally soybean meal (SBM) - whose inclusion levels may reach close to 50 %. While these high inclusions of SBM in principle ensure that most of the amino acid requirements are met, they come with several challenges.

One of the biggest challenges in the use of SBM in young turkey diets is their residual concentration of Anti-Nutritional Factors (ANF). In their unmodified form these ANF interfere with an efficient utilization of the soy product and the feed containing the soy product. Principal among these ANF are Trypsin Inhibitors (TI), Lectins (L), Phytic acid (PA), non-starch poly saccharides (NSP) and antigenic factors (AGF - glycinin and βconglycinin). Some of these ANFs are heat labile, and consequently, are partially destroyed or converted by a controlled heat treatment. This is notably the case of the TI, L and AGF which undergo dramatic changes during the toasting process as part of the routine crushing procedure and thus the production of SBM (Peisker 2001; Liener, 2000). Nevertheless, the residual levels of heat resistant ANF remain a potential obstacle to improved performance, notably in young poults. The significant negative effect of residual TI levels (and possible other ANFs) in turkey poults was recently again demonstrated using novel low-TI soybean varieties (Evans at al., 2021).

Contrary to the heat-labile ANF, the heat-stable ANF remain in the soy product at the same or higher concentration than in the original product. This is notably the case of NSP, more specifically the sugars raffinose, stachyose and verbascose. At the rate that SBM is currently used in complete feeds, the residual ANF in SBM do not pose a major problem to growing and mature animals. However, for young animals (poults) the level of residual ANFs maybe problematic and further treatment of the SBM is necessary to assure an additional reduction in ANF and this an improvement in digestibility.

A virtually complete reduction or removal of the ANF is possible through a process of extraction and precipitation to produce Soy Protein Isolates (SPI) or Soy Protein Concentrates (SPC) (Peisker, 2001). The resulting products have high levels of crude protein (> 65 %) and corresponding amino acids and only residual concentrations of the ANF with values for TI inferior to 1 TIU/g. In the case of SPI, sugars have been completely removed while concentrations of individual sugars in SPC have been reduced to less than 3%. Production of SPC and ISP requires advanced equipment and methods of control leading to a significant increase in cost. Thus, the use of these products being reserved for special and more expensive products.

Alternative methods have been proposed, and are increasingly used, to reduce ANF in SBM, principal among these are the enzymatic treatments or fermentation methods. These treatments have the advantage to remove much of the ANFs and improving protein or amino acid (AA) digestibility through a change in protein structure (e.g., Hong et al 2004; Chen et al., 2010; Opazo et al. 2012). This is especially the case for fermented SBM (FSBM) which - in the process – will also provide a source of probiotics. The reduction of ANF, the improved AA digestibility, and the added probiotic activity make FSBM an excellent potential candidate to replace high quality animal proteins and the above

mentioned ISP or SPC in diets for young poults or chicks where the high use of SBM can be problematic and may lead to a reduction in performance.

Production and nutritional characteristics of value-added soy products; FSBM.

Although FSBMs are classified as a separate and unique class of VA-soy products, their method of production varies greatly. Most FSBMs are obtained through a process of solid-state fermentation (SSF) which has been defined as the fermentation involving solids in the absence (or near absence) of free water (Pandey et al., 2000). Nevertheless, humidity levels applied during the production process can differ greatly.

Another important source of variation in the quality of the end-product is the inoculum used for the fermentation. Selection of the appropriate inoculum is key to the production of a specific end-product (corresponding to specific nutritional characteristic). In the solid-state fermentation process for SBM, bacterial and fungal strains are used – alone, or in combination. The main bacterial strains used are *Bacillus, Lactobacillus,* and *Bifidobacterium* while the dominant fungal strains are *Aspergillus* (*oryzae, usamii, niger or awamori), Saccharomyces, Rhizopus* and *Trichosporon* (Nout and Aidoo, 2002; Mukherjee et al., 2016).

Nevertheless, given the large array of factors that come into play to successfully achieve SSF of SBM, multiple combinations and procedures have been developed to produce FSBM. This naturally leads to a range of products that potentially differ greatly from one producer to another. To achieve a commercial product, the development of standard production processes resulting in a proprietary method of production and a characteristic FSBM of verified repeatable, standard, nutritional characteristics is necessary.

Table 1 provides typical ranges in nutritional values for some soy products, including FSBM allowing a comparison between FSBM and other soy products. The table also provides a general overview of the range in ANF concentrations for the various soy products including the differences between Hi-pro SBM and FSBM. Relative to SBM, all FSBMs show significant changes in nutritional composition but especially the concentration of ANF. Fermentation of SBM increases crude protein level by 5 – 10 % relative to SBM mainly due to a reduction in fermentable sugars (oligosaccharides) and some of the ANFs. The addition of microbial protein is possible but is generally considered small. With the increase in protein a re-arrangement of AAs concentration i.e. the AA profile of the fermented product may occur and attention needs to be paid t the specific AA profile of each FSBM.

Item	Units	Raw Soybean seeds	Full-fat Soybeans (FFSB)	SBM	Alcohol Extracted SPC	Fer-mented SBM (FSBM)
Dry matter	%	88 - 90	87- 90	88 - 90	92 - 94	89 - 91
Crude protein	%	33 - 37	33 -37	42 - 50	63 - 67	49 - 54
Fat	%	17 - 20	17 - 20	0.9 - 3.5	0.5 - 3.0	1.0 - 2.5
Ash	%	4.5 - 5.5	4.5 - 5.5	4.5 - 6.5	4.8 - 6.0	4.5 - 6.5
Oligosaccharides	%	14	14	15	< 3.5	< 3.0
Stachyose	%	4 - 4.5	4 - 4.5	4.5 - 5	1 - 3	0.04 - 0.08
Raffinose	%	0.8 - 1	0.8 - 1	1 - 1.5	< 0.2	0.01 - 0.04
Verbascose	%	--	--	0.3 - 0.4	--	--
Trypsin inhibitors (TIA)[3]	mg/g	25 - 50	1.0 - 14.0	1.6 - 5.0	2 - 3	<2.5
Glycinin	mg/g	150 - 200	--	20 - 70	< 0.1	<3.0
β-conglycinin	mg/g	50 - 100	--	3 - 40	<0.01	<1.0
Lectins[3]	ppm	2100 - 3500	< 1 - 20	20 - 600	< 1.0	< 50
Saponins	%	0.5	0.5	0.6	0	0.2
Phytic acid bound P	%	0.35	0.35	0.40 - 0.45	0.6	< 0.3

Note. SBM: defatted soybean meal; SPC: soy protein concentrate.

[1]Adapted from: van Eys and Ruiz, 2022. [2]All values as fed basis. [3]For trypsin inhibitors and lectins in FFSB and SBM these are typical commercial ranges, not recommended range

Together with the changes in AA profile, fermentation has a major effect on the crude protein fraction of SBM in terms of the structure of the proteins; more specifically a reduction in the size of the proteins. This leads to an increase in peptide levels (e.g., Chen et al., 2010). These changes in crude protein are associated with an increase in protein – and thus amino acid - digestibility.

More importantly however, ANF decrease significantly during the fermentation process. The decrease in ANF depends greatly on the criteria used to ferment the SBM (see above). But typical decreases in trypsin inhibitor and lectins are in the range of 50 to 75 %. Further decreases are possible if specific inoculums (notably *Aspergillus*) are used. The same is true for phytic acid and thus P digestibility. Fungal fermentation will reduce phytic acid by 50 to 95 %. The residual concentration of lectins is also drastically reduced in the fermented product.

The largest effect of fermentation of SBM is noted for the oligo-saccharides (Sucrose, Raffinose, Stachyose, and Verbascose). All oligo saccharides are reduced to very low concentrations and in the process important levels of organic acids are produced. Depending on the type of fermentation (especially the inoculum) significant quantities of lactic acid as well as acetic acid are produced (with – depending on the type of inoculum - minor productions of other organic acids such as butyric acid). Under practical conditions, the major organic acids can be detected at levels of 5 to 8 % in the fermented product. In addition to the organic acids and enzymes, the use of FSBM will add pro-biotics, especially if the fermentation is carried out with bacterial inoculum (*Lactobacilli* or *Bacilli* spp.). To emphasize this effect special pro-biotic spp. can be selected or included in the inoculum. However, it requires that the drying process is adequately adapted to assure bacterial survival. The addition of probiotics in combination with the organic acid concentrations obviously increases the effect of FSBM on gut health.

All these changes in FSBM relative to the original SBM lead to a special value-added ingredient of improved digestibility and gut- health of the animal especially of young animals with a developing digestive system. Although crude protein or AA levels may be lower than those of other value-added soy products (such as SPC or ISP) the additional attributes of FSBM (increased enzyme-, organic acids- and probiotics-concentrations) make this soy product especially suited for young birds.

Results of FSBM-based diets.

FSBM has been tested and is currently used in a range of specialty diets, among these pre-starters for poultry. In most trials looking at pre-starter and starter diets, FSBM has been incorporated as a complete or partial replacement of fish meal, animal proteins or even SBM when inclusion levels were relatively high and performance compromised. Using the appropriate formulation values adjusted to the type of fermentation used, the replacement has demonstrated significant improvements. These positive results are not limited to the pre-starter or starter phase although the effect in that period is the most pronounced with carry-over effects for the growing-finishing stages. For instance, fungal-fermented SBM replacement of untreated SBM in broiler chick diets during the starter and grower phases significantly increased average daily feed intake (ADFI) and average daily gain (ADG) while at the same time improving gut-health as shown by an increase in villus height, and decreased crypt depth of the jejunal mucosa. Enzyme production (trypsin, lipase, and protease) was also enhanced (Feng et al., 2007a, b; Wang et al., 2012). Changes in microbiota of the cecum has also been shown to be positively modified

Table 2. Growth performance of broiler chicks fed diets with fermented SBM products during 7 d after hatching[1].

	Control (SBM)	BF-SBM	LF-SBM	SPC	SEM	p-value
Feed Intake (g/bird)						
1 to 21 d	60.5	60.04	58.9	59.7	0.88	0.631
22 to 35 d	132.3	140.9	130.1	137.2	2.79	0.058
1to 35 d	88.7	91.5	86.5	89.9	1.26	0.08
Body weight gain; g/bird						
1 to 21 d	39.1^{c}	42.0^{ab}	38.5^{c}	39.9b^{c}	0.78	0.006
22 to 35 d	71.5^{b}	80.5^{a}	70.6^{b}	72.8^{b}	2.39	0.006
1to 35 d	52.0^{c}	57.7^{a}	52.0^{c}	53.5^{bc}	1.12	<0.001
FCR (Feed/gain)						
1 to 21 d	1.54	1.43	1.53	1.5	0.04	0.171
22 to 35 d	1.85^{ab}	1.75^{bc}	1.85^{ab}	1.89^{a}	0.05	0.05
1to 35 d	1.70^{a}	1.58^{bc}	1.66^{ab}	1.69^{a}	0.03	0.008

BF-SBM, Bacillus fermented SBM; LF-SBM 1, Lactobacillus fermented SBM; SPC, soy protein concentrate; SEM, standard error of the means.
[1]Adapted from Kim et al., 2016.

with higher concentrations of lactobacillus and Bacillus spp., and reduced concentrations of Coli-form bacteria in cecal contents. Table 2 provides an example of the effect of replacing SBM with low (3 %) levels of two types of fermented SBM, Bacillus and Lactobacillus fermented SBM. The results confirm the benefits of including FSBM even at a relatively low-level of replacement of SBM and they emphasize the importance between different types of FSBM. It also clearly demonstrates the effect of improvements in the starter diet due to inclusion of FSBM on the performance during the grower-finisher period. Birds receiving the BF-SBM diet during the first 7 days maintained better performance during the later stages of growth, both in terms of ADG as well as feed conversion.

A recently published meta-analysis of 16 studies (Irawan et al., 2022) looked at the use of FSBM from a range of fermentation (inoculum) types and with inclusion levels 1.5 to 36.1 % in broiler chicks. The result showed that FSBM inclusion increased final body weight of broiler chickens, particularly in the starter phase ($p < 0.01$). When the type of inoculum was taken into consideration, *Aspergillus oryzae* in conjunction *Bacillus subtilis* or Lactobacillus bacteria and a protease, had a significantly greater effect on ADG and FI than alternative sources.

FSBM use has also been proven effective in promoting health and performance in turkeys (Chachaj et al., 2019). In this case FSBM was obtained by fermentation with a *Lactobacillus plantarum* strain. Replacing SBM by FSBM at the rate of 7 – 10 % significantly improved weight gain up to 112 days and improved gut health as measured by jejunal villus height and crypt depth. In addition to these changes FSBM stimulated the immune and antioxidant systems.

Conclusions.

The production of pre-starter and starter rations for poults requires exceptional attention in terms of digestible nutrients as well as selection of ingredients. Many of these diets contain high levels of high-quality specialty ingredients such as animal products, fish meal or value-added plant proteins. In these diets the use of SBM (and many other plant proteins) is limited due to its significant concentration of ANF which interfere with the efficient utilization of nutrients by the young chick or piglet.

Solid state fermentation of SBM provides an excellent means to modify SBM through the reduction of ANF, increasing digestibility (especially of the AA), and providing gut-health-promoting compounds such as probiotics and organic acids. Multiple experiments have been completed and have demonstrated the important potential of FSBM to beneficially replace SBM or the more expensive specialty ingredients; generally reducing diet cost. Beside the potential for cost reduction, inclusion of FSBM in turkey pre-starter and starter diets permits improvements in health and performance during the early stages (days and weeks) with beneficial consequences for the later stages of growth.

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Feed cost saving in turkey production using enzymes and probiotics

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Feed cost reduction has become one of the leading strategies for improving profitability since the stark increase of ingredient costs due to global geo-political events. Feed costs account for approximately 70% of the total production costs in turkey production (Willems et al., 2013). Especially due to the need for higher quality ingredients and protein and energy dense raw materials, turkey diets specifically have seen a large price increase. Feed additives are known to reduce feed costs and cost of production by improving turkey performance (Ferket, 1992; Santos et al., 2014). These additives include, but are not limited to, enzymes (including phytase, carbohydrase, protease, and lipases) and probiotics or otherwise known as directly-fed-microbials. Enzymes have been utilized in animal nutrition since the early nineties. Since then, improved knowledge on substrate form and levels in diets has allowed scientists to create a better understanding of the mode of action and functionality of enzymes. This also includes improved tools for enzyme selection. Decision making processes and enzyme selection depend on amongst others animal species, age, stage of production, nutrient requirements, and feed materials costs. Like other poultry species, turkey diets depend on wheat or corn as main cereal ingredients and remaining diet is based on soybean meal, rapeseed meal, sunflower meal, wheat feed and others depending on ingredients prices and availability. An example of a turkey grower diet formulation is shown in Figure 1, including raw materials inclusion levels (left) and the enzyme substrates type and level (phytate phosphorus level, total arabinoxylans, soluble non-starch polysaccharides (NSP) and undigested crude protein fraction, right).

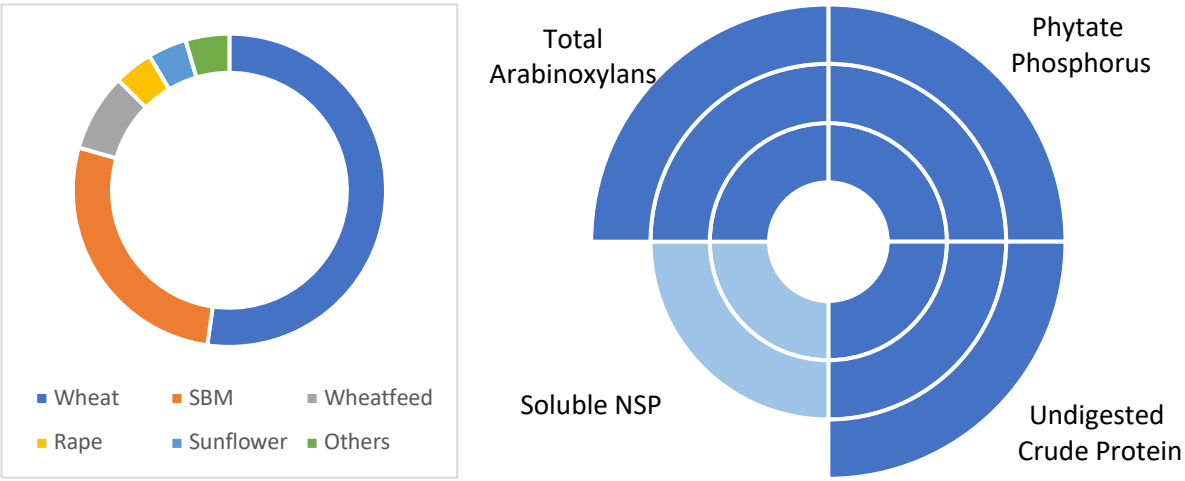

Figure 1. An example of a turkey grower diet formulation (left) and the overall levels of fiber fractions, phytate level and undigested crude protein (right).

In the above example, the indicative figure on the right shows that total arabinoxylans, phytate phosphorus and undigested crude protein fractions are high in this diet. Soluble NSP are medium to high. However, if the inclusion rates of ingredients like wheat feed, barley, rye are increased, the soluble NSP fraction will also increase. Based on this, inclusion of phytase, NSPase (such as xylanase) and protease in feed may help to improve nutrient digestibility and availability. Additionally, these enzymes can help to reduce feed cost by allowing higher inclusions of by products to replace main cereals and soybean meal.

Commercially utilized enzymes in reducing turkey feed costs

There are a few enzymes that have been known to reduce feed cost and either provide equal (when apply matrix) or improved (when added on top) performance. Out of many available enzymes, the most utilized enzymes are discussed here.

Phytase

Phytases have been used in poultry diets since the nineties. Recent developments in phytase have helped to produce more efficient enzymes that are quite effective in acidic environment of the gizzard and proventriculus (such as *Buttiauxella Sp* phytase; Li et al., 2016). Phytase helps breaking down phytate before it creates complexes with minerals and amino acids. A novel consensus bacterial 6-phytase variant at 2000 FTU/kg was able to replace 0.18% available P in turkey diets and maintain performance and bone ash (Bello et al, 2021). Replacement of monocalcium phosphorus by a *Buttiauxella* phytase did not affect tibia ash contents in turkeys of 21 days of age (Kwakernaak and Dersjant-Li, 2016). In general, phytases can either reduce or replace the need for inorganic phosphates. This can lead to approximately 8-10 euros/ton feed cost saving compared to a positive control (PC) situation where no phytase is added in the diet. Since phytase is added in almost every monogastric diet, using higher dose can help to reduce inorganic phosphates and reduce phosphorus excretion in broilers (Dersjant-Li et al., 2021). A commercial research trial conducted in France also showed using 2000 FTU of *Buttiauxella* phytase could reduce additional 5-6 euros/ton of feed cost when compared to using 500 FTU. These findings were also confirmed by novel consensus of bacterial 6-phytase variant (PhyG, the latest development in phytases) at 2000 FTU/kg with application of full matrix, that maintained performance compared to a nutrient/energy adequate PC and reduced feed cost per kg BWG (Bello et al., 2021, Figure 2).

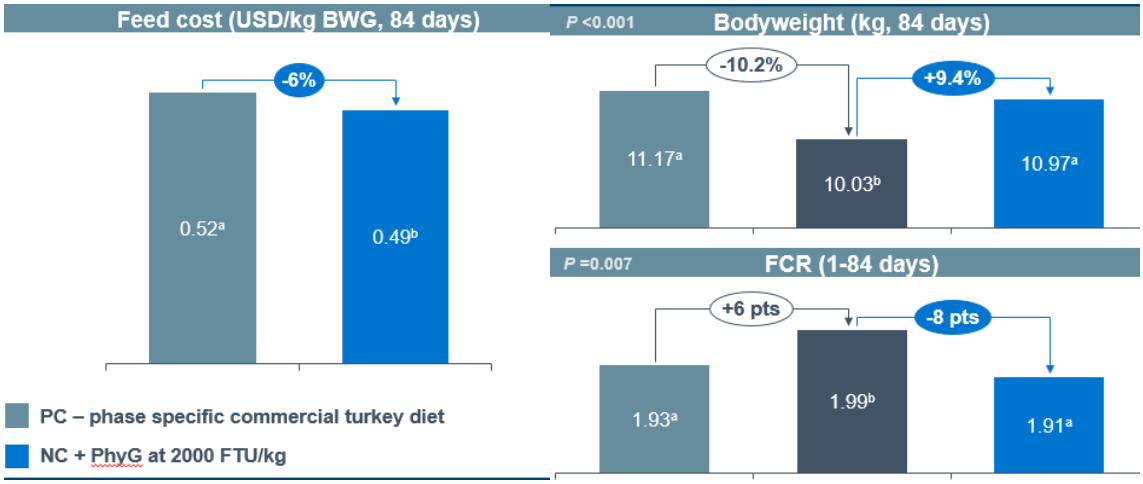

Figure 2. Effect of application of novel consensus of bacterial 6-phytase variant (PhyG) at 2000 FTU/kg with application of full matrix on performance and feed cost/kg BWG. PC: phase specific commercial turkey diet, NC: with reduced available P by 0.18% point (p), Ca by 0.21% p, Na by 0.05% p, dig AA by 0.02 to 0.06 p and ME by 74.5 kcal/kg.

Non starch poly saccharide (NSP) enzymes

NSP fiber fractions like arabinoxylans and beta glucans are present in cereals and various by products. Their presence can increase digesta viscosity leading to reduced digestibility and reduced performance. When appropriate NSP degrading enzymes are utilized they can reduce digesta viscosity in turkeys (Samu et al., 2005). It has also been suggested that combination of xylanase and beta-glucanase can reduce digesta viscosity and improved performance in turkeys (Mathlouthi et al., 2003). Alternatively, the optimal combination of enzymes can also help to allow by-products to be included in the diet, resulting in feed cost savings. Taking the same example as above, it is possible to reduce feed cost additionally by 9-11 euros/ton. This depends on substrate level, type of enzyme, its dosage and how matrix values are being utilized.

Protease or combination of protease with other NSP enzymes

As shown in the Figure 1, turkey diets are condensed diets with higher levels of crude protein. This may also increase the undigested crude protein fraction in the hind gut leading to overgrowth of non-beneficial microbial species.

Protease (*Bacillus Subtilis*) alone has been shown to reduce mortality by 5% and reduced pathogenic E. coli in the gut showing improving gut health (Kim et al., 2021). In earlier experiments xylanase alone did not show improvement in digestibility, while a combination with amylase (*Bacillus amyloliquefaciens*) increased nutrient digestibility (Ritz et al., 1995). However, in another study, a combination of protease, xylanase, and amylase enzyme (Avizyme®) showed to increase performance and nutrient digestibility in a dose dependent manner (Troche et al., 2007). As mentioned, the effect of enzyme is dependent on the type of enzyme, quality of the ingredients as well as substrates available for enzymes. There are a few studies that have suggested improvement with combination of enzymes and additionally feed cost savings as well. The Figure 3 shows additional savings of 6-8 euros/ton when using protease with combination of amylase and xylanase, depending on how matrix values are utilized.

A conservative approach of additional feed cost savings is shown in Figure 3.

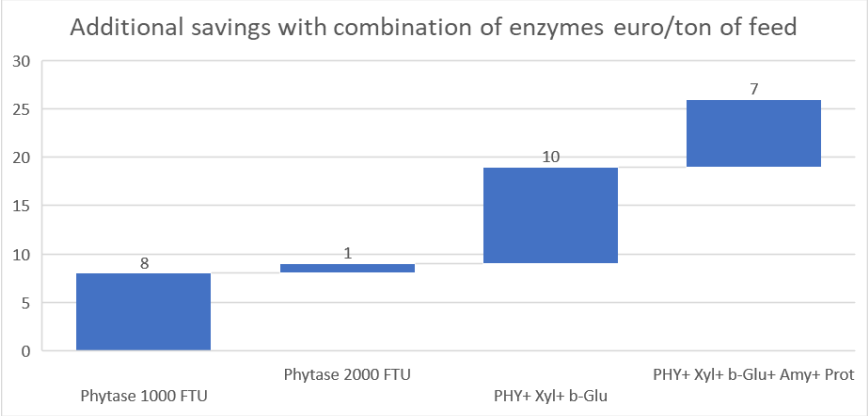

Figure 3. Savings per ton of feed when using combination of enzymes. PHY = phytase, Xyl = xylanase, b-Glu = beta-glucanase, Amy = amylase, and Prot = protease

The feed cost savings in Figure 3 shows a best-case scenario. However, this combination of *Buttiauxella* phytase (Axtra® PHY) with xylanase, beta-glucanase (Axtra®XB), amylase, and protease (Avizyme®) has been tested in a commercial research trial facility in the EU, where BUT line turkeys showed reduced FCR and feed cost savings (unpublished data).

Lipases

Lipase addition in the diet might help with feed cost savings considering the cost of energy and oils and fats. However, research in this area has shown the use and efficacy of lipases is limited. In a trial when lipase was studied in combination with xylanase, lipase addition did not improve turkey performance significantly at the age of 18 weeks (Santos et al., 2004).

Probiotics

There are many details related to the definition of probiotics, their function and effects on gut health and microbiota and performance improvement. These details have been summarized elsewhere (Abd El-Hack et al., 2020). Probiotics research in turkeys is scarce, especially related to cost reductions. Even though probiotics may not reduce the feed cost per ton directly in feed formulation, they can improve gut health and reduce mortality, which in turn can reduce the cost of turkey production. Probiotics have been shown to improve gut health by reducing leaky gut in turkeys as well as improving nutrient digestibility (Latorre, 2016). Probiotics addition can also help to reduce salmonella burden in turkeys (Wolfenden et al., 2011), which provides opportunities in the area of human food safety. The addition of *Bacillus Velezensis* probiotic reduced FCR by ten and seven points in starter and grower phase respectively in a recent experiment in turkeys (Hernandez, 2022). In this experiment, there was 5% improvement in FCR and more than 3% improvement in body weight gain.

Summary

Some of the feed cost increment can be offset by proper feed additives selection and application. Knowing substrates can define which enzyme to use. Phytase, NSPases and protease offer a great opportunity to reduce feed cost. A proper dose with application of full matrix can further help with feed cost savings while making sure to maintain the performance. Probiotics can further help improve gut health and reduce feed conversion ratio leading to reduced production cost and improved return on investment.

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Energy utilisation and inositol phosphate isomer excretion of turkeys fed diets formulated with rapeseed meal, supplemented with phytase and xylanase, alone or in combination

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Introduction

Rapeseed is the most widely grown oilseed crop in the UK/Europe. Rapeseed meal (RSM), a co-product of the rapeseed oil recovery process, is an attractive alternative protein source for poultry (Carré and Pouzet, 2014; Kasprzak et al., 2016). Although the majority of currently available cultivars are registered as "double zero" (00) due to their low erucic acid and glucosinolate content, RSM is still high in non-starch polysaccharides (NSP) and phytate (Watts et al., 2020, 2021). Thus, formulating poultry diets using RSM remains challenging as its nutritive value is reportedly lower and more variable than soybean meal (Khajali and Slominski, 2012). Application of dietary enzymes to facilitate phosphorus, protein, and energy utilisation is a common approach to quality improvements of RSM in poultry diets (Bedford, 2018; Watts et al., 2020). Most studies to date have been conducted in broilers (Pirgozliev et al., 2022). Thus, there is a scarcity of information as to the use of enzymes on RSM in turkey rations. In particular, little is known about the effect of the combination of phytase (PHY) and xylanase (XYL) on phytate degradation in RSM rich diets. In turkeys, this is an observation which is particularly pertinent given supplementary phytase appears to produce different results in chickens and turkeys (Pirgozliev et al., 2007; Olukosi et al., 2020). Therefore, the objective of this experiment was to examine the effect of supplementary PHY and XYL, alone or in combination, on the energy utilisation, mineral retention and phytate degradation when feeding diets containing 24 % RSM to young turkeys.

Animals and Experimental design

An experimental diet with the main ingredients being wheat (42%), RSM (24%) and soybean meal (23%) was produced (Table 1). The diet was split into four parts, with one part supplemented either with PHY (1500 FTU/kg) only, XYL (16 000 BXU/kg) only, and PHY + XYL (1500 FTU/kg + 16 000 BXU/kg) combination to create four dietary treatments, including the un-supplemented basal diet portion.

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. Birds were fed on a proprietary wheat–soybean turkey feed until 41 d of age. During the first eleven days all birds were fed the basal feed only (Table 1). Birds were randomly allocated to one of 40 cages (two birds per cage), with 0.36 m^2 floor area total. Each cage was equipped with a trough feeder and nipple drinker. Access to the feed and the water was *ad libitum*. There were ten cage replicates for each diet. The experimental house was equipped with a negative pressure ventilation system to meet commercial recommendations. Standard temperature and lighting programmes for turkeys were used (Aviagen Turkeys Ltd.). At 46 d of age, after 5 d adaptation period, the total excreta were collected for 4 d until the end of the study at 49 d age. Feed intake for the same period was recorded for the determination of dietary AMEn and total tract mineral retention coefficients.

Table 1. Composition of experimental turkey diet

Ingredients	**(%)**
Wheat	42.0
Maize-gluten meal	2.0
Rye	1.6
Rapeseed meal (RSM)	24.0
Soybean meal (Hi Pro)	23.6
L-Lysine HCl	0.28
DL-Methionine	0.28
L-Threonine	0.072
Soya oil	2.4
Limestone flour	0.8
Dicalcium phosphate flour	2.4
Salt	0.24
Vitamin/ mineral premix[1]	0.32
Calculated provisions %	
Oil	4.1
CP	26.2
ME	11.4
Lysine (available)	1.3
Methionine + Cystine	1.3
Ca	1.2
P (available)	0.7
Determined values	
Dry matter, %	88.3
Oil (g/kg)	28.4
CP (g/kg)	233
Ca (g/kg DM)	16.8
P (g/kg DM)	10.8
Phytate P (g/kg)	4.3
Na (g/kg)	1.24

[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

Table 2. Impact of enzymes phytase (PHY) and xylanase (XYL) on dietary N-corrected apparent metabolisable energy (AMEn), calcium (CaR), phosphorus (PR) retention coefficients, inositol phosphate isomers (IP) and Inositol concentration in excreta

	AMEn (MJ/kg DM)	CaR	PR	IP3	IP4	IP5	IP6	Inositol
PHY								
no	12.03	0.544	0.429	2828	4136	8562	39008	1319
yes	12.24	0.578	0.462	3559	7418	1635	7474	3133
XYL								
no	12.19	0.564	0.448	3056	5503	4978	22685	2178
yes	12.08	0.559	0.442	3331	6051	5218	23797	2273
SEM	0.102	0.0095	0.0108	185.5	449.7	220.0	754.0	396.2
DIETS								
Control	12.15	0.548	0.427	2507	4000	8560	38914	1140
PHY	12.24	0.580	0.569	3605	7007	1397	6456	3216
XYL	11.92	0.541	0.430	3149	4273	8563	39103	1497
PHY x XYL	12.24	0.577	0.454	3512	7829	1873	8491	3050
SEM	0.144	0.0134	0.0153	262.4	635.9	311.2	1066.3	560.4
Probability values								
PHY	0.161	0.017	0.043	0.010	<0.001	<0.001	<0.001	0.004
XYL	0.442	0.699	0.726	0.305	0.397	0.447	0.306	0.867
PHY x XYL	0.428	0.898	0.578	0.173	0.670	0.454	0.394	0.646

SEM= Pooled standard error of the mean

Results and discussion

There were no (P>0.05) PHY by XYL interactions for any of the variables presented in Table 2. Results in this study show that PHY supplementation influenced most of the studied variables. Dietary PHY increased retention coefficients of Ca and P ($P < 0.05$). Phytase supplementation also increased the concentration of inositol ($P < 0.05$), IP3 ($P < 0.05$) and IP4 ($P < 0.001$) isomers in excreta, but as expected, reduced the concentration of IP5 and IP6 isomers ($P < 0.001$). The increase in PR coefficient, IP3 and IP4 concentration in excreta agrees with previous research with turkeys (Pirgozliev et al., 2007; 2019; Olukosi et al., 2020). The reduced IP5 and IP6 excreta isomer concentrations were reduced in accord with Pirgozliev et al. (2019) and Olukosi et al. (2020). The lack of action of XYL independently or synergistically with phytase as hypothesised may be explained by the relatively short study period. For xylanase to be efficacious, it usually takes more than one week to condition the gut and start influencing nutrition related variables (Bedford, 2018).

The results in this study suggest that high levels of phytase are capable of reducing the IP5 and IP6 concentration in the excreta by 81%, even in relatively short periods of feeding. Both, IP6 and IP5, are highly potent chelators of minerals and may interfere with digestion of protein. The use of phytase in turkeys may therefore bring additional 'extra phosphoric' improvements beyond the release of dietary Ca and P observed in the present study, in keeping with the theory that a high IP6 concentration can chelate minerals and therefore reduce mineral availability.

Conclusion

In conclusion, the reported results confirm that supplementing PHY in turkeys is an effective strategy for improving the nutritional value of diets through the reduction of the anti-nutritional factors IP6 and IP5. Results indicate improvements in Ca and P retention coefficients. Experiments with prolonged length may be more suitable to assess enzyme impact on the studied variables, especially for the NSPase, as these may take extended periods of time to develop a response due to gut conditioning requirements.

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