Apr - Jun 2024
Vol 33 No 2
ISSN 2308-0647

AFMA
SAFE FEED FOR SAFE FOOD
Animal Feed Manufacturers Association

Matrix

Quarterly magazine of the Animal Feed Manufacturers Association

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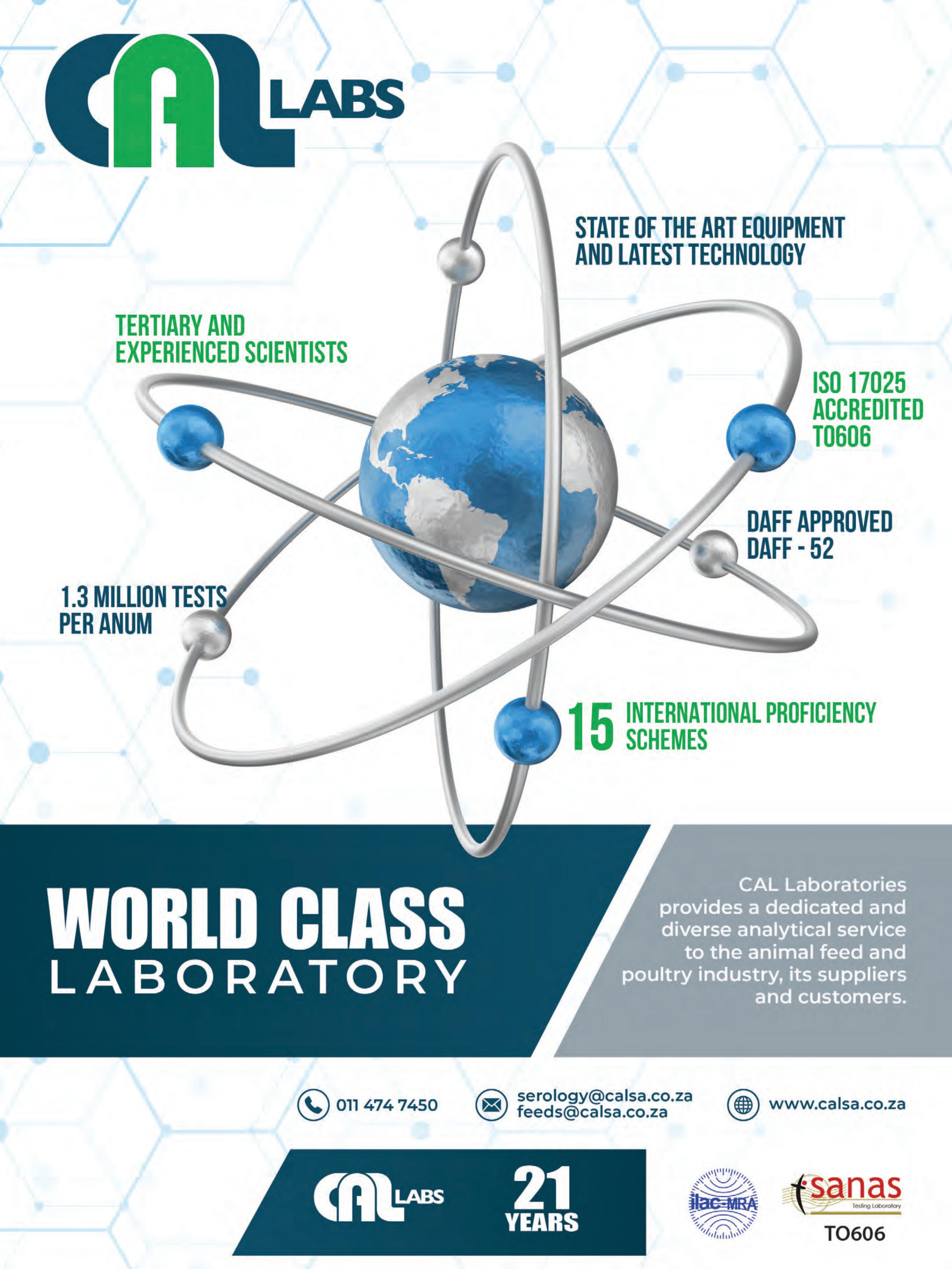
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Published on behalf of AFMA
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Looking forward necessitates a reflective glance backwards

By Liesl Breytenbach, executive director, AFMA

Future successes are intricately tied to past experiences. By embracing a forward-thinking approach that is informed by a thorough analysis of historical trends and challenges, businesses in the agricultural sphere can position themselves strategically to capitalise on emerging opportunities and mitigate potential risks.

Geo-political influences, including the wars in Russia-Ukraine and Gaza and a rise in global leadership changes, all have uncertain consequences for the growth of South African agriculture; coupled with an evolving squeeze on global natural resources through climate change, the markets remain volatile and under pressure.

Back home, South Africa is facing elections on 29 May, and 'coalition' is the word of the day – mixing in more uncertainty in an already challenging environment and forcing businesses to rethink their profit-making and production strategies. Finding solutions together with government, and playing an active part in implementing change, will pave the way for a better future, and agriculture can lead the way.

Need to navigate drier weather

Initially, it was estimated that the 2023/24 summer season crops would be at above average levels and South Africa would maintain its status as a net exporter of maize and soya beans. However, excessive heat and limited rainfall across the major crop-growing regions during February could result in lower yields and may even put pressure on local supply. This will culminate in increased animal feed ingredient costs, putting more pressure on feed manufacturers to deliver affordable feed for animals.

El Niño can be detrimental to rain-fed agriculture and result in decreased crop yields, reduced pasture growth for livestock, and water shortages for irrigation. Furthermore, it can result in higher food prices and economic hardship for producers. Being proactive and ensuring a continuous supply of maize and soya, including the provision for emergency importation of the commodities, is a fundamental part of forward thinking and should receive immediate attention.

In support of sustainability

South Africa's decision to regulate all new breeding technologies as GMOs will not only have negative implications for local producers to access the latest innovative technology in drought- and pest-resistance – it will also impact the ability of agro-processors to import the necessary commodities to address the shortfall due to asynchrony and asymmetry of regulatory approvals of those commodities.

In a joint statement by the South African National Seed Organisation (Sansor) and CropLife SA earlier this year, they reiterated the importance of innovative technology being a part of the solution to help meet national commitments in terms of food security, climate mitigation, and sustainability goals while ensuring the international competitiveness of the local agricultural sector. It is time for the sector to raise the bar and push for a proactive approach in agricultural policy and regulatory framework that supports the sector and mitigates the risks.

AFMA's strategic focus

Animal feed manufacturers are integral to the agricultural value chain and have a responsibility to contribute to the supply of animal proteins as a food source. Despite facing challenges from all sides of the value chain, such as fluctuating commodity prices, infrastructure limitations, global market dynamics, animal disease challenges, and a decrease in overall consumer spending on animal proteins, the sector continues to demonstrate adaptability and innovation.

This year the Animal Feed Manufacturers' Association (AFMA) will maintain its focus on addressing strategic areas relating to the price of feed ingredients, an improved regulatory framework for the manufacturing of animal feed, and supporting the expansion of export opportunities for animals and food products of animal origin.

AFMA will be addressing this via:

- An investigation into the potential impact of a reduction in import duties on soya and sunflower oilcake, and the listing of protein oilcake on the Johannesburg Stock Exchange (JSE).
- Support for a new regulatory framework that enables facility licencing under government authority and subsequently enhances food safety, stimulates exports, and reduces the regulatory burden for placing innovative animal feed products on the market.
- Actively encouraging the implementation of a robust traceability system and prudent use of antimicrobials.

Look out for the two-day Antimicrobial Resistance (AMR) Workshop that will be hosted in collaboration with the South African Animal Health Association in April. The workshop aims to stimulate discussions regarding AMR in South Africa between supplier and producer organisations, identify global AMR developments and drivers, sensitise stakeholders to current thinking around antimicrobial use, control, resistance and surveillance, and provide a platform for stakeholders to share progress in implementing their sector-specific programmes.

Working together

By acknowledging our failures and the paths we've taken, we can make informed decisions, avoid repeating mistakes, and clearly envision our goals. While working together may complicate decision-making in the short term, it is imperative for long-term success and will remain the basis of AFMA's collaboration going forward – working together with the soya value chain, the animal health and veterinary sectors, and the wider agricultural producer sector in addressing the strategic areas for improvement.

NEWS & views

Important dates on the AFMA calendar

4 April	16 April	6 to 16 May	12 June
AFMA General Meeting, Grain Building Auditorium, Pretoria	AFMA Student Outreach, KwaZulu-Natal	AFMA Feed Miller Short Course, Conclave Country Lodge, Pretoria	Technical Writing Skills Workshop, Centurion Country Club, Pretoria

Novus acquires BioResource International

Novus International recently announced it has completed the acquisition of Unites States-based enzyme company, BioResource International (BRI). Under the terms of the agreement, Novus becomes the owner of all BRI's products and intellectual property and takes control of the company's facilities.

"This move will allow us to serve our customers better and expand our innovation pipeline further," says Novus president and CEO, Dan Meagher. "Enzymes are vital tools for producers to ensure animal health and well-being and help deliver on-farm profitability. We're very excited to offer our customers more options, as well as aspire to develop new feed additives."

The relationship between the two companies isn't new. Novus has partnered with BRI since 2008 to manufacture its protease product, CIBENZA® Enzyme Feed Additive. Meagher says having full ownership and control of the product line and the option to expand Novus' portfolio beyond protease enzymes is a natural fit in the company's long-term strategic plans. – *Press release, Novus*

Positive shift in fishmeal prices anticipated

Anticipated improvements in anchovy fishing conditions during the first half of 2024 in Peru are expected to bring about a positive correction in fishmeal prices, as outlined in an industry note. Last year was marked by severe shortages of fishmeal and fish oil, with supply constraints intensifying during the initial six months of 2023.

Gorjan Nikolik, senior analyst in seafood at Rabobank, shared insights with Feed Navigator, coinciding with the release of Rabobank's comprehensive outlook on salmon, shrimp and fishmeal prospects for the first half of 2024. Nikolik highlighted that the enhanced fishmeal supply is likely to contribute to the normalisation of prices, especially with the moderation in prices of plant-based alternatives such as soya bean meal observed in the previous year.

The second Peruvian anchovy fishing season, spanning from November 2023 to January 2024, saw a catch rate of 75%, already prompting a corrective impact on fishmeal and fish oil prices. Nikolik anticipates a more substantial correction in these raw material prices to materialize in April. Despite the second season's catch quota of 1,682 million tons being considered good but below average, the positive catch rate has already influenced market dynamics. – *Feed Navigator*

Second-highest incidence of mycotoxins noted

Maize and other ingredients purchased in the past year could be subject to high levels of mycotoxin contamination, experts from DSM-Firmenich warned during a webinar earlier this year.

A total of 76% of 2023 maize samples from the United States (US) tested positive for fumonisin, falling just short of the record 78% seen in 2018, according to data from DSM-Firmenich. Globally, 86% of finished feed samples tested in 2023 were positive for fumonisin, nearly matching the 87% peak recorded in 2018 and matching high rates of contamination observed around the world since 2017.

Fumonisin is the most common mycotoxin found in US maize, but far from the only mycotoxin present in this past year's crop, said Paige Gott, DSM-Firmenich's mycotoxin risk manager for North America. Nearly three fourths of samples tested were positive for more than one mycotoxin, Gott said.

Gott also noted that 100% of dried distillers grains with solubles (DDGS) samples tested in 2023 came back positive for mycotoxins. – *Feed Strategy*

Astral sells its stake in Quantum Foods

The integrated poultry producer, Astral Foods Ltd (Astral), officially sold its 9,8% interest (equal to 19 550 855 shares) in Quantum Foods Holdings Ltd (Quantum). The total consideration for this transaction amounted to R141,7 million. The sale was carefully evaluated by Astral's full board of directors, and it was executed through a 'book over' process, effective 5 March 2024. No regulatory approvals were required by Astral for this transaction.

Chris Schutte, CEO of Astral, commented: "At the time Astral acquired the 9,8% equity stake in Quantum in June 2020, there were unmitigated risks the group had to manage. These risks centred around securing the supply of live broilers to Astral's County Fair operation in the Western Cape from Quantum. Quantum supplies Astral with approximately 600 000 broilers per week for further processing. That is approximately 35% of County Fair's total slaughter capacity." – *Press release, Astral Foods*

Nigerian poultry sector faces uphill battle

Driven by sky-high prices, two of Nigeria's leading feed manufacturers announced a temporary halt in buying maize and sorghum. This is the latest development in a long-running series of challenges for national food security in Nigeria, and for its population.

Premier Feed Mills reported it had suspended purchases of these two vital raw materials. The decision was driven by the prices of these feed ingredients, which it described as being artificially high. As a result, its finished feed prices for poultry and other livestock producers have become so expensive that national food security is under threat.

In January, the Poultry Association of Nigeria (PAN) called on the president's administration for help in managing the ongoing crisis. At issue is the massive hike in the cost of poultry feeds. The nation's poultry sector faces a total collapse, according to the secretary of one PAN local chapter.

Ongoing violence and insecurity in the maize-growing regions of northern Nigeria were blamed for the low domestic harvest, according to a report from the USDA Foreign Agricultural Service (FAS) published in October 2023. Other adverse factors it identified were speculative maize purchasing, rising costs of diesel and logistics, weakness of the local currency (naira), and widespread outbreaks of avian influenza in Nigerian poultry flocks.

FAS forecasts year-on-year reductions of 7% in Nigerian maize production, and 5% in consumption for this marketing year, which ends in June. Animal feed accounts for around one-quarter of maize used in the country, where it is a staple food for the human population. – *Feed Strategy*

Rainbow set to separate from RCL Foods

The board of RCL Foods has agreed to the formal separation of the company's Rainbow business, which covers its poultry and feed operations. The company's next steps will be to fully unbundle this business from RCL's value-added brands division to shareholders, and its listing on the Johannesburg Stock Exchange, RCL Foods reported.

Preparations for this development began in 2021 with a review of the business. Since then, they have explored different options for the separation of Rainbow. At the same time, a turnaround plan has been in progress to improve performance, with the aim for Rainbow to become a "sustainable, market-leading and low-cost producer," according to the company.

In support of the decision to unbundle Rainbow, the RCL board will allow the businesses to follow different growth ambitions and investment plans. The operations had already been separated. However, details are still to be completed, such as financing arrangements, separation of support services, and stakeholder engagement.

According to the board, it is "committed to transitioning the Rainbow business in a responsible manner which preserves the heritage and key strengths of Rainbow, and is in the best interests of Rainbow customers, staff and other key stakeholders."

Listing is expected to take place within a few months, according to RCL Foods' CEO, Craig Cruickshank. The move will allow shareholders to choose to invest in the poultry and feed business or RCL's value-added products. Cruickshank said the decision to separate is a win-win for both RCL and Rainbow.

RCL Foods will be able to pursue is value-added branded products strategy, while Rainbow will be listed as the benefits of breed transition are starting to take effect. – *Feed Strategy*

Agri gross value added experiences downturn

Statistics South Africa recently released figures indicating that South Africa's agricultural gross value added fell notably by 12,2% year-on-year.

According to Wandile Sihlobo, chief economist at Agbiz, South Africa's agricultural sector faced several challenges in 2023, but Agbiz did not anticipate that the overall annual performance would drop sharply. Along with the Bureau for Food and Agricultural Policy (BFAP), Agbiz expected a mild contraction in 2023 because of the animal disease challenges in the livestock and poultry sub-sector.

The headwinds in the livestock and poultry industry weighed on the sector more intensely than anticipated. The livestock and poultry industry, which accounts for nearly half of the sector's value, was hit by animal diseases such as foot-and-mouth, avian influenza and African swine fever. – *Press release, Agbiz*

Slowdown in pig production forecast

The latest Rabobank quarterly pork report signals a concerning trend of contraction in key regions' sow herds, casting a shadow over the global pork market. Despite facing significant challenges, the analysts note that consumption remains resilient.

The contraction in the sow herd is expected to result in a decline or flat production in China, the US, and some European countries throughout 2024, with disease pressures adding to the industry's challenges.

While some regions grapple with declining herds, others such as Brazil are on the rise, driven by global demand. Rabobank underscores the uneven growth across the globe, with African swine fever outbreaks and loss-making pressures accelerating breeding herd reductions in Asia, particularly China. – *Feed Navigator*

Analysis of the SA compound feed market

By Lucius Phaleng, trade advisor, AFMA

According to the United Nations' Food and Agriculture Organization projections, the global population is expected to reach 9,3 billion by 2050, necessitating a 60% increase in food production. The production of animal-based protein is anticipated to increase along with this growth. This surge in demand underscores the increasingly vital role of the compound feed sector.

The South African feed industry is a crucial component for fostering sustainable growth in animal husbandry. Compound feed holds significant importance within the food sector, serving as a vital source of safe and nutritious animal proteins.

Increases in animal protein production correspond with a need to ramp up feed manufacturing to ensure sustainable animal production. In 2023, South Africa's compound feed manufacturing totalled 6,9 million tonnes, marking a slight decline of 1,6% compared to 2022 (a trend mirrored by a 2% decrease in the European Union). This data from the Animal Feed Manufacturers' Association (AFMA) underscores the industry's response to challenges such as animal diseases, notably avian influenza and African swine fever, which have adversely affected the availability of raw materials and animal production capacity.

Furthermore, alterations in production techniques, along with fluctuations in demand stemming from evolving consumer preferences (impacted by food price inflation) and power outages, are influencing compound feed manufacturing in South Africa. Countries such as Germany, Ireland, Denmark, and Hungary also experienced an approximately 5% decrease in feed manufacturing.

The impact of avian influenza

In 2023, there was a decline in the manufacturing of layer and breeder compound feed, characterised by a negative growth rate, with a decrease of 14,5 and 2,7% respectively compared to 2022. This decline was primarily attributed to the significant impact of avian influenza on the poultry industry in 2023. However, broiler feed manufacturing saw modest growth of 0,8% during the same period, indicating that broiler production was relatively less affected by the disease. This growth in broiler feed manufacturing was partly fuelled by the expansion of broiler production in South Africa.

Figure 1: Compound feed per category comparing 2022 to 2023.

To counteract losses suffered due to avian influenza, the South African Poultry Association (Sapa) reported that approximately 21,5 million fertilised eggs were imported to replenish domestic flocks. Consequently, the negative trend in poultry compound feed manufacturing (for layers and breeders) was primarily driven by reduced feed demand resulting from decreased bird populations.

Feed manufacturing

Several other compound feed products encountered a decline in manufacturing growth, including pig feed (3,1%), maize-fed feed (6%), ostrich feed (34%), rabbit feed (18,8%), and other feeds (10,8%). This downturn in feed demand by the livestock sector is expected to lead to a temporary decrease in feed prices due to oversupply. Conversely, dog food, feed concentrates, and game feed witnessed the most substantial growth in production volume.

Additionally, other feed products such as dairy, cattle, and horse feed experienced moderate manufacturing growth. The rise in beef cattle feed manufacturing was fuelled by heightened demand for beef as an alternative protein source amid shortages in poultry meat supply.

Market outlook

The forecast for compound feed manufacturing and demand in 2024 presents a complex picture. Uncertainty prevails due to various factors including the repercussions of animal diseases, economic instability, sustained inflation in food and raw material prices, and the approval of poultry meat imports. These dynamics are exerting pressure on both local production and demand for feeds.

Feed manufacturers in South Africa will be required to adapt to these challenges, particularly the elevated costs of oilcake and electricity, alongside the persistent threat of animal diseases.

For enquiries, email Lucius Phaleng at trade@afma.co.za.

Train with a purpose!

By Learning Pathways and AFMA

Training is one variable that you can use to enhance your business activities, as trained employees lead to higher turnover and profit. In the animal feed manufacturing sector, skilled and well-informed feed milling operators play a vital role in the production and constant supply of good quality, safe animal feed.

The Livestock Feed Milling Operator Training Programme is recommended for people within the feed milling environment to prepare and equip them with knowledge and practical experience to function at an operator level in a livestock feed milling environment.

The training programme was initiated by the Animal Feed Manufacturers' Association (AFMA) in 2020 and consists of well-researched, world-class, practical content. For the past three years the programme has proven to be highly beneficial to both the students who have completed the programme and the feed businesses that have invested in the training of their feed mill operators.

A blended learning approach

The course is offered as a work-based integrated learning (WIL) programme and consists of two sections, namely theory (e-learning) and practical (work-based). Blended learning is one of the most successful methods of studying, since the retention of information is enhanced through theory as well as experience gained in the workplace.

To facilitate the theoretical modules, a user-friendly training platform have been developed that provides for constant evaluation and reporting. Multi-media is used to keep students' attention while assisting with understanding and retention of the programme contents.

The theoretical modules include:

- Module 1: Feed milling environment.
- Module 2: Material handling equipment.
- Module 3: Receiving, storage and warehousing.
- Module 4: Mixed feed production.
- Module 5: Feed processing.
- Module 6: Feed science.
- Module 7: Operational team leadership.

Practical modules

These modules are completed in collaboration with a workplace training facilitator that has been trained by Learning Pathways at the start of the training programme. Constant communication ensures that the facilitators are fully equipped to handle student training. Modules are designed with specific vocational applications in mind and are fully adaptable to the workplace.

The practical modules include:

- WIL 1: Bulk material intake and storage.
- WIL 2: Material warehousing and stock control.
- WIL 3: Material reclaim from storage.
- WIL 4: Mixed feed production.
- WIL 5: Feed processing.
- WIL 6: Team leadership.

By the end of the programme, students will be able to:

- Demonstrate an understanding of the animal feed industry, the mixed feed manufacturing process, the work environment, safe work practices, and the importance of a quality culture in the business.
- Demonstrate an understanding of material handling equipment, facilities, and the systems commonly used in handling material in a feed mill.
- Attend to processes related to the receiving of materials delivered in bulk and bags, storage of material, and release of material to the feed mill.
- Attend to mixed feed production processes and equipment operation (batching, mixing, additive/micro-ingredient addition, and particle reduction).
- Attend to mixed feed processing and equipment operation (conditioning, crumbling, pelleting, post pelleting treatments).
- Demonstrate an understanding of concepts that are related to animal nutrition, raw materials, additives, and ingredients.
- Attend to the supervisory roles and functions at a first line team leader level.

For more information regarding registration for this course, contact the AFMA office at admin@afma.co.za or 012 663 9097.

The hidden culprit: Unveiling recontamination risks in the feed mill

By Taylor dos Santos Serra, technical support specialist, Kemin

Recontamination of final feed is a concept that is well known, yet specifics regarding the management of recontamination are less well-defined. Recontamination is the re-introduction or transfer of unwanted organisms or material (for example pathogens) into feed after the feed has undergone processes to reduce the risk of contamination, and there is no additional mitigation step in the process (Munoz *et al.*, 2021).

Reducing the risk of recontamination is pivotal in the holistic approach to overall feed safety and, consequently, animal health and production.

Background of recontamination

Contaminants associated with feed safety can be divided into three groups:

- Microbial contaminants such as bacteria, fungi or moulds.
- Chemical contaminants such as mycotoxins or heavy metals.
- Plant compounds such as phenols or anti-nutritional factors (Australian Institute of Food Safety, 2023).

Each group of contaminants impact the quality of animal feed produced and, consequently, negatively affects animal health. It is critical to note that contaminants are not only found in the feed processing facility, but they also enter the facility through various raw materials, transport and people (Davies and Whales, 2010; Jones, 2011). It is often challenging to know and trace where and how the unwanted microbes or substances entered the feed.

Ensuring various measures are put in place in the feed production facility, can assist in decreasing the risk of contaminants entering the feed and can enhance traceability. Such measures include risk assessments and monitoring programmes.

Despite best efforts from feed producers and comprehensive monitoring programmes, it is not uncommon for recontamination to occur (Obe *et al.*, 2023).

Recontamination risk factors

It is vital to note that recontamination can occur throughout the feed processing facility, including the laboratory. There are many causes of recontamination and areas in the feed mill where recontamination is more likely to occur. However, inadequate cooling, cross-contamination, and environmental factors are the three most common causes (Parker *et al.*, 2022; *All About Feed*, 2017).

Understanding these causes of recontamination makes it easier to identify areas where recontamination is most likely to occur; consequently, ensure measures are in place to decrease the probability of pathogens re-entering the final products.

Inadequate cooling

Inadequate cooling occurs when the temperature of newly produced heat-treated feed (pellets) does not reach ambient temperature after production. If cooling is inadequate, coolers can often be areas where recontamination takes place. The coolers are responsible for cool, high airflow to lower the temperature of the freshly pelleted feed to ambient temperature before loading or bagging.

Inadequate cooling through insufficient airflow and temperature can result in condensation, providing moisture and optimal conditions for bacterial (e.g. *Salmonella*) proliferation and mould growth (Parker *et al.*, 2022).

Gosling *et al.*, 2022 demonstrated that in over 20 feed mills in the United Kingdom where the *Salmonella* prevalence was higher than 10%, there was substantial contamination in the coolers. Other studies which support the findings of Gosling *et al*. further mention that mitigation of pathogens found in the coolers can be extremely challenging due to the limited access to clean inside the cooler (Jones, 2011; European Feed Manufacturers Federation, 2014). This may result in pathogens becoming persistent over time with new feed constantly being produced and thus potentially becoming recontaminated (Podolak *et al.*, 2010).

Interestingly, recontamination through inadequate cooling can also occur in storage facilities, particularly bagged feed produced in warmer climates. When there is substandard ventilation or temperature control in storage facilities, the temperature and condensation of the bagged feed can start to increase within the bag.

Furthermore, the way in which the feed is stacked can aid in condensation, providing the required moisture for bacterial growth. With an elevated temperature and moisture present, microbes will start to proliferate which may lead to exothermic reactions and an increase in feed temperature.

Cross-contamination

Cross-contamination occurs when there is a transfer of pathogens into the final product with no additional mitigation step. According to numerous studies, cross-contamination most likely occurs through sampling and processing equipment (Pulido-Landínez, 2019; Parker *et al.*, 2022; Obe *et al.*, 2023). Samples are often contaminated in the laboratory due to the types of instruments used for sampling, which are inadequately prepared, and sample storage. The equipment in the laboratory should be cleaned and sanitised frequently.

In the feed processing facility, cross-contamination is most likely to occur in areas where residue or dust build-up is present and difficult to clean out. Dust is inherently produced during the feed production process. Nevertheless, it is known that the produced dust provides necessary nutrients, moisture and mobility for microbial growth, thus regular removal of dust is essential to mitigate cross-contamination after heat treatment (Gossling *et al.*, 2022). Environmental monitoring programmes often include analysing dust samples at critical control points over time to ensure the risk of pathogen contamination is decreased.

Understanding the life cycle or growth stages of pathogens, such as *Salmonella*, further iterates the need for frequent cleaning to ensure the probability of cross-contamination is lowered. Pathogens have a metabolising (growing) phase and a dormant phase (Gonzalez-Machado *et al.*, 2018).

Many pathogenic *Salmonella* serovars (*Enteritidis, Infantis* and *Typhimurium*) require a high moisture content for metabolism. When the pathogens are actively metabolised, the cells are more susceptible to mitigation through chemical and physical treatments. However, the dormant phase allows for the cells to be protected in a protein or mucous layer. This enables the pathogen to remain alive until there are favourable conditions for active growth.

The dormant stage is often referred to as the pathogens being encased in a biofilm. Currently, there are no known effective chemical treatment methods to break a bacterial biofilm. Therefore, the most effective way to alleviate potentially harmful biofilms is through physical means such as cleaning or heat treatment.

Research indicates there are *Salmonella* serovars that have adapted to be heat resistant and can form biofilms at rapid rates (Merino *et al.*, 2019). Furthermore, these serotypes are often found in the feed or oilcake processing environment on equipment and dust, thus highlighting the importance of dust removal.

Environmental factors

Environmental recontamination often refers to wild birds and rodents which may carry pathogens into the feed mill. Contamination from the environment can also be exacerbated by the weather – for example, prolonged rain or thunderstorms may cause excess moisture to be present in the processing facility, or strong winds may cause pathogens to enter the facility through dust blown in.

Possibly one of the easiest environmental factors to control is personnel on site. Factory workers and other employees carry a risk of bringing pathogens into the feed mill through their shoes, clothes and body. This risk can be easily mitigated through proper biosecurity and sanitising points.

Recontamination reduction

It is evident that recontamination can occur at many places through various mechanisms in the feed processing environment. Some processes can be in place to decrease the risk of recontamination. Monitoring programmes have proven to be one of the most effective methods in decreasing bacterial loads in the environment and final products (Gossling *et al.*, 2022).

Overall, a comprehensive feed safety monitoring programme in feed mills involves a combination of pre-production, production, and post-production activities (such as cleaning schedules and surface disinfection programmes) aimed at delivering safe and nutritious feed to animals. Monitoring programmes are customised based on a 'risk profile' of various critical control points, circumstances and environments.

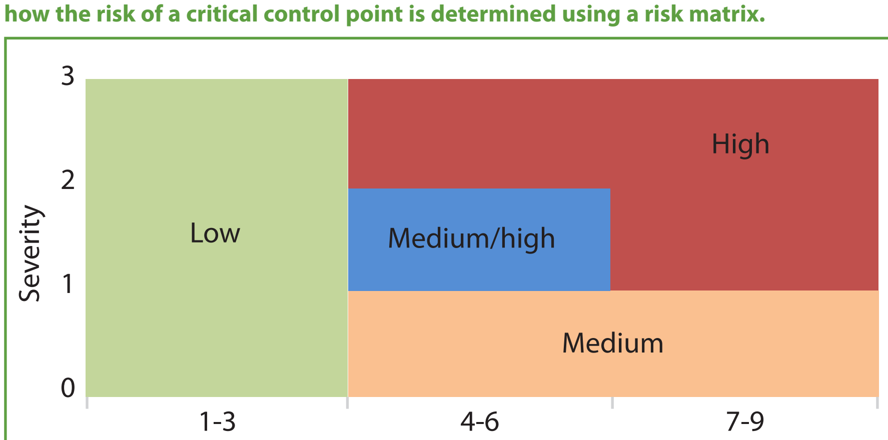
The higher the risk of contamination and the greater the severity thereof, the more comprehensive the pathogen mitigation and monitoring programme would be. For instance, breeder poultry flocks are at a higher risk of bacterial infections (Doughman, 2021). Breeder feed is often in the form of crumbles which go through heating, cooling and crumbling phases. The cooler is a high-risk area for pathogenic growth.

Thus, the cooler for breeder feed would need to be cleaned frequently and chemically treated to ensure the recontamination risk is lowered. Furthermore, the dust from the cooler would be analysed as part of the monitoring programme to ensure the risk of pathogen growth is improving over time. Evaluating the risk for each area where recontamination is likely to occur thus provides a plan of action to alleviate the risk.

The hazard matrix

The matrix works on the premise of probability versus severity based on a

Figure 1: Hazard matrix (adapted from Glöser *et al.*, 2015). The diagram explains how the risk of a critical control point is determined using a risk matrix.



hazard category. In this case, the hazard category is recontamination (Kovačević *et al.*, 2019). The likelihood (probability) of recontamination occurring on the x-axis along with the impact the recontamination will have (severity) will determine the risk of the area. For example, in a layer feed mill where bagged feed is produced, the bagging unit is highly likely to be a point of recontamination as it is the last point of contact of the feed and is challenging to clean (seven out of ten probability).

Layers are often more susceptible to *Salmonella* infections, indicating that if recontamination occurs, it could have a higher impact on the animal (two out of three severity). The bagging unit would thus be rated as 'high' indicating that a more extensive treatment and monitoring programme would be required.

Prevention protocols

Training feed mill employees is a vital element in managing recontamination. Training would involve communicating the dangers of residue, dust and bacteria with practical ways to remove the pathogenic material. Training would also involve how to sample correctly to ensure the samples are not recontaminated before analyses. Implementing strict hygiene protocols for entering and exiting the feed processing facility, as well as cleaning the equipment and plant will further assist in decreasing potential cross-contamination.

Certain measures can be put in place to ensure environmental recontamination is mitigated. For example, ensuring the cooler is on the correct temperature and pressure settings with enough airflow will decrease the amount of condensation in the cooler. Ensuring there is limited access for wild birds to enter the facility through mesh and gates will also assist in decreasing the microbial load. Ensuring that bulk bags and bagged final feed are stored in cool, dry environments, packed correctly and sealed is yet another mechanism to decrease possible contamination.

Ultimately, managing recontamination is comprised of various aspects. Knowing where and why recontamination can occur is the first step in mitigating the negative effects. Record-keeping plays a vital role in identifying high-risk areas where recontamination can occur. Controlling these areas through cleaning and treatment can further decrease the potential harmful effects.

Once record-keeping and controlling are in place, reviewing the areas and treatment options is essential to track progress and re-evaluate the high-risk areas. The implementation of the mentioned mitigation parameters can decrease the negative effects caused by recontamination. ❖

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Investigating the effect of pelleting on the particle size of commercial poultry feeds

By M Naeem, MR Bedford, EJ Burton and MR Azharz

Particle size is an important factor in feed quality because it may affect feed intake and gastric function and thus animal productivity, particularly in poultry (Panigrahi *et al*., 1987; Nir *et al*., 1994a). Particle size has been reported to be connected with feed intake and development of the gastrointestinal tract. Coarsely ground feed ingredients have been associated with longer retention time and improved development of the foregut, especially the gizzard, while excessively ground ingredients have been associated with a higher gastrointestinal passage rate, poor gut health, and low nutrient digestibility.

Feed ingredient particle size reduction can provide numerous advantages in feed processing. According to Goodband *et al*. (2002), decreasing feed raw material particle size can improve mixing characteristics by reducing segregation with other ingredients in the mixture, as well as pelleting capacity and pellet quality. Grinding also increases the available surface area for the digestive enzymes to interact with (Mavromichalis *et al*., 2000).

The particle size of poultry feed is measured by the sieving method where mash particle size is measured by dry sieving, while pellet particle size is measured by wet sieving (Amerah *et al*., 2007). Dry sieving of mash is an easier and less time-consuming (approximately 30 minutes per sample) process compared to wet sieving of pellets. Thus, the ability to predict pellet particle size from a predictive model based on the relevant mash particle size would be a very useful advance in understanding the importance of feed particle size.

The main aim of the current study was to assess the particle size of commercially produced poultry feeds in different forms, mash (pre-pellet) and pellets, and from this determine if the wet sieved particle size of pelleted feed can be predicted from the mash feed using a simple dry sieving approach.

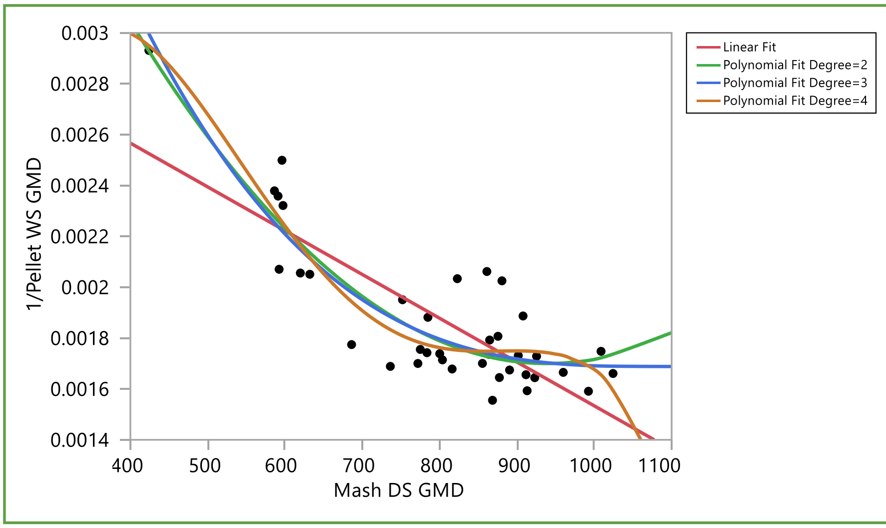
Results and discussion

The pelleting of feed significantly reduced ($P < 0,001$) the geometric mean diameter (GMD) of the original mash feed from 802 to 544,3µm. Pelleting significantly reduced GSD ($P = 0,037$), calculated minimum and maximum GMD ($P < 0,001$), and calculated GMD range ($P < 0,001$). However, the sample proportion of fraction size >4mm was not affected significantly ($P = 0,242$) when the samples were dry (mash) or wet (pellets) sieved. However, the sample fraction of mash 2 to 4mm, 1 to 2mm, and 0,5 to 1mm was significantly higher ($P < 0,001$) than that of pellets. Sample fraction <0,5mm was higher ($P < 0,001$) for pellets compared to mash.

The Pearson correlation analysis showed that there was a strong positive correlation ($r = 0,8052$) between the particle size of mash and pellet, whereas on the conversion of pellet particle size to reciprocal, the correlation was marginally improved and negative ($r = -0,8117$). The quartic model showed the highest correlation ($R^2 = 0,804$) and returned the lowest root mean squared error (RMSE) value of 0,0001. In the analysis of the training and validation data subsets, there was no statistical difference (pellet wet sieved GMD, P = 0,753; mash dry sieved GMD, P = 0,507), confirming no biases in the data sets.

The graphical representation of the fit models is shown in *Figure 1*. The intra- and intercomparison of the models showed that the quartic model for the training data set resulted in the highest R^2 value of 0,8410 with a root average squared prediction error (RASPE) value of 0,0001. However, this R^2 value was reduced to 0,6597 and the RASPE value increased to 0,0002 for this model in the validation data set. The closest R^2 and prediction error values were found in the linear models in both data sets: training and validation.

Figure 1: Fit models between mash dry sieved (DS) geometric mean diameter (GMD) and reciprocal of pellet wet sieved (WS) GMD.



Effect of the pelleting process

The reduction of particle size in pellets compared with the originating mash feed is clearly a result of the process itself. Many factors could be considered such as the addition of moisture in the form of steam, and increased temperature, both of which would make the ingredients softer and more susceptible to the physical stress encountered in pelleting due to the augur of the conditioner and the rollers and die of the pellet press.

The ring die pressure, ranging from 75 to 600kg/cm^2 (Hastings and Higgs, 1980), is quite considerable and combined with the compression/grinding effect of the roller and forcing of the ingredients through the pellet die, this likely is the principal cause of this size reduction.

The current study found that pelleting reduced GMD by approximately 32%. Though particles larger than 4mm were not significantly reduced in pellets, all the particle classes including those larger than 4mm, between 2 and 4mm, 1 and 2mm, and 0,5 and 1mm decreased by approximately 25, 32,35, 44,44, and 64,85%, respectively, in pellets. However, pelleting significantly increased particles smaller than 0,50mm in pellets by approximately 123,75%.

This increase in the proportion of particles smaller than 0,5mm in the pellets may be due to the profound partial grinding effect of pelleting on smaller particles to a higher extent than on the bigger ones, with the help of easier penetration of steam in the smaller particles compared with the larger particles.

Another factor may be that moving water during wet sieving brings smaller particles to the lower sieves. In wet sieving, using water can prevent the clogging of particles. However, it should be noted that wet sieving is more complex and takes much longer than dry sieving. This reduction in particle size may increase the availability and digestibility of feed nutrients due to the thermal treatment of feed. So, the reduction of particle size as a consequence of pelleting a feed must be considered when evaluating a diet's performance.

As mentioned, smaller particle size has been associated with a higher gastrointestinal passage rate, poor gut health, and low nutrient digestibility. Amerah *et al*. (2007) observed that in mash diets coarse grinding of wheat improved weight gain and feed-to-gain ratio compared with medium grinding while pelleting had a negative effect ($P < 0,05$) on nitrogen-corrected apparent metabolisable energy.

Some studies assessing the effect of feed particle size on broiler performance reported that the pelleting process evened out particle size distribution of poultry feeds (Amerah *et al*., 2007; Abdollahi *et al*., 2011) as reduction of particle size due to pelleting was also observed here.

Increase in smaller particles

The results of the current study also agreed with the previous findings of others who have reported that the proportion of particles less than 0,05mm increased and that of larger particles decreased after pelleting (Amerah *et al*., 2007) and this was noted in both finely and coarsely ground diets (Engberg *et al*., 2002).

Further support for the current observations was provided by Svihus *et al*. (2004) who noted a significantly increased proportion of fine particles following pelleting, and that hammermilled mash diets that had 40 to 50% particles smaller than 0,2mm before pelleting resulted in 50 to 60% of particles in this range after pelleting. In the current study, the particles larger than 4mm were not significantly affected by pelleting the mash compared with particles smaller than 4mm, which may be due to the easier penetration of moisture and steam in smaller particles than the bigger particles.

The findings of the current study were in agreement with the findings of Abdollahi *et al*. (2011) and Engberg *et al*. (2002) as these researchers found particles larger than 2 and 1mm reduced in the pellets while particles smaller than 0,075mm were increased. Peron *et al*. (2005) also observed a decrease in the particle size on pelleting the diets. It was observed in the current study all the classes of particle sizes except those smaller than 0,5mm were decreased in the pelleted diets. However, Svihus *et al*. (2004) speculated that larger particles are more prone to be reduced during pelleting.

Other researchers also confirmed that the particle size of the feed reduces with the pelleting process (Liesner *et al*., 2009;

Vukmirović *et al*., 2016). Dirkzwager *et al*. (1998) found that the fraction of fine particles (<600 microns) increased while the proportion of coarse particles (>600 microns) decreased in both mash and pellet when they were wet sieved compared with dry sieved, suggesting wet sieving may reduce particle size, which may be due to hydrolysis of particles or movement of running water.

Indeed, in the current study, the fraction of particles smaller than 0,5mm was reduced in mash compared with the subsequently derived pellets. Thus, the results noted in the current study may be partly due to differential methodologies applied to mash and pelleted feeds.

Increased digestibility

One traditional view is that the smaller the particle size is, the more surface area is available for the interaction of digestive enzymes (Preston *et al*., 2000). On the other hand, it has been reported that poultry prefers larger particle sizes, particularly as they age (Parsons *et al*., 2006; Chewning *et al*., 2012). Although performance has not been studied here, it has been reported previously that the reduction in particle size of feed has brought many benefits to the birds in terms of increased digestibility (Lyu *et al*., 2020).

However, the previous studies evaluated the effect of feed form on the performance and gastrointestinal tract of broilers fed diets without considering the effect of pelleting on particle size. For example, Nir *et al*. (1994b) observed reduced gizzard relative weight and length of jejunum and ileum in birds fed pellets compared with mash, as did Nir *et al*. (1995) with regard to proventriculus weight and Engberg *et al*. (2002) on gizzard and pancreas weight. Amerah *et al*. (2007) also observed lower weight of intestine segments on feeding pelleted diets.

Thus, it appears from the findings of the current study that a reduction in particle size during the process of pelleting may be partially responsible for differences in animal performance on mash and pelleted diets noted in previous work. Engberg *et al*. (2002) reported that the impact of feed form is much more important than that of grinding in broiler growth performance but in their work, they did not measure particle size reduction due to pelleting even though they acknowledged it likely occurred.

Composition of diets

The composition of feed may also affect particle size during pelleting as a high-temperature stream is added which increases moisture level and has variable effects on ingredient physical integrity depending upon their composition (Van der Poel *et al*., 2018). The current study used wheat-soya diets, however, there are no studies available to compare with using the same or different compositions of diet where the impact of pelleting has been studied.

> Pellet particle size (GMD) can be predicted with reasonable confidence and less prediction error from a measure of mash feed particle size (GMD), using the proposed linear model in the current study.

Other factors may also come into play such as the hardness of the main ingredients (e.g., cereals), the moisture level of the feed ingredients, the wear and tear of grinding mills (roller or hammer mill) and the size of the roller gap in the pellet mill. Additionally, conditioning temperature and duration, pellet mill size or production rate, and fat level added may also affect the particle size of the pellets during pelleting. All these factors remain the limitations of the current study, which need further investigation.

Grinding effect

To maintain particle size in pelleting, the grinding intensity of the ingredients can be lowered (Vukmirović *et al*., 2017). However, particle size or grinding has a direct association with pellet quality. It has been reported that grinding plays an important role in overall pellet quality in addition to formulation, conditioning and die size. Methodology to measure the particle size may also have an impact on the results as some studies measuring the particle size have used an agitating object or ball in the sieves (Fahrenholz *et al*., 2010).

Because particle size should ideally be fine to obtain higher pellet quality, a nutritional conflict arises, particularly in the case of poultry feed. To achieve good animal performance, coarse particles are required for chickens for adequate gizzard development. However, because these coarse particles have lesser binding properties, the pellets break more easily, resulting in fines and thus increasing feed loss. In most cases, the grinding effect during pelleting is ignored, but this may influence digestion as much as it does in mash feeds. However, particle size is determined commercially only after grinding, and this work confirms that the particle size in the pellets is likely smaller than that measured in mash.

Conclusions and applications

For wheat-soya-based commercial feeds, the particle size as GMD is reduced significantly by about 32% when the mash is pelleted.

Pellet particle size (GMD) can be predicted with reasonable confidence and less prediction error from a measure of mash feed particle size (GMD), using the proposed linear model in the current study. It can help the poultry feed industry in assessing the pellet particle size as a function of mash particle size. Further work is needed to determine whether a similar relationship exists in maize-soya-based diets as this constitutes the bulk of global animal feed.

Analysis of particle size in pellets is much more likely to yield guidelines for range and maxima and minima for optimum performance, and this work introduces a potentially simple route to start this process, although more work is needed to determine if the choice of ingredients significantly changes the models generated here.

This article has been condensed for publication in AFMA Matrix. For the full article, visit *doi.org/10.1016/j.japr.2023.100390* or email M Naeem at naeem.naeem@ntu.ac.uk.

Bugging out: Insects as fish meal alternatives

Anton Paar

This study explores the viability of insect meal as an economical and sustainable raw material for fish feed. Utilising Torque Tec Instruments, the research focusses on optimising feed composition through rheological analysis. The methodology includes moisture determination and gelatinisation property analysis, enhancing insights into fish feed digestibility.

In light of the escalating expenses and ecological repercussions associated with fish meal production, the utilisation of insect meal emerges as a promising alternative for the cost-effective generation of raw materials in the manufacturing of fish feed. This potential transition is underscored by the remarkable attributes of insect meal, including its elevated protein content and biological valence, which are pivotal elements in the development of sustainable feed formulations.

Anton Paar Brabender Instruments, through its innovative technologies, offers a feasible solution for the exploration and optimisation of fish feed production on a smaller laboratory scale. This entails not only the meticulous analysis of rheological properties in the final product, but also provides a platform for refining the composition of raw materials and enhancing manufacturing processes.

In essence, the integration of Anton Paar Brabender Instruments into the production workflow facilitates a comprehensive approach towards achieving both economic viability and environmental sustainability in the evolving landscape of fish feed manufacturing.

Materials

Three batches of pelleted fish feed were produced using the Brabender twin screw extruder KETSE 20/40 (*Table 1* and *Figure 1*). For manufacturing, the contained fish meal was replaced with no-fat black soldier fly (*Hermetia illucens*) larvae meal in concentrations of 0,50 and 100% (*Figure 2*).

Method

The investigation into the digestibility of fish feeds, specifically in relation to their gelatinisation properties, was conducted through a systematic approach involving key procedures. The initial step involved the determination of moisture content in the pellets. This was carried out using the MT-CA method, a reliable technique known for its precision in moisture analysis.

Following the moisture determination, the extrudate underwent a grinding process utilising the Break Mill SM4. This crucial step aimed to achieve a consistent and homogenous particle size, preparing the samples for subsequent analysis.

The gelatinisation properties of the meals were then comprehensively examined using the MVAG (*Figure 3*). This analytical tool facilitated a thorough exploration of key characteristics associated with gelatinisation, providing valuable insights into the thermal transitions and behaviour of the feed components.

These procedures were systematically executed to unravel the intricate relationship between fish feed digestibility and its gelatinisation properties. The selected methods were chosen for their precision and reliability, ensuring accurate

Figure 1: Screw configuration, KETSE 20/40.

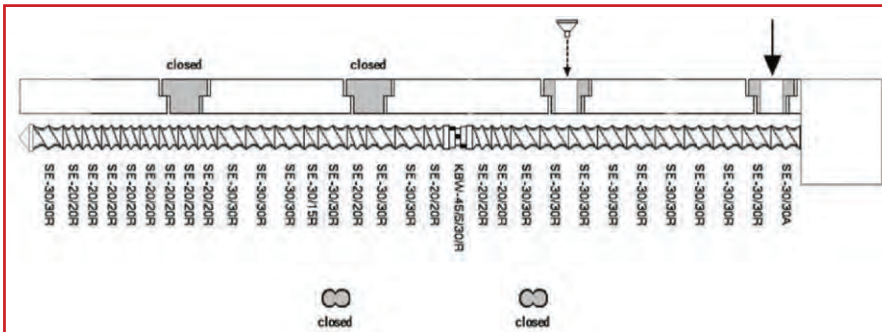


Table 1: Process conditions, KETSE 20/40.

Screw speed	400rpm
Temperature profile	Hz 1: 40; Hz 2: 65; Hz 3: 90; Hz 4: 115; Hz 5: 135
Feed moisture	24%
Throughput	7,5kg/h
Nozzle diameter	2,5mm

Table 2: Amount of fish meal substituted by black soldier fly larvae meal.

	0%		50%		100%		Pressure value
	Mean value	Standard deviation	Mean value	Standard deviation	Mean value	Standard deviation	
Expansion ratio (%)	117,0	± 7,5	138,7	± 16,2	142,8	± 11,2	0,0001
Bulk density (g/l)	278,5	± 1,7	236,5	± 1,3	231,0	± 1,3	0,0000
Breaking force (N)	384,2	± 6,5	370,2	± 10,9	319,0	± 7,0	0,2023
Durability index (%)	98,7	± 0,1	98,5	± 0,1	98,7	± 0,1	0,0254
Die pressure (bar)	129,0	± 7,9	114,6	± 6,8	114,1	± 3,7	0,0000
Specific mechanical energy (Wh/kg)	92,4	± 4,9	93,5	± 9,5	97,4	± 6,5	0,1525
Product temperature (°C)	135,0	± 0,0	135,0	± 0,0	135,0	± 0,0	1,0000

assessments and contributing to the overall robustness of the research findings.

Results and discussion

The results of the MVAG measurements (*Figure 3*) have shown that a higher concentration of insect meal lowers the residual gelatinisation of starch, which is directly related to the digestibility of fish.

Considering the results shown in *Table 2*, it can be said that using insect meal increases the expansion ratio and specific mechanical energy of the batches, while decreasing bulk density, breaking force and die pressure.

Summary

Trials showed that insect meal can be used as a substitute for fish meal in fish feed manufacturing. It should be noted that the extrusion process is subject to significant influences and that product property changes have to be determined depending on the intended use of the feed.

With regard to further investigations, changing the process conditions (e.g. varying the extruder screw speed) might optimise the product properties. Feeding trials are needed to evaluate the feed acceptance and nutritional value of the product. Studies indicate that the nutritional value of black soldier fly larvae meal is lower than the nutritional value of fish meal. Therefore, higher quantities might be required for a proper diet.

Figure 2: Extruded fish feed (whole and ground).

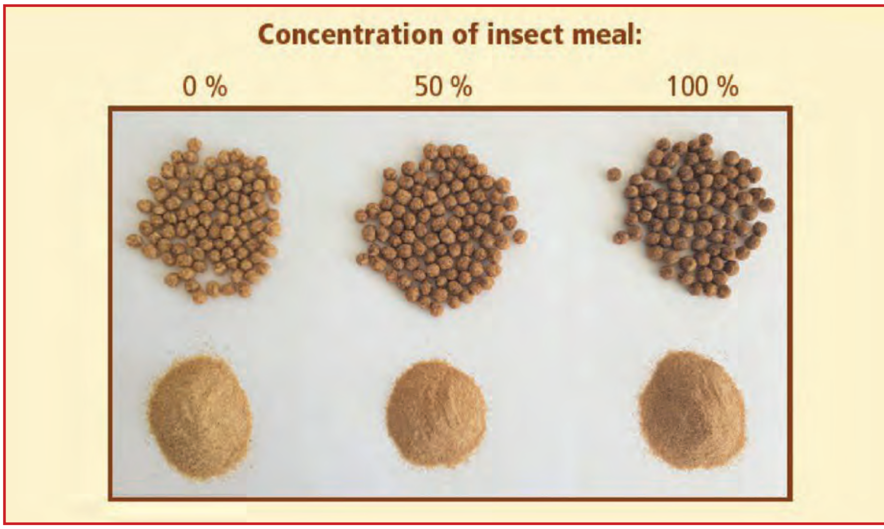
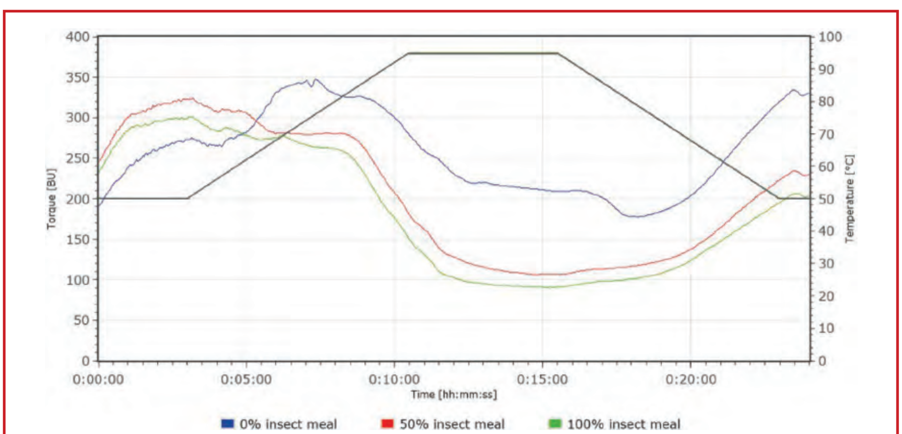


Figure 3: MVAG measurements.



Further research is needed in order to improve the omega-three fatty acid spectrum of the fish-free product. The addition of micro algae with high omega-three contents might be a solution.

However, the extrusion process must be adapted when fish meal is substituted by insect meal. Should black soldier fly larvae meal become cheaper than fish meal, it could prove to be a viable option to reduce the environmental impact of aqua culture in the future. ❖

References available on request. Contact Jocelyne Abreu, business unit manager for characterisation, Anton Paar Southern Africa, on +27 82 808 6945 or at Jocelyne.abreu@anton-paar.com, or visit *www.anton-paar.com/za-en/products/details/brabender-mt-ca/*.

Challenges and opportunities for upcycling food waste to animal feed

By Zhengxia Dou, Ellen S Dierenfeld, Xiaozhong Wang, Xinping Chen and Gerald C Shurson

Although awareness has increased along with widespread efforts to reduce it, the scale and magnitude of global food loss and waste (FLW) have not abated. Compared to earlier estimates of 1 300 million tonnes (Mt) (FAO, 2011), recent studies using updated FLW parameters reported global FLW to be roughly 1 640Mt (Wang *et al*., 2023) to 2 500Mt (WWF, 2021), which represents 32 to 40%, respectively, of the food meant for human consumption.

Many factors contribute to FLW and affect the effectiveness of reduction efforts, especially infrastructural constraints and consumer food behaviour (Zurek *et al*., 2022; Cattaneo *et al*., 2021; UNEP, 2021). For the enormous amounts of food waste continuously generated, a recovery-treatment-reuse approach is essential for achieving a circular food system for a more sustainable future.

Food waste upcycling to animal feed offers the greatest value compared to all other options down the hierarchy (e.g., Dou *et al*., 2018, 2021; Shurson *et al*., 2020, 2022c). By feeding food waste biomass that is unfit for human consumption to meat, milk and egg-producing animals, multiple sustainability benefits of improved food security, climate mitigation and resource conservation can be achieved.

Resource and climate burdens

The production of uneaten food contributes significantly to climate change, unsustainable resource extraction, biodiversity loss, land degradation, depletion of finite essential resources such as phosphorus (P) and aggravating reactive nitrogen (N) problems, among other unwanted environmental consequences (e.g. Crippa *et al*., 2021; Rosenzweig *et al*., 2020; Rockström *et al*., 2009; Leinweber *et al*., 2018; Chen *et al*., 2016; Reis *et al*., 2016).

Although many of the adverse environmental consequences are not readily quantifiable, a few key impact metrics have been defined quantitatively. For resource use, about 30% of arable land, 20% of freshwater withdrawals, and 38% of total energy consumption are used in vain to produce food that is lost and wasted globally (FAO, 2013).

Results from the most recent studies published in 2023 have shown no improvement in reducing FLW trending and associated carbon footprint. The amounts of global FLW have not decreased from a decade ago (Wang *et al*., 2023; FAO, 2011), and nor have country-level per capita food waste estimates declined (EPA, 2023a).

Animal-based upcycling

Unparalleled by any other options, upcycling food waste to animal feed reduces the need for conventional feedstuffs such as grains and oilseed meals, which in turn brings about a cascade of resources and environmental co-benefits such as spared land, and fertiliser and avoided N and P losses (e.g., Dou *et al*., 2021; Nath *et al*., 2023, Nakaishi *et al*., 2022).

However, not all food waste is suitable for use in animal feeds. High-quality materials that contain at least modest amounts of energy and/or protein with negligible feed safety risks are suitable for thermal treatment and conversion to animal feed, whereas poor quality or high potential feed safety risk biomass should be treated via composting or anaerobic digestion.

Better than landfilling

Making feed from food waste is not carbon neutral, as food waste collection, transport and treatment processes involve energy consumption. A few studies have evaluated the carbon footprint using a lifecycle assessment approach for real-world food-to-feed operations. Kim and Kim (2010) reported that the collection-transport-processing of one tonne of food waste in South Korea resulted in a carbon footprint of 61kg CO_2-e for making wet feed, but 200kg CO_2-e for dry feed, compared with an estimated emission of 1 010kg CO_2-e if food waste was landfilled.

By and large, the carbon footprint of converting food waste to animal feed for safe feeding is relatively small (roughly 5 to 20%) of the emissions compared with landfilled food waste. This is important information for policymakers as well as on-the-ground decision-making personnel.

Supermarket waste in swine diets

Maize and soya bean meal are major feed ingredients used in modern animal production around the world. Studies have found that supermarket food waste has nutritional attributes comparable to that of maize and soya bean meal in swine diets (Jinno *et al*., 2018; Fung *et al*., 2019).

The amount of land attributed to the production of feed from food waste is negligible and considerably less than the land required for maize and soya bean production. In fact, the production, processing and use of conventional feed grains and oilseed meals in animal feed are major factors contributing to the overall environmental burdens associated with animal agriculture (Andretta *et al*., 2021; Van Zanten, 2022).

For global regions or countries that rely heavily on imported feed ingredients to support their animal production, such as China and many European countries, using locally sourced food waste in circular agrifood systems can offer great sustainability benefits.

Citrus and lactating dairy cows

Many studies have reported the utility of incorporating food waste into diets and documented animal productivity performance. While focussing on animals and their performance metrics, rarely did those studies provide quantitative information on resource use and environmental impacts.

A recent study by Baker *et al*. is unique in that it used an integrated and systems approach to examine the incorporation of culled citrus from a processing centre, as a source of food waste, into dairy cow diets to determine the impact on milk production and quality, feed cost, use of conventional feed ingredients, and environmental benefits.

Cows fed the diet containing 7,1kg fresh citrus waste per cow/day had production parameters (milk yield, milk fat and protein) similar to those of cows fed the control diet, while reduced use of conventional feed ingredients resulted in a daily feed cost savings of 37 cents/cow/day. On a whole herd (160 cows) basis, the amount of citrus waste to be upcycled as lactating cow feed would total 415 tonnes annually.

This would allow reducing the amounts of maize, maize silage, soya bean meal and other feed ingredients in the diet, which in turn would spare 14ha cropland, 944kg N fertiliser, 480kg P fertiliser, 40kg herbicides and 83 040m³ water for growing the feed crops (*Table 1*). Estimated carbon mitigation capacity would total 387 360kg CO_2-e, much of which was attributed to emission offset with citrus waste diverted away from landfills and towards the farm as cattle feed. The fertiliser and herbicides spared would not only reduce on-farm feed production costs, but also bring about a cascading effect such as decreasing nutrient losses to water and mitigating other environmental consequences.

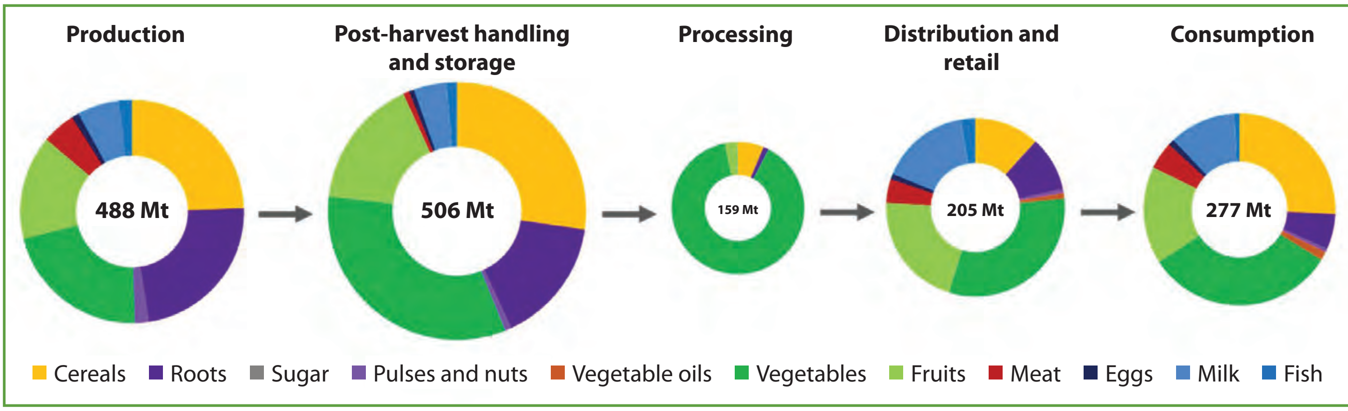
The water-sparing effect is equally significant because of the effects of climate change on rainfall patterns and increased frequency of drought conditions in major agricultural regions of the United States. In addition, ensiling fresh citrus waste with grass hay was used to prevent fresh citrus

Table 1: Reduction of conventional feed ingredients in treatment diet with the incorporation of fresh citrus waste (7,1kg fresh weight/cow/day), relevant resources to spare due to the reduced feed ingredients, and GHG emission mitigation capacity from spared fertilisers and herbicides as well as landfill emission offset. All data are for per cow per year[1].

Feed reduction		Resources to spare					Emission mitigation	
	Kg DM cow^{-1} yr^{-1}	Land ha (x 10^{-3})	Water m³	N fertiliser kg	P fertiliser kg	Herbicides kg	Reduced fertiliser etc., kg CO_2-e	Landfill emission offset kg CO_2-e
Maize silage	182	11,54	167,9	1,93	0,45	0,06		
Rye silage	29	46,87	123,5	2,10	0,62	0,04		
Grass hay	11	2,43	10,5	0,25	0,10	0,01		
Shelled maize	73	7,46	67,2	1,25	0,58	0,04		
Soya beans	110	20,28	150,1	0,39	1,27	0,11		
Total[2]	405	88,58	519,20	5,91	3,01	0,25	104	2 421[3] (7 264)

[1]Data adapted from Baker et al. (in this SI). [2]Total may not add due to rounding. [3]Based on global warming potential of methane, 28 CO_2-e for 100-year timescale, 84 for 20-year timescale (in parenthesis).

Figure 1: Distribution of global food loss and waste at different stages of the food supply chain, as 11 major food groups.



Data used for calculating the relevant amounts included: (i) primary food production (FAO Food Balance Sheet, 156 countries, 2017 data), (ii) food flow and partition across the supply chain, as described by Wang et al. (2023), and (iii) food loss and waste parameters synthesised from the literature, as reported in Wang et al. (2023). Aggregated global total food loss and waste amount (1 640Mt) has been reported in the recent study of Wang et al. (2023) investigating global food nutrients availability.

from spoiling and preserve its feeding value during long-term storage, and supported optimal milk production when fed to dairy cows on a commercial farm.

Potential environmental impacts

Despite prevailing European Union (EU) legislation restricting food waste feeding to animals, there is no lack of European interest in the economic and environmental benefits of using food waste in animal feed. Zu Ermgassen *et al*. (2016) estimated that changing the EU legislation to allow safe use of food waste in animal feeding (e.g., employing the Korean or Japanese models) could spare 1,8 million hectares of agricultural land, reducing the land use attributed to EU pork production by 20%.

Salemdeeb *et al*. (2017) used a hybrid consequential lifecycle assessment method to estimate a broad range (14 indices) of environmental and health impacts of converting food waste to animal feed. They concluded that diverting food waste to animal feed had the least environmental and health burdens for almost all indices, compared with alternative treatments of composting and anaerobic digestion. Most of the environmental benefits were attributed to the substitution effect of using food waste to partially replace conventional feedstuffs in diets.

More recently, Boumans *et al*. (2022) explored the prospect of food waste feeding to support EU pig and poultry production if the current legislation was modified to allow such use. Other researchers have suggested that because animals fed conventional diets consume more human-edible protein than they produce, the land on which feed crops are grown could have yielded more human-edible protein if food crops were grown instead (Van Zanten *et al*., 2016; Hennessy *et al*., 2021).

Food waste that is not suitable for human consumption can be thermally processed and diverted for use in pig or poultry diets to reduce food-feed competition and enhance food system efficiency and sustainability.

Food waste sources

Optimal animal performance and health are achieved by knowing the nutrient requirements of the animals to be fed, the energy and digestible nutrient composition of the specific feedstuffs to be fed and using this information to formulate complete diets. Therefore, the key to effectively using various types of food waste in animal feeds is knowing the nutritional composition and digestibility of the sources fed.

However, due to high variability in nutrient composition within sources, nutritionists frequently either limit diet inclusion rates to <10% of the complete diet or add a 'safety margin' as insurance to avoid underfeeding energy or nutrients when less nutritionally defined ingredients are used in commercial diets.

Accelerating upcycling

For the massive amounts of food waste continuously generated, the potential of capturing some of the embedded nutrients via animal feeding is huge. Japan has created an effective working model for converting food waste into animal feed that could be used in many countries globally.

Through government policies and incentives, Japan has succeeded in achieving zero (organic) waste at landfills, and the development of its Eco-feed programme has helped to reduce reliance on imported feed ingredients by collecting and processing locally produced food waste into animal feeds (Suguira *et al*., 2009; Nakaishi and Takayabu, 2022). The success of this programme is a direct result of enabling legislation, a well-developed regulatory framework, strong public-private partnerships, and stakeholder participation.

Globally, the path towards greater adoption of food waste upcycling via animals for multi-sustainability benefits starts with enabling policies. It also requires de-risking strategies to address feed safety and animal health concerns and fill knowledge gaps.

Enabling policies

Policies that indiscriminately prohibit the use of food waste materials as animal diet ingredients are the greatest barrier to achieving widespread adoption of food waste upcycling into animal feed. Prevailing EU legislation (since 2002) is known for its strict ban on the use of food waste from animal feeding due to risks of disease transmission.

Government regulations must be adapted to incorporate new evidence and knowledge to address the urgent need to improve the circularity of our global food system. The European Commission's

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circular economy action plan (EC, 2015, 2020) is an opportunity to create more in-depth discussions to re-examine these opportunities. Recently, an amendment was made to *Annex IV in Regulation No 999/2001* to allow processed animal proteins derived from pigs to be fed to poultry and vice versa (EFPRA, 2021). Further modifications are needed to allow and stimulate broader use of food waste as circular feeds for sustainable food production (Boumans *et al*., 2022).

Similarly, in other regions or countries, the regulatory framework should not be the limiting obstacle for developing more circular food systems that encourage the use of appropriate, low-risk food waste sources in animal feeds. Further, for food waste materials that are suitable for animal feeding, policies that prioritise lower-hierarchy alternatives such as composting or anaerobic digestion cause lost opportunities to capture economic benefits and have a greater reduction impact on the environmental footprint of food production.

Ambiguity or lack of appropriate policies can also impede the adoption of safely upcycling food waste to animal feed.

De-risking strategies

Concerns over risks of disease transmission are a major factor limiting the use of food waste in animal feeding (Georganas *et al*., 2022). The concerns are valid and understandable, although there has been no direct evidence that implicates properly treated food waste causing animal disease outbreaks.

The scientific basis is clear regarding the temperature and duration of thermal treatment needed to inactivate various infectious disease agents (Shurson *et al*., 2020). Field-based evidence is abundant and shows that feeding properly treated food waste is safe for animals and public health risks are minimal. Nevertheless, feed safety and animal health are of utmost importance; additional de-risking strategies and practical measures besides thermal processing can help mitigate potential problems.

The guiding principle should be to treat food waste biomass not as garbage to discard, but rather as valuable feedstocks that can be recycled into animal feed to enhance sustainable animal production. The material flow and handling of food waste biomass, from collection to storage to transport to processing until feed is produced, should adhere to this principle.

Towards this outcome, source separation is a critical control point. When and where food waste is generated, it should be separated from non-food items into designated clean containers, stored properly (e.g., cold storage or immediate dehydration for perishables, if conditions allow), and transferred in a timely manner to minimise spoilage.

Further, the entire food-waste-to-feed operation should be a closed-loop system for the prevention of physical, chemical and biological contamination. Implementation of the 'not-garbage but feedstock' principle every step of the way is essential to successful and sustainable upcycling, as exemplified by the Japanese Eco-feed programme.

Species-specific feeding is a strategy that allows matching food waste types/sources with animal species to support maximal use of the biological value of nutrients while minimising disease risks (Dou *et al*., 2021; Shurson *et al*.).

Market unsellable and post-consumer food wastes are usually mixtures of plant- and animal-source materials. Since prions (associated with bovine spongiform encephalopathy) are only infective in ruminants, and the African swine fever virus is only infective for pigs, cross-species feeding of such food materials should not be an issue where adequate processing controls and monitoring programmes are in place to ensure feed safety (Shurson *et al*.).

On the other hand, plant-based biomass free of animal tissues (generated in pre-consumer stages) but high in fibre are most suitable for ruminants because of their digestive capabilities of using fibre as an energy source. Moreover, ruminants are generally resistant and less susceptible than monogastrics to feed spoilage (Gallo *et al*., 2015).

Reducing risks via dilution adds another layer of protection for the safe use of food-waste-derived feeds in animal production. This may be achieved by limiting the diet inclusion rate for any animal species (Section 3,2; Shurson *et al*., 2022b). Also, in the feed supply sector, the incorporation of processed food waste at low inclusion rates into the formula of compound feeds can provide an effective buffering solution to address quality (feed safety) and nutrient variability concerns. Developing accurate calibrations and the use of near-infrared spectroscopy can also be an effective way of dynamically determining nutrient concentrations of highly variable food waste streams to enable precision feed formulation in near-real time.

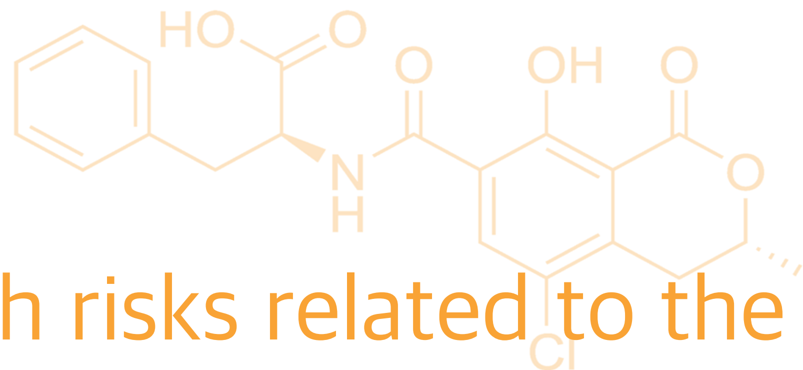
The safety of feeds derived from food waste for animal-based upcycling requires cooperation among food waste generators, collectors, handlers and processors, feed suppliers, farmers and their nutrition and animal health advisors. Regardless of the type or source of feed ingredients, there is no single method or approach that is sufficient for dealing with every possible feed contamination threat (Čolovíc *et al*., 2019a, 2019b). Integrating de-risking strategies and good management practices, combined with proper regulatory and technical control mechanisms, can make the use of food waste in animal feeds safe and viable.

Information gaps

Strategically, the high-level questions are: Where did all the lost and wasted food go? How much can be recovered and diverted to safe animal feed? As previously mentioned, up to half of the global FLW may be accounted for in municipal solid waste streams, whereas much of the rest remains an enigma. Going forward, understanding the food waste landscape of 'what (type), where (source), and how much (amount)' is a necessity for designing policies and directing resources to make meaningful changes.

Comprehensive data on physical, chemical and biological safety measures of various food waste sources used in feeds are scarce, despite abundant feeding trials demonstrating the safety and utility of various food waste materials.❖

This article has been condensed for publication in *AFMA Matrix*. For the full article visit *www.sciencedirect.com/science/article/pii/S0921344924000120* or email douzheng@vet.upenn.edu.



Animal health risks related to the presence of ochratoxin A in feed

By EFSA Panel on Contaminants in the Food Chain: Dieter Schrenk, Margherita Bignami, Laurent Bodin, James Kevin Chipman, Jesús del Mazo, Bettina Grasl-Kraupp, Christer Hogstrand, Laurentius (Ron) Hoogenboom, Jean-Charles Leblanc, Carlo Stefano Nebbia, Elsa Nielsen, Evangelia Ntzani, Salomon Sand, Tanja Schwerdtle, Christiane Vleminckx, Heather Wallace, Jürgen Gropp, Gunther Antonissen, Guido Rychen, José Ángel Gómez Ruiz, Matteo Lorenzo Innocenti, Elena Rovesti and Annette Petersen

Following a request from the European Commission, the European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain (CONTAM Panel) evaluated the risks to animal health and transfer from feed to food of animal origin related to the presence of ochratoxin A in feed. The previous assessment relating to the presence of ochratoxin A (OTA) as an undesirable substance in animal feed was published by EFSA in 2004.

The *2004 Opinion* was used as starting point; nevertheless, since 2004 new scientific information has become available on the risks to animal health and transfer to food of animal origin related to OTA in feed, which was incorporated in the present assessment. Information from the *2020 CONTAM Panel Opinion* on OTA in food was also used for this *Opinion*.

Role of temperature

OTA is produced by several fungi of the genera *Aspergillus* and *Penicillium*, including *P. verrucosum*, *A. ochraceus* and *A. carbonarius*. OTA is described as a heat stable toxin; nevertheless, OTA reduction during heating at 175°C and the formation of its degradation products have been shown. Feed materials and compound feed undergo, during production, different steps by which temperature impacts feed (e.g. conditioning, pelleting, expansion, extrusion, solvent extraction). No data on OTA isomers in feed have been identified.

Numerous analytical methods are available for OTA in food (both 'single-toxin' and 'multi-toxin' methods). The most used analytical methods for food are usually also used for feed. Reference materials for OTA in feed are commercially available and proficiency tests are offered by the European Union (EU) reference laboratory for mycotoxins and plant toxins, as well as by private providers.

Plasma protein binding

OTA is rapidly and extensively absorbed in the gastrointestinal tract in most animal species. In general, OTA is characterised by strong plasma protein binding. Inter-species differences have been observed, with sheep having lower plasma protein binding for OTA compared to other ruminants; turkeys had the highest plasma protein binding of different poultry species and donkeys had lower plasma protein binding compared to pigs.

In contrast to other animal species, very low oral bioavailability and lower plasma protein binding has been observed in fish.

OTalpha

In cattle and sheep, it has been suggested that ruminal microbiota play a major role in the extensive hydrolysis of OTA into ochratoxin alpha (OTalpha). Moreover, OTalpha has been detected at low amounts in the urine of pigs. *In vitro* data showed that OTA in chickens, pigs, goats and cows is metabolised via hydroxylation and dechlorination. OTA is excreted both via urine and faeces in all animal species.

Possibility of OTA transfer

Transfer to products of animal origin was described in the *2004* and *2020 CONTAM Panel Opinions*, with very similar conclusions. Newly available studies confirm the conclusions from the previous *Opinions* that there is a low transfer of OTA from feed to milk in ruminants and donkeys, as well as to eggs from poultry. In pigs, OTA has been found mainly in the liver and kidneys. For all other animal species, no information is available about transfer from feed to food of animal origin.

Animal products used as feed materials in animal nutrition could contain OTA (from the transfer from feed to organs/tissues). Of certain relevance are the kidneys and blood which could contribute, in combination with naturally contaminated plant feed materials, to the exposure of animals, particularly

carnivore species; nevertheless, this contribution is likely to be low, based on the limited use of these feed materials.

Toxicity and resistance

Studies in piglets indicate that OTA impairs the function and structure of the kidneys and liver, while in growing pigs, long-term exposure affected the growth performance at the only tested level. A number of new studies were available on OTA toxicity in poultry.

In growing chickens (chickens for fattening, reared for laying and breeding), OTA caused an increase in liver and kidney weights, a decrease in thymus weight, and was associated with liver lesions. Immunosuppression and a depression of zootechnical performance (e.g. bodyweight gain, feed/gain ratio) were found. A comparable picture results from studies concerning OTA in laying hens with significantly reduced egg mass production and deteriorated feed-to-egg ratio.

A decrease in the growing performances of weaned rabbits was identified following exposure to OTA. No experimental data on OTA toxicity in horses was reported in the *2004 EFSA Opinion*. Although it has been suggested that, like other monogastric species, solipeds might be more susceptible to OTA than ruminants, no relevant reports documenting adverse effects of the mycotoxin in those species were retrieved from the recent literature.

From the fish data available, the salmonids appeared to be relatively resistant to OTA with no measurable effects observed up to the highest feed concentration dose tested (2mg OTA/kg feed). Depression of zootechnical performance, increased intestinal permeability and alteration of hepatopancreatic tissue were found in various juvenile herbivorous fish species.

Established reference points

The CONTAM Panel considered the following as reference point (RP) for animal health adverse effects:

- For pigs: 0,01mg OTA/kg feed.
- For growing chickens and hens: 0,03mg OTA/kg feed.
- For rabbits: 0,01mg OTA/kg feed.
- For ruminants, the CONTAM Panel concluded that it is not possible to derive an RP due to the lack of information. Nevertheless, several studies assessing the effects of OTA in castrated adult male sheep, calves, lactating ewes and goats demonstrate the protective function of the ruminal microbiota. This was shown for levels up to 3,5mg OTA/kg in complete feed for sheep.
- For herbivorous fish: 0,5mg OTA/kg feed.
- Although an indication of toxicity in dogs and farmed mink is given, the CONTAM Panel could not derive RPs for these animal species and neither for cats, where no information could be retrieved.

OTA levels and risks

A total of 10 757 analytical results on OTA in feed were initially extracted from the EFSA database (sampling years 2012 to 2021). After assessment, data cleaning and conversion based on dry matter (DM), data on a total of 9 184 samples were made available. The highest OTA levels were reported for 'horse beans' and 'lucerne meal'. Among cereal grains, the highest levels were reported in 'barley grain', and complete feed for pre-ruminant calves among the compound feed samples.

Dietary exposure was performed using different scenarios based on either model diets composed of feed materials or compound feed (complete and/or complementary). Forages were also included for ruminants and horses. Exposure was performed using either a mean or a high-exposure scenario (using the highest reliable percentile based on the number of samples available).

Risk characterisation was performed for those animal species for which an RP could be identified, namely chickens for fattening, laying hens, weaned pigs, pigs for fattening, sows and rabbits. Exposure was derived for salmonids, but an RP could only be derived for herbivorous fish, therefore the risk for fish could not be characterised.

The CONTAM Panel characterised the risk comparing the exposure against the relevant RP and expressing the exposure as a percentage of the RP. A percentage below 100 was considered a low risk. For weaned piglets the exposure amounted to 14 to 86% of the RP, for sows it was 11 to 49%, while for growing pigs it was 11 to 54% of the RP.

For chickens for fattening and hens, the exposure amounted to 2 to 17% of the RP, and for rabbits it came to 16 to 55%. The intervals ranged between the lowest lower bound (LB) and highest upper bound (UB) for two exposure scenarios. The CONTAM Panel considers that this indicates a low risk for adverse health effects.

Uncertainty analysis

Uncertainty analysis was performed for the assessment. The uncertainties were identified and prioritised by the experts based on their potential input on the risk assessment output. For the animal species for which it was possible to characterise the risk (poultry [hens and chickens for fattening], pigs and rabbits), the CONTAM Panel considers that the risk related to OTA in feed for adverse effects is very likely (95 to 99% certain) to be low.

Recommendations

The CONTAM Panel concluded with a few recommendations. Further information is needed on OTA TK in animal species, particularly in solipeds, dogs, cats and farmed mink. In addition, further data are required on the adverse effects of OTA in ruminants, solipeds, dogs, cats and farmed mink.

Regarding the submission of OTA occurrence data to EFSA, it is urged to provide adequate information on the feed samples analysed. This refers to reporting at least information on the expression of results and the moisture content (if the results are expressed in whole weight), and sufficient details on the samples analysed (e.g. target animals for the complete/complementary compound feed).

The use of the most sensitive methods for the analysis of OTA in feed materials is recommended to reduce the uncertainties linked to the LB-UB estimations.

This article has been condensed for publication in *AFMA Matrix*. For the full article, visit *doi.org/10.2903/j.efsa.2023.8375* or email feedco@efsa.europa.eu.

Physical effectiveness of maize silage fractions stratified with the Penn State Particle Separator

By FA Piran Filho, JM Bragatto, CS Parra, SMS Silva, PJ Roco, LF Ferraretto, MN Pereira and JLP Daniel

Providing adequate physically effective neutral detergent fibre (peNDF) in dairy rations is required for promoting chewing, saliva production, ruminal mat formation and rumen motility (Allen, 1997; Humer *et al*., 2018), decreasing the risk of sub-acute ruminal acidosis (SARA) (Zebeli *et al*., 2012).

Despite the benefits of providing peNDF by feeding long forage particles, excess peNDF limits dry matter intake (DMI) and lactation performance (Yang and Beauchemin, 2007; Zebeli *et al*., 2009). Therefore, dairy nutritionists are often challenged with the task of formulating diets that maintain high intake and productivity without compromising rumen health.

Although NDF concentration is measured by chemical analysis (Mertens, 2002), the physical effectiveness factor (PEF) can be determined from animal responses (Armentano and Pereira, 1997) or by the fraction of feeds retained on a set of sieves (Mertens, 1997).

The Penn State Particle Size Separator (PSPS), composed of 19 and 8mm-diameter sieves and a pan, was proposed by Lammers *et al*. (1996) to monitor the particle size of feeds and total mixed rations (TMR). Although other versions have emerged, with the addition of 1,18 (Kononoff *et al*., 2003) and 4mm sieves (Heinrichs, 2013), many nutritionists still use the first model (two-sieve model; NASEM, 2021).

Whole-plant maize silage (MS) is the main forage source for high-producing cows worldwide, and in most diets, MS is a primary source of peNDF in the diet. However, to the best of our knowledge, the physical effectiveness of NDF from MS fractions retained on each PSPS sieve is unknown. Integrating the PSPS with an evaluation of animal responses to determine PEF could aid in elucidating the true effectiveness of MS particles and would be a practical tool to balance the particle size and peNDF concentration of dairy rations.

Therefore, this study aimed to compare the physical effectiveness of MS fractions stratified with the PSPS for lactating dairy cows. We hypothesised that the NDF from particles greater than 19mm has equal or lower physical effectiveness than the NDF from 8 to 19mm particles, due to the mastication of particles before swallowing (Schadt *et al*., 2012) and sorting behaviour against long particles (McCarthy *et al*., 2018). In addition, despite the smaller particle size, MS particles <8 mm may provide some physical effectiveness, but to a lesser extent than particles >8 mm.

Treatments

The four experimental diets were: (1) CON (control): 17% forage NDF (fNDF) from MS (basal roughage); (2) PSPan: 17% fNDF from MS + 9% NDF from MS particles <8mm stratified with the PSPS; (3) PS8: 17% fNDF from MS + 9% NDF from MS particles 8 to 19mm stratified with the PSPS; (4) PS19: 17% fNDF from MS + 9% NDF from MS particles >19 mm stratified with the PSPS.

Results and discussion

Particle size of feeds

Particle size distribution of experimental diets reflected the inclusion of the respective MS fractions, with differences in particle size distribution being more noticeable for the PSPS than the dry sieving procedure. Compared with the PSPS, dry sieving promoted a displacement of particles from the 8mm sieve to the 1,8mm sieve and pan. Such divergence between sieving methods was likely due to the differences in moisture content (as fed versus dry sample), fragmentation of dry particles during handling, type of agitation method, pore shape and thickness of screens (PSPS versus Ro-Tap).

Actual particle lengths of MS fractions, measured manually with a scanner, highlighted that the MS fraction passing through the 8mm sieve (pan) had sizes ranging from 1 to 11mm, with a greater proportion of particles between 1 and 8mm, and mean, mode and median values of 4,67, 4,5 and 4,4mm, respectively. The MS fraction retained on the 8mm sieve had the highest proportion of particles between 5 and 30mm, with a 13,2mm mean, 10mm mode and 11,5mm median.

The CS fraction retained on the 19mm sieve had mean, mode and median values of 37,9, 30 and 30mm, respectively. The large range found for MS >19mm (1–175mm) was due to the occurrence of extreme sizes.

Milk yield and composition

Cows fed CON had lower fat-corrected milk (FCM) and energy-corrected milk (ECM), in addition to milk fat concentration and yield, compared with PS8 (*Table 1*). Also, the milk fat-to-protein ratio was lower than CON. Diets with lower forage NDF and greater starch concentration (i.e., CON treatment) often induce milk fat depression (Swain and Armentano, 1994; Clark and Armentano, 1999; Piperova *et al*., 2000), likely due to the accumulation of ruminal fatty acid (FA) biohydrogenation intermediates that inhibit fat synthesis in the mammary gland (Griinari *et al*., 1998).

Under conditions of low ruminal pH, as verified for the CON treatment, PUFA are only partially biohydrogenated, producing bioactive FA, such as trans-10, cis-12 CLA, and trans-10 18:1, which can reduce milk fat (Griinari *et al*., 1998; Colman *et al*., 2010; Dewanckele *et al*., 2020). Compared with PS8, CON tended to reduce ECM yield per unit of dOM intake because of the reduced ECM.

Cows fed PS19 had lower yields of milk, FCM, ECM, fat and protein and a tendency for lower lactose yield than cows fed PS8. The lower productive performance is partially related to reduced feed intake observed for PS19, compared with PS8, as ECM/DMI did not differ across treatments. In contrast, PSPan led to intermediate yields of milk and components, compared with CON and PS8, except for milk-fat yield, which was greater for PSPan than CON.

Overall, lactation performance results highlight the vital interaction among fNDF, starch concentration and digestibility, and dietary particle size and distribution (NASEM, 2021). Higher starch concentrations in low-forage diets or coarser particles in high-forage diets were detrimental to performance.

Milk concentrations of protein, lactose, TS, SCC, BW, and BCS did not differ among treatments, but MUN tended to be lower for CON, compared with PS8 and PS19. Of the urea produced by the liver, a part is excreted in the urine and milk, and the remainder is recycled to the gut (Reynolds and Kristensen, 2008).

Nutrient intake

The DMI was greater for cows fed PS8 than CON and PS19, and PSPan did not differ from the other three treatments. Compared with diets containing MS fractions, the CON diet reduced intake of NDF and uNDF, while increasing starch intake. In addition, compared with PS8, CON had a lower intake of DM and NDF >8mm.

Adverse conditions in the ruminal digesta induced by the lack of forage fibre (i.e., SARA) might have contributed to lower DMI in CON, compared with PS8 (Krajcarski-Hunt *et al*., 2002; Khafipour *et al*., 2009; Plaizier *et al*., 2022). In addition, hepatic oxidation of propionate may have contributed to this effect, compared with PS8, as propionate may increase Krebs cycle anaplerosis in hepatocytes, signalling the satiety centre to regulate DMI (Allen *et al*., 2009).

Feeding PS19 decreased the intake of DM, NDF, uNDF and starch, compared with PS8. Lower DMI for PS19, compared with PS8, is probably due to a greater amount of long particles (>19 mm) in the swallowed bolus and rumen digesta in PS19. Approximately one third of particles >19 mm in the treatment PS19 reached the reticulorumen upon swallowing the masticated ration (i.e., 6,04 and 18,2% respectively), suggesting that chewing does not completely standardise particle size.

Despite the lower DMI, PS19 had similar ruminal pools of NDF and uNDF, compared with PS8. Grant *et al*. (2018) demonstrated that combining particle size, NDF and its indigestibility improved predictions of feed intake and productive responses in dairy cows. In our study, although PS8 and PS19 were balanced with the same contents of NDF and uNDF, the greater proportion of

Different sized Penn State Particle Separators.

Despite the benefits of providing peNDF by feeding long forage particles, excess peNDF tend to limit dry matter intake and lactation performance.

Table 1: Effect of maize silage fractions on dairy cow lactation performance.

	Treatment[1]					
Item	**CON**	**PSPan**	**PS8**	**PS19**	**SEM**	**P-value**
Milk yield, kg/d	26^{ab}	$26,3^{ab}$	$26,8^{a}$	$24,8^{b}$	1,26	0,05
3,5% FCM, kg/d	$24,7^{b}$	26^{ab}	$27,2^{a}$	$25,2^{b}$	1,22	0,03
ECM, kg/d	$24,7^{b}$	$25,8^{ab}$	$26,9^{a}$	$24,8^{b}$	1,17	0,04
Fat, %	$3,18^{b}$	$3,43^{ab}$	$3,62^{a}$	$3,46^{ab}$	0,082	0,01
Fat, kg/d	$0,827^{c}$	$0,901^{ab}$	$0,962^{a}$	$0,889^{bc}$	0,0418	0,01
Protein, %	3,37	3,27	3,28	3,30	0,072	0,30
Protein, kg/d	$0,865^{a}$	$0,857^{ab}$	$0,873^{a}$	$0,798^{b}$	0,0338	0,02
Lactose, %	4,75	4,77	4,84	4,81	0,046	0,46
Lactose, kg/d	$1,24^{xy}$	$1,26^{xy}$	$1,30^{x}$	$1,20^{y}$	0,065	0,10
TS, %	12,3	12,5	12,6	12,6	0,09	0,11
TS, kg/d	$3,20^{xy}$	$3,28^{xy}$	$3,38^{x}$	$3,13^{y}$	0,147	0,08
MUN, mg/dL	$10,3^{y}$	$11,2^{xy}$	$11,5^{x}$	$12,1^{x}$	1,08	0,07
Log_{10} SCC	2,04	2,20	2,16	2,22	0,245	0,72
ECM/DMI	1,19	1,21	1,23	1,19	0,047	0,44
ECM/dOMI[2]	$1,99^{y}$	$2,10^{xy}$	$2,12^{x}$	$2,04^{xy}$	0,110	0,08
BW, kg	597	600	605	600	37	0,53
BCS	3,24	3,24	3,29	3,27	0,089	0,67

[a-c]Means within a row with different superscripts differ by Tukey-Kramer test ($P \leq 0,05$). [x,y]Means within a row with different superscripts differ by Tukey-Kramer test ($0,05 < P \leq 0,10$). [1]CON = control; PSPan = TMR containing maize silage fraction retained on the Penn State Particle Separator (PSPS) bottom pan; PS8 = TMR containing maize silage fraction retained on the PSPS 8mm sieve; and PS19 = TMR containing maize silage fraction retained on the PSPS 19mm sieve. [2]dOMI = intake of digestible OM.

long particles in PS19 may have increased its filling capacity.

Sorting behaviour

In all treatments, cows selected against particles >19mm (i.e., PSI <100%), but refusal decreased in CON and PSPan. Several studies have reported that TMR particles retained on the 19mm sieve are more likely to be refused than shorter particles (DeVries *et al*., 2008; Zebeli *et al*., 2009), even when cows were under SARA conditions (i.e., CON treatment). However, in this experiment, sorting was less evident in diets deficient in NDF (CON) or with extremely processed fibre (PSPan).

Lactating cows can drastically change their TMR preference depending on the particle size distribution and diet composition (Maulfair *et al*., 2013; Andreazzi *et al*., 2018). Zebeli *et al*. (2009) also observed selection against particles retained on the 19mm sieve (mean PSI = 74%) for diets containing CS with different particle lengths (three different theoretical particle lengths: 14, 8,1 and 5,5mm).

In our study, PS19 increased the selection against diet NDF and uNDF, but in favour of starch, consequently changing the composition of the actual consumed TMR, compared with the offered TMR. Feeding diets that discourage selection can positively affect energy intake throughout the day while maintaining rumen function, as it reduces the variation in the nutritional value of the diet remaining in the feed bunk, especially in the initial hours after feed delivery (DeVries *et al*., 2005).

Ruminal fermentation

The CON treatment had a lower mean ruminal pH, a longer duration of pH <5,8, a lower proportion of isovalerate and caproate, and a higher ruminal concentration of lactate than diets with MS fractions. In addition, CON had a lower minimum pH, acetate proportion and acetate:propionate ratio, but an increased molar proportion of propionate, compared to PS8.

Cows fed CON might have experienced SARA, as the daily mean rumen pH was below six (Khorrami *et al*., 2021) and the duration of pH below 5,8 was greater than 5,2h/d (Zebeli *et al*., 2008). A greater proportion of propionate and a higher concentration of lactate in CON may have been an outcome of greater activity of amylolytic bacteria.

Streptococcus bovis, for instance, is known to produce lactic acid as a fermentation end product when substrate availability is high and pH is low (Hungate *et al*., 1952; Owens *et al*., 1998; Gill *et al*., 2000). Simultaneously, lower pH conditions might have decreased the activity of lactate-consuming bacteria, such as *Megasphaera elsdenii* and *Selenomonas ruminantium* (Russell and Dombrowski, 1980; Goad *et al*., 1998).

In our study, ruminal lactate concentration peaked six hours after morning feeding in the CON treatment, but the peak of total (d- + l-) lactate was much lower than the values of d-lactate reported by Harmon *et al*. (1985) in acute acidosis-induced steers. Meanwhile, the mean lactate concentration in the rumen fluid of CON animals was close to those reported by Khafipour *et al*. (2009; 2,29 versus 2,27m*M*) for SARA-induced cows, indicating that our CON cows experienced SARA, rather than acute acidosis. Cows receiving MS fractions had a lower lactate concentration (mean = 1,65m*M*) than CON, and the values were similar to those reported by Khafipour *et al*. (2009) for cows not experiencing SARA (mean = 1,33m*M*).

Total-tract digestibility and faeces

No differences were observed among MS fractions for total-tract digestibility of nutrients or faecal traits. However, CON had lower ether extract and starch digestibility, faecal pH and faecal score and a greater faecal starch concentration. The greater proportion of starch from dry maize, combined with a less consistent rumen mat in CON, might have allowed more starch to escape from the rumen. In our study, the CON diet contained a greater proportion of starch from dry ground maize, which is less digestible than ensiled starch (Ferraretto *et al*., 2013) and likely contributed to lower total-tract starch digestibility.

Ferraretto *et al*. (2013) found that a single unit decrease in total-tract starch digestibility corresponded to a 3,4 percentage unit decrease in ruminal starch degradation. Decreased apparent digestibility of starch indicates that post-ruminal digestion did not compensate for lower ruminal degradation of starch in the CON treatment.

The lower starch digestibility observed in CON agrees with the increased faecal starch and possibly explains the lower faecal score and faecal pH in the CON treatment. Upon greater passage of nutrients to the large intestine, greater post-ruminal fermentation occurs, which increases acid production and osmotic pressure. This ultimately increases water inflow to the lumen of the intestine and decreases faecal score (Huber, 1976).

Conclusion

Feeding different MS particle fractions stratified with the PSPS had different effects on DMI, digestion, blood metabolites, milk production and composition. The inclusion of MS fractions in a low-forage fibre diet reduced signs of SARA.

Rumination per kilogram DM and rumen pH suggest some effectiveness of fNDF, even when MS particles are smaller than 8mm, but total chewing per kilogram NDF and blood LPS imply that NDF of MS particles <8mm is less effective than NDF of MS 8 to 19mm particles. However, compared with MS 8 to 19mm particles, MS particles >19mm decreased milk and component yields and total chewing and promoted sorting against long particles and diet NDF.

These results highlight that this fraction should be considered with caution when estimating the physical effectiveness of MS for dairy cows and used for diet formulation only in combination with frequent sorting measurements. Further research is warranted with MS fed in combination with other forages varying in particle size, fragility, and NDF digestibility to determine the physical effectiveness of subfractions of particles <8mm (e.g., stratified with a 4 or 1,18mm sieve).

This study reinforces the importance of proper silage harvest management and monitoring to achieve adequate particle size for dairy cattle feeding.

This article was condensed for publication in *AFMA Matrix*. To read the full article, visit *www.journalofdairyscience.org/article/S0022-0302(23)00382-X/pdf* or email JLP Daniel at jlpdaniel@uem.br.

Improving energy release from fibre in dairy rations

By Dr Derek McIlmoyle, ruminant market champion and sales director South Africa, AB Vista

Fibre is a key component in ruminant diets due to its role in maintaining rumen function and cow health. It is an extremely important energy source, with a substantial part of the energy for milk production extracted from fibre.

Dairy cows and other ruminant species use microbial fermentation to derive energy from fibre, which contributes up to 70% of their total energy pool. Fibrolytic bacteria attach to fibre surfaces and, with the aid of enzymes produced by the microbial biomass, they extract energy in the form of volatile fatty acids.

One of the overarching goals in dairy nutrition is to maximise milk from forage. Though it is often perceived that fibre is well digested by ruminants, plant cell wall digestibility is in fact under 50% (which implies there is still significant room for improvement).

If the digestibility of the fibre is not maximised, then a good proportion of the best value energy in the diet can be easily lost, with knock-on effects for milk yields, growth rates, feed costs and overall profitability.

In high yielding dairy cows, butterfat and milk protein must be maintained together with optimal rumen function. With the ongoing volatility in feed ingredient prices, this becomes a major challenge for the dairy producer. Therefore, in today's economic environment, producers may need to look for new opportunities to optimise production.

Maximising fibre utilisation

Highly digestible forage is one of the most cost-effective solutions within ruminant systems and supports both animal health and production. By improving rumen microbes' ability to digest fibre, it is possible to help the animal to better utilise fibre, which can have an overall positive effect across the entire farm and improve production margins.

Having access to the best quality forage possible should always be the aim of dairy producers, whether that is through growing and ensiling their own forage or purchasing forage from other sources.

Forage growing, harvesting and ensiling techniques have improved over the years, but there are still climatic and regional challenges which will have a big effect on the quality of the forage available.

Some other technologies that have been studied and applied to improve fibre digestion and feed efficiency have included various feed additives such as ionophores, direct-fed microbials, probiotic live yeasts and buffers to help manipulate rumen microbes and ruminal fermentation.

Pre-treatment of rations

Another recent innovation in ruminant nutrition is the pre-treatment of the ration with novel fermentation extract technology to assist in extracting more energy from home-grown forage. This process of pre-treatment roughens the fibre structure by forming pits on its surface, speeding up the microbial attachment and colonisation, reducing lag time leading to increased fibre digestion, improved performance and feed efficiency.

This process can release previously unavailable energy to help improve the opportunity to produce more milk from forage and increase overall animal productivity. In field conditions, adequate fibre supply ensures the best responses in the herd. This pre-treatment technology can be applied directly to the total mixed ration (TMR) and allows pre-digestion of the feed prior to consumption by the animal.

A recent *in vitro* study performed by a leading research facility in Canada has shown improved fermentation parameters, such as improved microbial biomass – a 'gold standard' parameter associated with higher milk production – when TMR was pre-treated with such a fermentation extract, compared to the control group. It demonstrated greater fast pool (gas produced from starch and soluble fibre) and improved slow pool (gas produced from fibre, primarily hemicellulose and cellulose).

Also important is NIR analysis, which can be used to monitor the variability in forage, analyse how much more energy can be extracted from forage and calculate

Table 1: Potential energy from grass silage when treated with novel fermentation extract (n=74).

N=74	NDF (%)	ADF (%)	Soluble CHO (%)	D-value (%)	ME value (MJ/kg DM)
Average value before pre-treatment	47,9	30,5	4,13	64,9	10,8
Average value post-treatment	38,9	24,9	5,22	69,7	11,6
Overall effect	**-9**	**-5,6**	**+1,09**	**+4,8**	**+0,8**

the potential reduction in the cost of bought-in feed. For example, grass and maize silage samples were analysed with near-infrared (NIR) spectroscopy before and after treatment with a novel fermentation extract.

The results (*Tables 1* and *2*) clearly demonstrate the mode of action of the fermentation extract and shows the extra energy value available, which can be accounted for when formulating the diet, averaging 0,8 MJ/kg dry matter (DM).

Table 2: Potential energy from maize silage when treated with novel fermentation extract (n=76).

N=76	NDF (%)	DOMD (%)	ME value (MJ/kg DM)
Average value before pre-treatment	44,8	65	10,4
Average value post-treatment	30,7	69,8	11,17
Overall effect	**-14,1**	**+4,8**	**+0,77**

Monitoring animal response

Manure quality is often the first parameter where we start to notice positive results of improved fibre digestibility, and monitoring this using NASCO sieve analysis can be an effective tool. Animal response to increased dietary energy supply will depend on various factors, including lactation stage, body condition, and metabolic state.

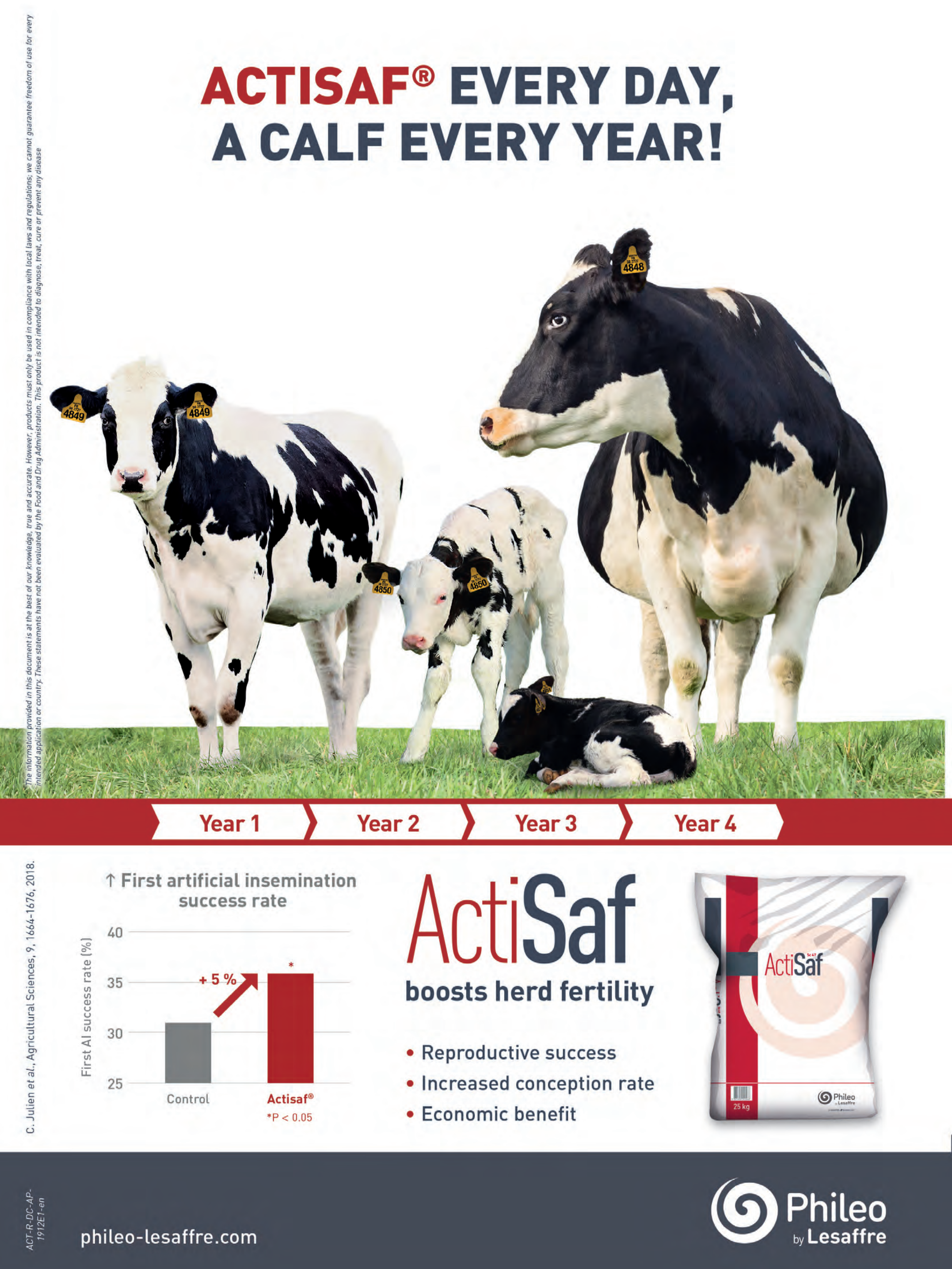
A great way of monitoring the efficacy of any product added to the diet is to examine milk yield and milk solids, intakes and fertility records. Energy deficient animals, such as primiparous cows that are still growing, often replenish the deficiency first, reflected in improved body condition and fertility parameters, after which they start to show improvements in milk yield.

Conclusion

Producers, while faced with ongoing environmental and commercial pressures, are required to increase performance and harvest more energy, all while reducing emissions. Extracting more energy from feed and particularly forage is critical to maximise feed efficiency, reduce costs and lower emissions – and consequently improve farm profitability. In turn, this ensures the sustainability of the farm and the longevity of the herd.

Moreover, maximising the energy from fibre is rumen friendly and reduces the need to use excessive starchy cereal grains, which can cause acidosis. This results in additional cost savings and health benefits for the animal as well.

For more information, contact Avipharm Feeds on 033 3427041/2.



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Dietary fibre effects and the interplay with exogenous carbohydrases in poultry nutrition

By Michael R Bedford, Birger Svihus and Aaron J Cowieson

The first report of the use of an exogenous enzyme blend being added to the diet of commercial poultry was by Hervey (1925) and was followed quickly by Clickner and Follwell (1926). In both these cases, the enzyme mixtures were crude and the considerable benefits to animal live performance that were generated probably did not stem only from exogenous enzyme activity.

Nonetheless, these authors opened interest in a new category of feed additive and stimulated almost 100 years of continuous research and development that has generated a global biotechnology industry with value in excess of US$1 billion per annum. Feed enzyme use is estimated to save the global animal protein industry an estimated US$6 billion in nutritional input costs (an average feed cost reduction from the combined use of phytase, protease and carbohydrase).

The nutritional feed enzyme market today comprises three major segments: carbohydrases, proteases and phytases.

Carbohydrase categories

The carbohydrase category is, of course, not homogenous, but comprises an array of mono-component and multi-component carbohydrases across multiple activity classes. Despite this diversity, most exogenous carbohydrases used in commercial swine and poultry production are based on xylanase (endo-1,4-β-xylanase; EC 3.2.1.8) and/or glucanase (endo-1-3(4)-β-glucanase; EC 3.2.1.6).

These two activities are the most widely accepted, most frequently guaranteed by enzyme suppliers from a regulatory perspective, most comprehensively studied, and dominant in around 70% of global carbohydrase products. Other notable activities include amylase (Cowieson *et al*., 2019), various pectinases, β-mannanases and α-galactosidases, as well as a host of minor and side activities with variable effects on carbohydrate sidechains and amorphous regions in lignified cellulose.

Except for crude enzyme products with diverse side-activities, most carbohydrases used in commercial swine and poultry production are endo-acting (i.e. randomly cleaves interior linkages within the backbone of the fibre structure) and do not generate appreciable concentrations of monomeric sugars. This is partially by design as monomeric sugars from non-starch polysaccharide (NSP) are not universally beneficial for non-ruminant animals. Xylose, for instance, a five-carbon sugar, is readily absorbed from the intestine of chickens but is not compatible with the Krebs cycle and so must be excreted in the urine at net cost to the host (Schutte, 1990).

Helpfully, reaction products from commercial carbohydrases are typically small oligosaccharides of varying molecular weight and the resulting benefit to the host is usually derived subsequently from fermentation by the intestinal microbiota.

The success of carbohydrases in commercial pig and poultry production stems from an appreciable feed cost saving and this is largely associated with increases in metabolisable, digestible, and net energy. Typical responses range from 30 to 150 kcal/kg, an increase of between 1 and 5% relative to the control diet (Adeola and Cowieson, 2011).

Adding this nutrient release value to the enzyme, or increasing the energy density of one or more feed ingredients in the least cost formulation ingredient matrix allows nutritionists to capture value by displacing more expensive energy sources from the ration. In addition to this 'direct' effect of carbohydrase, there are a range of secondary benefits to animal performance that are generated from improvements in gut health (partially

mediated via favourable changes in fermentation patterns in the microbiome), pollutive nature of the faeces, net energy and nutrient requirements, gut motility, development, and the lumen environment, etc.

Challenges

The substrate for exogenous carbohydrases is heterogenous and not well defined (Aftab and Bedford, 2018; Choct, 2015a, b; Nguyen *et al*., 2021a). This lack of clarity creates challenges for optimisation of existing carbohydrase products, e.g. in dose or product selection or in aligning enzyme choice with growth stage, diet type, or animal species. These are not unique to carbohydrases, but offer a more complex challenge than is the case for phytase which enjoys a more well-defined substrate-enzyme relationship.

Despite the challenges in clarifying the role of fibre per se and the interaction between fibre type, origin, solubility profile, molecular weight and exogenous carbohydrases, considerable progress has been made on many of these topics.

Optimising the fibre fraction of non-ruminant production animal diets to elicit more consistent live performance outcomes and improving the magnitude and consistency of the effect of exogenous carbohydrases will bring appreciable benefits to the industry, both fiscally and in sustainable animal production.

What is dietary fibre?

Fibre is a term used to describe an incredibly complex and heterogeneous group of structures which differ markedly in the effects they have on the environment of the intestinal tract. Even small variations in composition can have significant biological effects. As a result, the description of a fibre source needs to be in much greater detail if we are to fully understand the benefits and the limitations that any one source can bring to the diet. At present, nutritionists tend to calculate the fibre contents of the diets they formulate but they rarely change formulations based on the numbers they generate.

More advanced analytical methods routinely enable nutritionists to understand the composition, size, degree of branching and solubility of the fibre which directly relates to its functionality are needed to allow its full potential to be realised (Schäfer *et al*., 2019).

Nutritional benefits

The nutritional benefits of exogenous carbohydrases vary depending on individual product recommendations, animal species and growth stage, diet type, enzyme dose and the presence of additional feed enzymes such as phytase or protease (Cowieson and Bedford, 2009; Cowieson *et al*., 2010; Cowieson, 2010). However, typical nutrient release values for NSP degrading enzymes would include a metabolisable, digestible, or net energy value of 50 to 150kcal/kg and a modest digestible amino acid value of 1 to 3%.

The energy release values are usually higher in diets based on wheat, barley, oats, rye, and triticale and lower in diets based on maize or sorghum. This is principally associated with the lower concentration of high molecular weight soluble pentosans in maize and sorghum (Bach Knudsen, 1997) and, axiomatically, the relatively lower metabolisable energy concentration of wheat compared with maize though particle size and animal age play important roles (Amerah *et al*., 2008; Khalil *et al*., 2021).

One of the most important factors that can promote or demote the mean nutrient release value of carbohydrase (and indeed other feed enzymes) is the relative nutrient digestibility in the control group (Cowieson and Bedford, 2009; Cowieson, 2010; Cowieson and Roos, 2014). In a meta-analysis of the effect of carbohydrases on the ileal digestibility of amino acids in pigs and poultry, the authors noted that around 65% of the variance in enzyme effect size could be explained by the digestibility of amino acids in the control diet (Cowieson and Bedford, 2009).

Indeed, the effect of carbohydrase on amino acid digestibility declined by around 50% for every 10% increase in the digestibility of the control group. Furthermore, this relationship has also been confirmed for exogenous protease (Cowieson and Roos, 2014) and exogenous phytase (Cowieson *et al*., 2017a, b).

Importantly, the association between the relative digestibility of the control diet and the magnitude and consistency of nutrient release by feed enzymes is not linear, but typically follows a second-order polynomial curve (Cowieson and Bedford, 2009). This implies that an increase in the digestibility of the control group from e.g. 70 to 80% will create a relatively more substantial headwind for enzyme effect size than a change from 80 to 90%. A similar relationship between the inherent digestibility in the control diet and enzyme effect size has been noted for energy metabolism.

Energy and digestibility

Douglas *et al*. (2000) noted an equivalent relationship between the inherent ileal digestible energy of soya bean meal and the effect of a blend of xylanase, glucanase and protease on the same. Soya bean meal with a low digestible energy responded more readily to the supplemental enzyme than soya bean meal with a high digestible energy value, with an enzyme response range from -174 to +299 kcal/kg and an average of +56 kcal/kg. Thus, the use of generic or static matrix values for carbohydrases for either energy or amino acids will generate variable outcomes in carcass composition and animal live performance phenotypes.

Most precise estimates may be delivered by articulating raw material quality surveillance with feed enzyme selection and dosing (Cowieson, 2010), for example, via the use of near-infrared spectroscopy or similar laboratory testing.

Animals with a lower digestive capacity, more sensitivity to dietary antinutrients, poor disease or general health status or with compromised immune or microbiome function, or exposed to environmental or other stressors will typically deliver below-average digestibility values and may, in turn, benefit more from exogenous feed enzymes.

It can be concluded that exogenous carbohydrases reliably generate increases in the metabolisable or digestible energy value of diets, and have the capacity to increase amino acid digestibility and modify animal live performance. However, substantial heterogeneity exists in these responses, and this is associated largely with the inherent nutritional value of the diet or feed ingredient to which the enzymes are added.

Systematic analysis and reporting of raw material quality, substrate concentrations and awareness of the relative health and nutritional status of the cohorts of animals that will receive the diets will bring more

precision in enzyme selection, dosing and nutrient release value assignment.

Health benefits

Reduction of antinutritive effects

Exogenous carbohydrases perform multiple roles which ultimately have health-giving benefits for the host. The activity of these enzymes likely loosens fibre structures and facilitates reduction of particle size in the gizzard provided the diet has been stored in the crop prior to entry into the proventriculus gizzard. This will facilitate more rapid digestion of cellular contents and, as a result, deprive the microbiota of nutrients which the host can utilise directly. The depolymerisation of viscous fibre structures also accelerates nutrient removal from the intestine by the host and again reduces the potential for excessive microbial growth in the small intestine.

It also results in improved miscibility of the contents of the intestinal trace which increases the oxygen content of the digesta, providing a further barrier to invasion by opportunistic facultative anaerobic pathogens (Moran and Bedford, 2022). In this regard, exogenous carbohydrases reduce the challenge posed by that portion of fibre which is detrimental to animal health.

Provision of beneficial effects

Exogenous carbohydrases can produce smaller fibre fragments from the larger, more anti-nutritive fractions, and may even produce stimbiotic oligosaccharides directly. In essence, they result in a progressive movement of fibre from the insoluble to the smaller soluble fractions.

This can be very significant, particularly when antibiotics are not routinely administered since many intestinal resident bacteria preferentially ferment carbohydrate and produce short-chain fatty acids which are beneficial for intestinal health and energy status, or, if fibre is limiting, putrefy protein to amines and indoles which are detrimental to intestinal and ultimately host health (Apajalahti and Vienola, 2016).

This becomes more of an issue as the bird ages and the small intestinal microbiota matures, removing the most rapidly fermentable fibre prior to it reaching the caeca, thereby restricting the caecal carbohydrate supply. The use of carbohydrases increases the supply of fermentable NSP, ultimately through dissolution and depolymerisation of insoluble NSP, thereby reducing the likelihood of protein putrefaction taking place.

Some exogenous carbohydrases are able to reduce soluble fibre to oligosaccharides which may have direct stimulatory activity on the fibre-degrading microbiota.

Future opportunities

The positive effect of coarse insoluble fibre exerted through stimulation of gizzard functionality indicates that a certain level should be added to poultry diets. However, the level of fibre needed is still unclear and should be targeted in future research. Adding to the complexity of this issue is that both the chemistry of the insoluble fibre and the content of other components such as coarse cereal particles affect the gizzard-stimulating effect.

Jimenez-Moreno *et al*. (2013), for example, observed a much stronger gizzard-stimulating effect when the insoluble fibre was in the form of oat hulls than when it was in the form of soya bean hulls. This may be due to differences in structural rigidity and resilience to grinding, although this is far from certain. Further, as the level of oat hulls in the diet increased above 5% of the diet, no further increase in relative gizzard weight occurred, indicating that 5% was enough when this fibre source was used.

The effect of insoluble fibre will very much depend upon the coarseness of cereal particles in the diet. Although the relatively fine grinding and the additional grinding effect of the pelleting effect would probably result in insufficient gizzard stimulation by coarse cereal particles in most diets, more drastic feeding practices such as the use of whole wheat would certainly diminish the beneficial effect of insoluble fibre.

Thus, there seems to be an unexplored possibility for the inclusion of a certain level of insoluble fibre in poultry diets. However, further studies are needed to set specific targets for different fibre sources, where the coarseness of the cereal used in the diet is also taken into consideration. Taylor *et al*. (2021) demonstrated that broiler chickens given pelleted diets were able to maintain weight gain through an increased feed intake when oat hull levels increased to as high as 30% of the diet.

Thus, when sources of insoluble fibres are available, the risk of oversupplying the diet with these fibres at least seems to be low.

Conclusion

Fibre is the macro-ingredient in poultry diets for which there are the most significant gaps in our understanding. The role of fibre in animal health, welfare, microbiome function, behaviour, nutritional status, gut physiology, and environmental sustainability are not entirely clear. However, the concept that fibre is a relatively simple nutritional diluent or even a feed component that influences feed intake or digestive dynamics in the gut of poultry, has been replaced with a much more nuanced understanding of the functional effect of fibre.

However, more research is needed to explicitly associate high-value phenotypes with specific fibre composition and concentration and to create more consistent and reproducible effects of exogenous carbohydrases.

Given the recent increases in attention to sustainability, it is likely that the concentration and complexity of fibre in the diets of poultry will increase over time as nutritionists turn to locally sourced raw materials and make more complete use of by-products and other novel feed ingredients. This, coupled with new insights related to the value of fibre on gut function, microbiome modulation, animal health and welfare, will create significant impetus to optimise the fibre fraction of feed.

It is likely that exogenous carbohydrases will make a critical contribution to success in the next chapter of macro-nutrient optimisation of poultry diets.

This article has been condensed for publication in *AFMA Matrix*. For the full article visit *www.sciencedirect.com/science/article/pii/S2405654523001440* or email Michael Bedford at mike.bedford@abvista.com.

The next generation of water-soluble probiotics: Ecobiol® Fizz

By Natasha Davison, business manager, Evonik Africa

Animal production faces a number of challenges which compromise profitability. These challenges include feed changes, vaccination, stress and the presence of enteric pathogens such as *Salmonella*, *Escherichia coli* and *Clostridium perfringens*. The presence of pathogens places immense pressure on the gut by causing inflammation, disease and subsequently poor growth performance.

When animals are suffering from a digestive disturbance, the consumption of feed is significantly reduced, thereby limiting the potential of in-feed additives. The supplementation of probiotics through drinking water can assist in introducing beneficial bacteria into the gut to rebalance the microbiome.

Introducing Ecobiol® Fizz

Ecobiol® is a single-strain probiotic containing *Bacillus amyloliquefaciens* CECT 5940, and supports the maintenance of an intestinal microbial balance in poultry and swine, compatible with the commonly applied water additives such as organic acids, chlorine and hydrogen peroxide. Ecobiol® has been shown to reduce pathogenic microflora in the intestine and improve animal performance.

Ecobiol® exhibits a number of unique modes of action – such as secondary metabolite production, lactic acid production, immune modulation and quorum quenching – to effectively inhibit harmful bacteria in the gut, thereby preventing their over proliferation and disease.

Ecobiol® Fizz is the new innovative product from Evonik which provides a fast and easy way to supplement probiotics in the drinking water, thereby supporting a naturally balanced microbiota. Ecobiol® Fizz is an effervescent tablet which can be supplemented at one tablet per 1 000ℓ of water. The tablet ensures complete homogenous distribution without stirring, and does not cause biofilm formation or clogging of the water tanks and drinking lines.

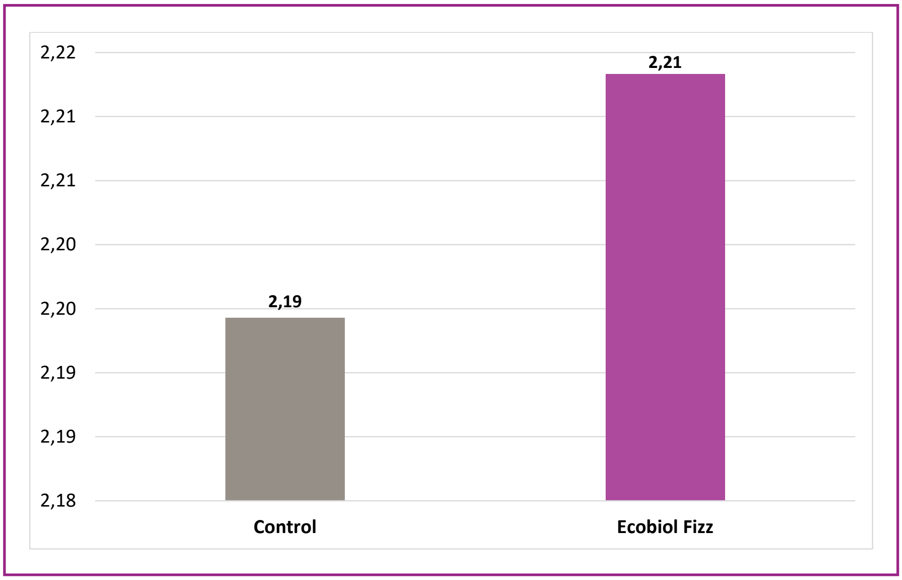
Ecobiol® benefits in field trials

Ecobiol® Fizz can either be used continuously in the drinking water

Benefits at a glance

- Ecobiol® Fizz can be directly applied in drinking water on farm.
- Supports the establishment of healthy intestinal microflora when feed intake is low.
- Delivers fast effects on microbiota in stressful periods.
- Flexible usage of probiotics, regardless of current feed formulation.
- Easy dosing and handling of highly concentrated tablets.
- No sugar-based carrier or additives that impact the formation of biofilm in the water tanks and drinking lines.
- Homogenous distribution of probiotic spores in the water line.
- Compatible with other feed and water additives.

Figure 1: The improvement in slaughter weight (kg) after intermittent supplementation of Ecobiol® Fizz.



of animals to promote the presence of beneficial bacteria, or can be used sporadically in times of increased stress, such as at the placement of day-old broilers, at weaning for piglets, and after antibiotic treatment.

The following trial was conducted at Optifarm in the United Kingdom on a total of 730 000 broilers. The drinking water was supplemented with Ecobiol® Fizz on days 1 to 3, 10 to 12, 21 to 23 and 27 to 29, to assist in the most stressful time periods the birds experience. With the improvements observed in performance, in particular bodyweight at slaughter (*Figure 1*), the return on investment (ROI), taking into account the cost of application versus the additional income for the farm, was 6:1.

In addition to the benefits on performance improvement, welfare parameters such as hockburn, pododermatitis, factory rejects, and farm mortalities can also be reduced with Ecobiol® Fizz.

In the following trial, broilers were supplemented with Ecobiol® Fizz during the entire cycle and their performance compared to their counterparts not receiving supplementation. It can be observed in *Table 1* that Ecobiol® Fizz not only improved bodyweight gain, but improved the welfare parameters as well. For this specific trial, the ROI was calculated to be 5.4:1.

Table 1: Improvement in performance of broilers supplemented with Ecobiol® Fizz throughout the entire cycle.

Parameter	Control	Ecobiol® Fizz	
Av bodyweight gain (kg)	2,23	2,26	+34g/bird
Mortality %	6,51	5,31	-1,20%
Hockburns %	29,72	24,58	-5,14%
Pododermatitis %	25,3	19,82	-5,48%
Factory rejects %	0,49	0,38	-0,1% ~ 1 095 birds

As the challenges in animal production continue to rise, and pathogens continue to become ever-present on the farm, strategies to support a healthy microbiome need to be considered. Ecobiol® Fizz has consistently demonstrated its effectiveness against pathogenic bacteria and the promotion of beneficial bacteria in the gut, and can therefore assist in improving overall gut health and performance on poultry and swine farms.❖

References available on request. Email natasha.davison@evonik.com for more information.

Nutritional aspects of hemp-based products and their effects on the health and performance of monogastric animals

By D Lanzoni, E Skrivanova, L Pinotti, R Rebucci, A Baldi and C Giromini

Sustainable agriculture is the production of agricultural products at an environmental cost that does not jeopardise food/feed security and animal health. For this reason, scientific research is investigating new alternatives to classical protein sources to ensure these principles. This objective is strongly pursued by the European Union through the One Health approach, and subsequently with the Green Deal, dated 1 December 2019, which recognise that human, animal, and environmental health are interconnected, promoting efficient use of resources through a circular and clean economy (Wolf *et al*., 2021).

Among the possible alternatives, *Cannabis sativa* L, commonly known as hemp, is gaining increasing attention in the food/feed sector, not only due to the high nutritional and functional properties of hempseed, but also for the role it plays in environmental protection. As well as being able to grow rapidly in different agro-ecological conditions (it can reach 4m in height) while requiring limited amounts of water and herbicides, it is an excellent candidate for carbon sequestration (nine to 13 tonnes/ha).

To ensure sustainable agriculture, one possible approach involves recycling nutrients from residues and unusable crop co-products and adding them to the diet of livestock; among these, hemp co-products are attracting great interest, in particular the whole plant, leaves and flowers (obtained before/after the cannabinoid extraction process), stems, stalks, whole seed heads, and seed hulls, as reported by Kleinhenz *et al*. (2020), Vastolo *et al*. (2022) and Ely and Fike (2022).

However, the last report by the European Food Safety Authority (EFSA) on the use of hemp-based products in the feed sector, entitled *Scientific opinion on the safety on hemp* (*Cannabis genus*), for use as animal feed, dates back to 2011 (EFSA, 2011). More precisely, in this report EFSA suggests inclusion levels based on the work published until then. As might be expected, the number of scientific publications investigating the use of hemp-based products in the diets of monogastric animals has increased since 2011.

For this reason, the objective of the following review, in addition to outlining the nutritional profile of hempseeds and co-products, aims to investigate their use in the monogastric sector (pigs, broilers and laying hens) by summarising the main works in the literature up to 2023, investigating the effects on animal health and performances.

Nutritional value of hempseed

Hempseed is the main component of the plant used in the food and feed sector due to its very promising nutritional and functional properties. It is an achene, enveloped by a thin and hard pericarp characterised by a high amount of fibre, in particular 40 to 50% neutral detergent fibre (NDF), 30 to 35% acid detergent fibre (ADF), and 10 to 15% acid detergent lignin (ADL), 23 to 30% proteins easy to digest and rich in essential amino acids (AAs), 25 to 35% lipids with balanced fatty acids and 5% of ashes (Farinon *et al*., 2020; Fike, 2019; Lanzoni *et al*., 2023). These values are similar to those of flaxseeds, an important matrix in the food/feed industry.

However, as reported in the literature, these percentages depend on the plant genotype, environmental growth factors and, above all, the type of technological treatment the seed is subjected to (Fike, 2019; Leonard *et al*., 2020; Lanzoni *et al*., 2023). The fibrous fraction of hempseed is characterised by a soluble:insoluble fibre ratio of 20:80, a similar value to that of other food/feed sources, such as flaxseeds (Callaway, 2004; Farinon *et al*., 2020). However, due to treatments such as heating and extrusion, it is possible to concentrate fibre fractions, making them more appreciable for food and feed applications (Amaducci *et al*., 2008).

Table 1: Chemical composition of hemp co-products used in the monogastric feed sector. Values are expressed in % dry matter (DM). (Source: Kleinhenz *et al*., 2020; Vastolo *et al*., 2022; Ely and Fike, 2022).

Item	Whole plant	Leaves	Stalks	Seed cake	Seed heads	Chaffs	Extracted flowers	Whole plant SHB	Stems and leaves SHB	Hemp hulls
CP	6,9	13	5,3	30 – 34,4	23	21,2	24,5	22,4	19,2	21,9
Fat	2,7	8,9	1,2	10,2 – 12,4	13,2	4,6	3,2	4,3	7,5	23,5
ADF	60,8	20,8	64,6	32,1 – 39,5	29,6	18	18,1	32,3	17,6	37,9
NDF	81,6	44,7	84,4	39,3 – 51,9	53,2	27,9	30,9	40,1	23,4	57,1

SHB: Spent hemp biomass.

In hempseeds, the proteins are mostly located in the inner layer of the seed, with a low fraction in the hull. To date 181 proteins have been identified, the most common of which are edestin, albumin and β-conglycinin (Farinon *et al*., 2020).

Hemp co-products

The use of co-products is a valid strategy to ensure the sustainability of the feed sector. The main hemp co-products investigated in the scientific literature are whole plant, leaves, flowers, stems, stalks, seed cake, chaffs, seed heads and hulls (*Table 1*). The percentages of these nutrients vary depending on the cultivation techniques, environmental conditions, and genotype analysed.

In particular, the crude protein content varies from 5,4 to 34,4%, with higher values reported for hempseed cake (30 to 34,4%), due to the high level of the protein content following the oil extraction process. At the same time, as reported by Vastolo *et al*. (2022), this product has a high protein digestibility, tested following the *in vitro* digestion process, showing values highly comparable to tobacco coproducts.

Interesting values include seed heads (23%), chaffs (21,2%), hulls (21,9%), and exhausted biomasses (Kleinhenz *et al*., 2020; Vastolo *et al*., 2022; Ely and Fike, 2022). As reported by Ates (2021), the total protein content of co-products, obtained as a result of the cannabinoid extraction process, is similar to lucerne, with higher values for the lipid content.

Hemp co-products are characterised also by high levels of fibre (*Table 1*). The NDF ranges from 23,4 to 84,4%, which is lower for co-products rich in leaf and flower components. The latter has higher digestibility than maize stalks, oat or barley straw. However, as reported by Kleinhenz *et al*. (2020), such co-products are poor sources of energy within livestock diets, suggesting a different use as a source of dietary fibre, especially in ruminants.

However, at the same time, hemp co-products have some limitations. These include the high variability of nutrients due to different processing methods and the need for essential preservation treatments to stabilise the product, especially those with high moisture and lipid values (Ely and Fike, 2022).

Monogastric animal applications

The use of hempseed and hemp-based products in the swine sector is still at an early stage. The main studies concern the use of these products in diets for lactating sows and piglets, which are key steps for the success of the entire production cycle. Weaning piglets represent the most delicate and crucial time for the health of these animals. During this phase, the animals face environmental (separation from the mother), social (union of different broods), and nutritional (transition from mother's milk to solid feed) changes.

All these factors act as stressors, simultaneously causing both a decrease in feed intake and the development of gastric and intestinal problems, resulting in severe physiological and immunological disorders (Palade *et al*., 2019; Vodolazska and Lauridsen, 2020). For this reason, the energy and nutritional intake provided through breast milk must be high, to overcome this critical phase. Although piglets are born with an energy deficit and low-fat reserves, the absorption of lipids and fatty acids from colostrum and milk is efficient (Palade *et al*., 2019; Vodolazska and Lauridsen, 2020).

For this, animal producers supported by research are trying to intervene through diet to safeguard their health. One of the possible alternatives, still little explored in the literature, concerns the use of hemp-based products in sow rations to study how piglets' performance varies.

The addition of hempseeds (up to 50g/kg) in the diets of lactating sows resulted in positive results on the growth performance of piglets. This result, as reported by Habeanu *et al*. (2018), is related both to:

- The direct consequence of lipid supplementation in the diet, which allows for an increase in milk fat and thus a higher energy intake.
- The high nutritional profile, especially the lipid content of hempseed.

Positive results were obtained with the inclusion of 50g/kg HS oil in the diet (Vodolazska and Lauridsen, 2020). Vodolazska and Lauridsen (2020) found high concentrations of ALA (C18:3, n-3), arachidonic acid (C20:4, n-6), and stearidonic acid (C18:4, n-3), resulting in an even lower n-6/n-3 ratio (4,3) in the milk of treated sows. Positive growth performance was also observed with a lower (16g/kg) inclusion level of hempseed oil (Idricenau *et al*., 2021).

Sow and piglet health

As previously reported, in the pig sector, particular attention is focussed on the weaning period. However, at this stage, according to the literature, sows can lose an average of 15 to 40kg during the entire lactation period (Hansen, 2012). For this reason, it is necessary to select a diet that guarantees the health of both the piglets and the sows.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Although, the addition of two different concentrations of hempseed oil resulted in the same positive effects on piglet performance, the inclusion of 50g/kg hempseed oil affected the total weight of the lactating sows (320 ± 0,18kg), compared to the control group (346 ± 11,4kg) (Vodolazska and Lauridsen, 2020). This trend was not observed with the inclusion of 16g/kg hempseed oil (Idricenau *et al*., 2021). Most probably, while a high lipid content in the diet of lactating sows allows an improvement in the growth performance of piglets, high milk production with a high lipid content affects the performance of sows, resulting in weight loss (Hansen, 2012).

Another determining factor in weight loss is the oxidative stress faced by sows mainly related to the process of lipid peroxidation, which alters the antioxidant status of the animal. In particular, oxidative stress affects the ability of sows to produce milk which, as previously reported, negatively limits the growth and performance of piglets. Persistent stress conditions lead to the formation of a large amount of free radicals and reactive oxygen species, which can affect the endogenous antioxidant system. In these situations, poor nutrient intake may be one of the main causes of systemic oxidative stress in sows (Palade *et al*., 2019).

In the study conducted by Palade *et al*. (2019), however, the inclusion of a dietary treatment with HSs (20g/kg hempseed meal before farrowing; 50g/kg during lactation) led to an improved antioxidant profile in the plasma of sows and piglets. According to the same authors, the positive correlation between hempseed and antioxidant activity is due to the high content of polyunsaturated fats (PUFAs), which are known not only for their high nutritional profile but also for their ability to increase gene expression and the activity of several antioxidant/detoxifying enzymes (Palade *et al*., 2019).

Broiler results

Interesting results were reported for broilers. More precisely, as observed in the study by Khan *et al*. (2010), the inclusion of 20g/kg of hempseeds resulted in a better feed conversion ratio (FCR) with a higher slaughter weight compared to the control group (2 087,2 ± 10,25 and 1 861,4 ± 32,2 g/kg). As explained by the authors, this trend is due to the high lipid and AA profile of the hempseeds, confirming the results reported by Parr *et al*. (2020), where the inclusion of 20g/kg proved to be better than 10g/kg, 30g/kg, and 40g/kg to modulate the growth performance of broilers.

However, the inclusion of 40g/kg, as reported by Skřivan *et al*. (2020), resulted in comparable results in terms of performance and product quality to flaxseeds, but added at 60g/kg, confirming the high nutritional profile of HSs. This trend was also observed for bone health, a key parameter for animal welfare, especially under intensive livestock conditions.

Most probably, as reported by Skřivan *et al*. (2020), this result is positively correlated with the presence of α-tocopherol, which is higher in both the diet and meat of animals that received the hempseed diet. Indeed, α-tocopherol supplementation tends to induce an increase in osteogenic bone mass in the vertebral secondary cancellous bone, where active bone remodelling occurs (Skřivan *et al*., 2020).

However, although these results showed that an inclusion of up to 40g/kg of hempseeds in broiler diets leads to positive results, it is necessary to consider how in the first weeks of the production cycle, the high fibre and mineral content could affect the health of the animals. Indeed, as reported by Vispute *et al*. (2019), at this stage, chickens have underdeveloped mucosa, unable to produce sufficient enzymes capable of digesting high cellulose content.

Laying hens

The use of hemp-based products has been widely explored in the laying hen sector. In fact, the major studies focus on the functional and nutritional characterisation of eggs as well as the evaluation of qualitative and quantitative parameters. In parallel, aspects such as production performance and animal welfare have been investigated.

Regarding the functional aspect of eggs, although Mierliță (2019) observed no differences in the total cholesterol content of egg yolk from hens treated with hempseeds (80 and 200g/kg), studies by Shahid *et al*. (2015) and Skřivan *et al*. (2019) reported different trends. More specifically, Shahid *et al*. (2015) reported that increasing the inclusion of hempseeds (150, 200, and 250g/kg) reduced the cholesterol content with significant differences (16,91 ± 0,01mg/g; 14,29 ± 0,01mg/g; 11,65 ± 0,01mg/g) compared to the control (maize and soya bean meal) (19,27 ± 0,01mg/g), respectively.

The use of hemp-based products has resulted in largely positive results in laying hens, both in the functional and nutritional aspects of eggs, animal health and growth performance. These positive effects were recorded with different levels of hempseed inclusion (up to 250g/kg). In parallel, the use of hempseed oil increased the nutritional profile of eggs by raising the PUFAs present, with an inclusion of up to 300g/kg. Interesting results were also reported for hemp co-products, in particular hempseed cake (up to 150g/kg) improving laying hen production.

Conclusion

The inclusion of hemp-based products in the diets of monogastric animals has led to different results depending on the species studied. As reported, the use of these products needs further investigation, especially in pigs and broilers, due to limited studies. However, for these species, this review increased the data shown in the last EFSA report by reporting for the first time, considerations for the use of new matrices such as hempseed oil.

For laying hens, on the other hand, the large number of scientific studies has made it possible to identify and confirm hemp-based products as effective and safe matrices, able to positively modulate animal health and performance, while simultaneously enhancing the nutritional and functional profile of eggs. However, even in this case, the choice of the optimal inclusion of hemp-based products requires further evaluation.❖

This article has been edited and condensed for publication in *AFMA Matrix*. To read the complete original article, visit *doi.org/10.1016/j.animal.2023.101058* or send an email to carlotta.giromini@unimi.it.

Fertility, the key driver to a successful cattle operation

By Alretha Naudé

The main goals of any beef cattle operation are to ensure that cows conceive, deliver healthy calves, and wean productive calves at an annual rate – all at a reasonable cost to remain economically viable. A critical component to reaching these goals are keeping the herd as close to a 365-day calving cycle as possible. In this regard, it is key to shorten the postpartum anoestrus interval and increase first-service conception rates to reduce the need for cattle rebreeding.

Both nutrition and body condition affect anoestrus and conception, dictating cows' ability to rebreed successfully. Therefore, to meet their goals, it is vital for cow-calf producers to recognise that reproductive traits are twice as important than growth traits. This should also be considered when selecting replacement heifers.

Boost fertility from the get-go

Oftentimes producers are not aware of the significant impact of infertility on their operations. Producers may only become aware of the problem when it is too late, as correcting cows with infertility issues between the tight margins of calving and breeding is no easy task. Therefore, the best option is to set the cow up for success before breeding and calving by ensuring her nutritional needs are met.

Fertility is essentially a function of fleshing ability (a measurement of the adaptability of cows based on resources that they must produce). Fleshing ability is a function of low maintenance requirements. Reproduction cannot take place until maintenance requirements have been met and cows are storing energy reserves as fat. This means that a cow with little fat reserves (slim or lean in appearance) will not be very fertile. Since fleshing ability and maintenance requirements are heritable traits, fertility is also a heritable trait.

Prevent nutrient deficiencies

Using body condition scoring is vital in determining whether the cow is at an optimal plane of nutrition with sufficient reserves. Assessing the physical appearance of the cow is often a good indicator of energy and protein levels in the diet. However, apart from energy and protein, it is important to also remember trace minerals such as copper, zinc, manganese, and selenium. These minerals play key roles in health, metabolism, fertility, and general nutritional requirements of cows.

Trace minerals are involved in the synthesis of reproductive hormones, the reduction of free radicals and the improvement of the uterine microenvironment for embryonic implantation, as well as foetal growth and development. Subclinical deficiencies of trace minerals have been shown to lead to reduced cyclicity and diminishing reproductive health.

It has been shown that deficiencies of single trace minerals rarely occur, and combinations of mineral deficiencies are more common. This can be explained by the variation of trace mineral levels between raw material to raw material, area to area and season to season. As such, adding supplemental trace minerals to cows' feeding programmes are fundamental in bridging the gap between trace minerals available in forages and cows' nutritional requirements.

Inorganic vs organic minerals

The form in which these trace minerals are supplemented determine how the cows respond to the trace mineral programme. Inorganic forms of trace minerals are often the byproducts of the mining industry and are typically inexpensive. These byproducts are essentially sulphates, oxides, chlorides, and hydroxides.

As these trace minerals are mined underground, cows have not been exposed to forms of inorganic trace minerals in their own natural diets. Have you ever seen a cow go mining for their minerals? What this means is that cows are not adapted to optimally use inorganic forms of trace minerals. It is for this reason that inorganic trace minerals are added in excess to animal feeds, to avoid deficiencies. However, this system often leads to mineral-to-mineral antagonism, a higher degree of degradation of other nutrient elements in the feed and higher levels of mineral excretion into the environment.

Recognising the aforementioned issues, organic minerals were developed to mimic trace minerals found in plants, which form part of the animal's natural diet. Thus, organic minerals are optimally absorbed and utilised by the animal with less wastage. Expectedly, this also means that organic minerals can be fed at lower levels than inorganic minerals.

Mineral programme

Implementing a proper mineral programme and timing of supplementation is therefore essential. This is especially so when maintaining optimal reproduction with early conception rates, while boosting calf health and growth.

Preparing for breeding and calving go hand in hand. Supplementing three months before breeding allows time for nutrients to be absorbed, metabolised, and take effect in the cow to support reproductive soundness and breeding-related stresses. The last 50 to 60 days of a cow's gestation period is known to be critical for colostrum quality, and calf health and growth. For this reason, it is important to maintain a mineral programme.

Producers only get one calf per year; not meeting their cow reproduction and calf growth goals requires a lot of time before corrections or improvements can be made. In this way, choosing a mineral programme for cows can be a proactive choice in ensuring their nutritional diet supports the annual fertility goals of the cattle operation.

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Effect of herbal extracts as feed additives in animal nutrition

By Wondimagegn Tadesse Alem, Department of Animal Science, Kebri Dehar University, Ethiopia

Factors contributing to negative animal performance include inadequate nutrition, high incidence of disease, suboptimal genetic resource development and management, and failing market infrastructure. Feed resources with inadequate nutrients for the existing livestock population, poor quality of available feed, seasonal variations in feed availability and water shortages are the main elements that contribute to the low production and productivity of cattle in Ethiopia.

Feed additives are compounds introduced to feed to improve feed quality, enhance animal health, and optimise overall performance. Among these additives, plant herbal extracts have been investigated by scientists to enhance both animal health and production. Extracts include cinnamon, garlic, ginger, yucca, turmeric, and other herbs.

Research has shown that incorporating natural plant extracts in animal diets significantly impacts animal performance, carcass traits, and biochemical and immune responses. Certain plants, including cinnamon, peppermint, cloves and nutmeg, possess antioxidant compounds (polyphenols, flavonoids and terpenoids). These compounds play a role in preventing oxidative stress and associated diseases.

Phytogenic feed additives, also known as secondary plant compounds and metabolites, have beneficial implications on animal health and production by improving feed quality and animal products.

The levels of bioactive compounds in plant-derived animal feed components may vary based on the plant parts that are used, the harvesting season and geographical origin. Natural extracts hold several benefits for animals including increased digestive secretions, improved nutrient digestibility and absorption, enhanced gastrointestinal micro-organisms, immune system stimulation, antibacterial properties, coccidiostat effects, as well as anthelmintic, antiviral, anti-inflammatory and antioxidant properties.

As a result, the purpose of this research is to study the effects of herbal extracts as feed additives in animal nutrition.

Literature review

Plant extract properties

Plant extracts are plant-derived substances added to animal feed to improve overall performance and product quality. These extracts, including spices, essential oils and oleoresins are classified based on their origins and processing methods (*Table 1*).

Plant extracts include various materials and the active secondary plant metabolites are typically isoprene derivatives and flavonoids. The type and level of plant extracts' biological activities depend on several factors including geographical location, time of collection, soil conditions, harvesting period, moisture content, drying method, storage conditions and post-harvest processing.

The levels of active compounds in herbal extracts can vary significantly based on the specific plant part used, plant maturity stage, preservation methods, storage duration and extraction methods.

Plant extract as feed component

Feed additives and nutrients play a strategic role in the management of farm animals. In the last decade or so, there has been a growing interest in natural plant feed additives which led to research on plant extracts, essential oils and by-products derived from plants as alternatives to synthetic vitamins in cattle nutrition to enhance animal health and productivity.

Secondary metabolites found in plant extracts, such as tannins, saponins, flavonoids and essential oils have the potential to alter digestive tract metabolism and enhance animal production performance. DM Samadi (2019) investigated numerous local herbs in the Aceh province in Indonesia including *Vernonia amygdalina* Del., *Calotropis gigantean*, *Syzygium oleana* and *Syzygium cumini* L, and concluded that each phytogenic may be used as a feed additive because of its antimicrobial and antioxidant properies.

Effect on animals

Antimicrobial effect

Due to improved digestive system health, animals are less susceptible to toxins of microbiological origin. Rumen microbial activity indicates that the utilisation of plant extracts and secondary plant metabolites offers an opportunity for ruminal modifiers because of their ability to enhance the efficient utilisation of energy and protein within the rumen. Secondary metabolites found in medicinal plants have antibacterial properties that assist in resistance prevention.

Herbs including *Allium sativum*, *Angelica dahurica*, *Sanguisorba officinalis*, *Artemisia argyi*, *Coptis chinensis*, *Dictamnus dasycarpus*, *Fraxinus rhynchophylla*, *Geranium thunbergii*, *Hydrastis canadensis*, *Phellodendron amurense*, *Polygonum cuspidatum*, *Scutellaria baicalensis* and *Sophora flavescens* contain important flavonoids such as baicalin, limonene, cinnamaldehyde, carvacrol or eugenol – these compounds exert antimicrobial effects.

When combined with other complementary herbs, they demonstrate antibacterial activity against *Salmonella* spp, *E. Coli* and gram-positive micro-organisms such as *Staphylococcus* spp and *Streptococcus* spp. Additionally, these phytobiotics have been investigated for their antibacterial, anti-inflammatory, antioxidative and anti-parasitic effects.

Ethanol extracts from the leaves of *Withania somnifera* L, *Ageratum conyzoides* L, and *Becium obovatum* (E. Mey. Ex Benth. In E. Mey) leaves, in addition to *Pentas lanceolata* root, exhibit antibacterial action against gram-positive bacterium, *S. aureus*, but not *E. coli*. For instance, *Pentas lanceolata* ethanol extracts demonstrated the largest growth inhibition zone against *S. aureus*, with inhibition zones of 15,67 and 18,67mm at concentrations of 50mg/mℓ and 100mg/mℓ, respectively.

Extracts derived from *Citrus sinensis* (orange) show remarkable antibacterial activity against both gram-positive and gram-negative micro-organisms. In addition, they display antifungal activity against *Candida albicans*.

Antioxidant impact

Natural extracts showed an antioxidant impact by upregulating the expression of antioxidant proteins, including heme oxygenase-1 (HO-1), superoxide dismutase (SOD) and catalase (CAT).

Antioxidants play a crucial role in inhibiting lipid oxidation, reducing rancidity and preserving nutritional quality when added to food products. Flavonoids and phenolic compounds in plants exhibit a range of beneficial effects, including antioxidant, anti-inflammatory, antimicrobial and antiallergic properties. Phytogenic feed additives, comprising of individual components or combinations thereof provide promising antioxidant capacity as feed additives.

Consuming natural ingredients can boost serum antioxidant enzyme activity including glutathione peroxidase levels. The addition of rosemary to layer diets seems to have boosted the antioxidant capacity in the birds. Rosemary contains a variety of beneficial phenolic compounds, such as carnosol, carnosic acid, and rosmarinic acid, in addition to other compounds that exhibit antioxidant, anti-cancer, and anti-inflammatory properties.

A subcutaneous injection of saffron petal extract at a dose of 25mg/kg bodyweight significantly reduces low-density lipoprotein cholesterol levels within the lamb plasma compared to those fed a basal diet only.

Anti-inflammatory impact

Plant extracts were used as herbal therapeutic agents to combat inflammation, which is characterised by an overproduction of inflammatory mediators such as reactive oxygen species and pro-inflammatory cytokines. Curcuma, crimson pepper, black pepper, cumin, cloves, nutmeg, cinnamon, mint, and ginger extracts have been identified for their anti-inflammatory properties. Phenols, terpenoids, and flavonoids play a key role in reducing inflammation.

Aloe Vera has antibacterial and antiprotozoal properties that make it particularly effective in managing coccidiosis in poultry. Flavonoids and genins present in the plant exhibit an anti-edematous impact during the acute phase of inflammation. When administered at graded doses of 200 and 400mg/kg, a notable reduction in the weight of granulomas was observed.

In a study on the antinociceptive and anti-inflammatory effects of *Urtica dioica*, it was found that the *U. dioica* leaf extract, when administered at doses of 100, 200 and 400mg/kg, inhibited abdominal twitches by 41, 64 and 81%, respectively.

Herbs as immune stimulants

Natural plants with immune-modulating properties offer an alternative approach to standard chemotherapy for various disorders, in particular the ones involving immunodeficiency. Flora such as echinacea, liquorice, garlic, and cat's claw include chemical substances that stimulate the immune system. These herbs can boost lymphocyte and macrophage function, promote phagocytosis and induce interferon production.

Tocopherols, lutein, beta-carotene, and retinol concentrations in the plasma and tissue of broilers were raised by the

Table 1: The active components and functions of several herbal feed additives. (Source: *Importance of medical herbs in animal feeding: a review*)

Active compounds	Herbal plant	Utilised parts	Function
Eugenol	Clove (*Syzygium aromaticum*)	Flower	Appetiser, digestion stimulant, antiseptic
Cinnamaldehyde	Cinnamon (*Cinnamomum zeylanicum*)	Leaf	Appetiser, digestive stimulant, antiseptic
Linalool	Coriander (*Coriandrum sativum*)	Leaf-seed	Appetite enhancer, digestive stimulant
Anethol	Anise (*Pimpinella anisum*)	Fruit	Digestive stimulant, galactagogue
Phthalides	Celery (*Apium graveolens*)	Fruit and leaf	Appetiser, digestive stimulant
Capsaicin	Capsicum (*Capsicum annuum*)	Fruit	Reduce pain and swelling
Zingerone	Ginger (*Zingiber officinale*)	Rhizome	Appetiser, antioxidant, stress reducer
Allicin	Garlic (*Allium sativum*)	Bulb	Enhance gut activity, promote growth in livestock and poultry
Cineol	Rosemary (*Rosmarinus officinalis*)	Leaf	Anti-bacterial, antimicrobial and neurological protection
Thymol	Thyme (*Thymus vulgaris*)	Whole plant	Antimicrobial, antioxidant, anti-carcinogenesis, anti-inflammatory and immune stimulant

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presence of oxidized fat. Essential oils extracted from medicinal flowers boost the immune system and can regulate the mucosa of the duodenum, resulting in significant benefits for animals.

The Nile tilapia fish, when treated with turmeric, rosemary and thyme plant extracts, exhibited increased levels of haematocrit, leukocrit and nitro blue tetrazolium activity compared to the control group. These medicinal herbs may have a positive impact on immune stimulators.

Feed palatability and digestion

Herbal extracts have been proven to have positive effects on the digestive tract, including prevention of diarrhoea and methane emissions, stimulation of digestive secretions and accelerated enzyme activity. BS Yahya (2017) stated that feed intake is not only influenced by the palatability of the feed, but also by the smell, feel, texture and colour of the feed and the balance of the dietary content.

Capsaicin has been proven to improve pancreatic and intestinal enzyme output in non-ruminants via salivation (producing amylase). Cinnamaldehyde stimulated pancreatic enzymes and prolonged feed retention time in the stomach of pigs because it decreased gastric motility. As a result, increased digestive enzymes improved nutrient digestibility and availability.

Flavonoids, phenols, glycosides, coumarins, saponins, terpenes, alkaloids and anthracene, among other biologically active compounds found in herbs, have a multilevel effect on the body. Herbs can make feed more palatable. Evidence has also shown that products that is primarily plant-based can manipulate *E. coli* populations inside the ileum of pigs and *L. intracellularis* in pig faeces. However, when utilised in excessive concentrations, essential oils may have strong or overbearing smells.

The addition of herbs to pig feed has the immediate impact of stimulating appetite. It was determined that menthol (*Mentha arvensis*) improves protein and amino acid digestibility, thus feed efficiency, in weaned piglets, and black pepper improved broiler performance.

Aloe vera herbal products will increase the digestion process, helping to combat bacteria and viruses, and will increase the antibody titers to combat Newcastle disease and coccidiosis in broilers. A natural bioactive combination comprising lemon, onion and garlic juice promoted health, egg weight, number of eggs/bird, feed conversion efficiency, and decreased the blood cholesterol of laying hens. Feed conversion efficiency in layers is improved by way of a higher dose of garlic powder supplementation in diets.

Recently, it was discovered that the administration of red ginger and brotowali extracts either separately or added to drinking water resulted in excellent frame weight, feed conversion ratio, carcass percentage, belly fat percentage and cooking-loss percentage in broilers.

Rumen microbial activity

The rumen is an essential digestive organ in farm animals, which houses a complicated environment composed of a wide variety of microbial species, normally along with bacteria, protozoa and fungi. According to studies, plant extracts can act as rumen modifiers because of their antimicrobial activity against micro-organisms.

The vast body of research on plant extracts applied to decrease ruminal methane emissions supports their position as rumen modifiers. Plant extracts such as garlic powder at dose rates of 250 and 300mg/kg frame weight enhance basic feed intake, frame weight, body condition score and feed conversion efficiency in buffalo calves.

Supplementing feed with medicinal and aromatic natural plants such as caraway (*Carum carvi*), fennel (*Foeniculum vulgare*) and lemon balm (*Melissa Officinalis*) significantly enhances growth, *in vitro* dry matter digestibility, *in vitro* organic matter digestibility, metabolic efficiency, overall methane emissions, unsaturated fatty acid levels and rumen fermentation. These effects are particularly pronounced at a 7% supplementation level compared to the control group.

Supplementation with rosemary extract appears to enhance milk yield and milk lactose yield, probably by promoting propionate production within the rumen. It improves the antioxidant status of high-producing dairy cows, and decreases the relative prevalence of *Prevotella* bacteria while increasing the prevalence of numerous genera associated with nutrient degradation and fermentation.

The fermentation product was extensively accelerated at low levels of supplementation with tea saponin (10g/livestock/day). These changes are likely linked to changes in the relative prevalence of microbial species involved in carbohydrate decomposition within the LT treatment group, along with actinobacteria, *Saccharomyces* and *Aspergillus*.

Saponin supplementation in sheep also enhances the digestibility of organic matter, neutral detergent fibre and acid detergent fibre by 9,6 and 27,9 and 38%, respectively. Because of their selective effect on specific rumen micro-organisms, essential oils reduce protein and starch degradation in addition to suppressing amino acid degradation in the rumen.

Conclusion and future perspective

Feed additives are substances which can be added to animal feed to improve its quality and the performance and health of the animals. Plant extracts are one of the feed components utilised in farm animals' feed.

They may be crucial to improving growth performance and because they are used for their antioxidant, antimicrobial, anti-inflammatory, anti-coccidial and anthelmintic properties in addition to enhancing ruminal microbial activity, diet palatability and stimulation of digestion.

Bioactive chemical substances produced in nature, consisting of flavonoids, glucosinolates and isoprene derivatives, are frequently responsible for the traits of plant extracts. The quantity of active compounds in plant extracts varies notably due to natural components, geographic origin, herbal maturity level, maintenance strategies and length, storage and extraction processes.

This article has been condensed for publication in *AFMA Matrix*. For the full article or a list of references, visit *doi.org/10.1016/j.heliyon.2024.e24973* or email Wondimagegn Tadesse Alem at wondie659@gmail.com.

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AFMA INTERVARSITY WRITER'S CUP 2024: WINNER ROUND 2 / LITERATURE REVIEW

The importance of understanding phosphorus digestibility in poultry production

By Jamie Fourie, Chemunique

Phosphorus (P) is an essential micromineral for poultry, and is involved in major functions such as bone, egg, muscle, membrane and nucleic acid formation. It is also a co-factor in various biochemical processes.

Monogastric diets often require inorganic P (P_i) supplementation, as there is very low P available to the bird. This is because of the high cereal and cereal by-product content in the diet, and a subsequently high phytate concentration, to which P is bound and unavailable unless acted upon by exogenous phytase enzymes.

Even with this, however, P_i is still globally supplemented in feeds in the form of rock phosphates (e.g. monocalcium phosphate [MCP] and monodicalcium phosphate [MDCP]) to reach the birds' requirements for optimal growth.

This literature review aims to focus on the importance of understanding P digestibility, with a particular focus on the poultry industry.

Phosphorus terms

Literature commonly refers to P using at least five different terms, namely total phosphorus (total P), phytate phosphorus (PP), non-phytate phosphorus (nPP), available phosphorus (avP), and digestible phosphorus (digP). *Table 1* elaborates on these terms – and others – commonly used in research studies. There are, however, limitations in these widely accepted definitions.

For available P, the P content is assumed to be 100% available from P_i sources and animal-based sources and 30% available from plant sources (NRC, 1994). This assumption does not account for the large variability in the P availability of different feed ingredients and therefore lacks accuracy (Angel, 2013), which presents difficulties for nutritionists and animal scientists around the world when aiming for hyper-accurate nutrition. These differences in the digestibility and/or availability of various P_i ingredients are further elaborated on in *Table 3*.

Current Ca an P requirements

Literature tends to differ with regard to recommendations for the requirement of calcium (Ca) and P, as studies have shown that the NRC (1994) recommendations are often higher than those of more recent studies (Li *et al*., 2016; Angel *et al*., 2022). This presents issues of potential overformulation, or unnecessary addition of expensive ingredients in diets, both of

Table 1: Phosphorus terms in common literature.

Term	Definition	Reference
Total P	The total amount of P in the feed.	NRC (1994)
Phytate P (PP)	P that is bound to the phytate molecule and assumed to be 100% unavailable to the bird.	NRC (1994)
Non-phytate P (nPP)	Used interchangeably with available P (avP). NRC (1994) defines this as the total P less the phytate P, and it is assumed to be completely available to the bird.	NRC (1994)
Available P (avP)	The sum of the total P is from inorganic sources as well as animal sources, and 30% of the P in plant-based sources.	NRC (1994)
Digestible P (digP)	The dietary P recovered in the distal ileum.	Doeschate *et al*., 1993
Retained P (rP)	Used interchangeably with total tract P digestibility, representing the dietary P recovered in the excreta. Since this would include uric acid in poultry, it would not be an appropriate measure for P utilisation in birds.	Ven der Klis, 1994

Table 2: Ca and P requirements of layers and broilers.

Parameter (g/kg)					Reference
Layers					
Age (weeks)	*18 to 20*	*20 to 60*			NRC, 1994
Ca	32,5	35,6			
nPP	3	2,5			
Age (weeks)	*18 to 35*	*36 to 55*	*56 to 74*	*75 to 91*	Hy-Line, 2016
Ca	40,8	39,1	4,09	44	
AvP	4,5	3,8	3,5	3,3	
Age (weeks)	*19 to 50*	*50 to 70*	*70 +*		Lohman, 2017
Ca	28,5-37,2	30,6-39,9	31,25-40,9		
AP	2,9-3,8	2,8-3,6	2,7-3,4		
Broilers					
Age (days)	*0 to 21*	*22 to 35*			NRC, 1994
Ca	10	10			
nPP	4,5	3,5			
Age (days)	*0 to 10*	*11 to 24*	*25 +*		Aviagen, 2022
Ca	9,5	7,5	6,5		
AvP	5	4,2	3,6		
Age (days)	*0 to 10*	*11 to 16*	*17 to 24*	*25 to 32*	Angel *et al.*, 2022*
Total Ca	9,4	8,9	7,5	6,5	
Digestible P	5,3	4	3,2	2,8	

**Diets were supplemented with a given matrix value of phytase.*

which are detrimental to the bird or the formulator.

Rock phosphate issues

The majority of P_i supplemented in diets originates from rock phosphates. This, however, poses an issue due to various reasons, the first being the issue of affordability. Rock phosphate prices have shown to be volatile in nature, dramatically increasing in times of conflict, such as during the Russia-Ukraine war, where prices doubled between the time of invasion and April 2023 (Shanini *et al.*, 2023).

This is not only due to international discord, but also other factors such as supply chain challenges heightened by the Covid-19 pandemic, recent unfavourable climate conditions, and global shipping crisis (Pope *et al.*, 2023). These all pose an issue to animal nutritionists when formulating diets on a least-cost basis, especially as feed costs are estimated to account for an estimated 70% of total production costs.

Secondly, there are environmental concerns surrounding the use of high P_i inclusion levels. Rock phosphates are a finite source, expected to be depleted in 50 to 100 years (Cordell *et al.*, 2009). Their over-supplementation in the diet leads to an increased P content in excreta, which may contaminate water sources, causing eutrophication and threatening the existence of aquatic fauna and flora (Pope *et al.*, 2023).

A higher P content in excreta is also associated with a higher incidence of wet litter, which creates a welfare issue due to the increased occurrence of footpad dermatitis and hockburn (Collett, 2012). These have been the motivating factors behind the recent global focus towards reducing dependence on rock phosphate.

Maximising P utilisation

To reduce the dependence on rock phosphate, one needs to focus on

maximising the P already in the diet. The inclusion of exogenous phytase is now widely practised in the poultry industry. This allows for PP from the diet to be better utilised by the bird, minimising the need for P_i supplementation (Applegate *et al.*, 2003; Li *et al.*, 2021). Although the addition of phytase has been proven to be undoubtedly effective, exogenous enzymes are expensive and reliant on the PP content of the diet, so phytase cannot be considered the sole solution to improving P digestibility (Li *et al.*, 2016).

Optimal formulation and feeding of P should also be implemented by considering the differences in P availability of different raw materials, and, more importantly, creating an accurate model of the P requirements of modern poultry genotypes (Rodehutscord, 2013). In modelling this system, *in vivo* methodology utilises a low-P basal diet and pre-caecal digestibility methods using a regression approach (Rodehutscord, 2013).

Studies have also shown that Ca chelates phytate at pH values above four, rendering both Ca and PP unavailable (Tamim *et al.*, 2004). This further emphasises the

Table 3: Available and digestible P values from various inorganic P sources.

Inorganic P feedstuff	AvP (%)	DigP (%)	Reference
MCP[1] (hydrous)		85	CVB, 2021
DCP[2] (hydrous)		78	
DCP[2] (anhydrous)		55	
MDCP[3]		79	
MSP[4] (hydrous)		91	
MCP[1]	21,2	85	Rostagno *et al.*, 2017
DCP[2]	18,5	70	
MDCP[3]	19,6	85	
MCP[1]		81	Angel *et al.*, 2022
DCP[2] (hydrous)		76	

[1]MCP: Monocalcium phosphate; [2]DCP: Dicalcium phosphate; [3]MDCP: Monodicalcium phosphate; [4]MSP: Monosodium phosphate.

importance of including an exogenous phytase in the diet, allowing P to be liberated from PP in the proximal digestive tract before chelation with mineral cations occurs. To further manage this chelation with phytate, dietary management of these ions (including Ca) is essential, and accidental over-supplementation of these ions must be avoided at all costs. This is because, when Ca is supplied in excess, it acts as an anti-nutrient (David *et al.*, 2021). Consequences of this overinclusion (when supplemented in the form of limestone) include reduced broiler performance (David *et al.*, 2021), as well as reduced Ca and P digestibility (Tamim and Angel, 2003; Tamim, 2004; Plumstead *et al.*, 2008; Abdollahi *et al.*, 2016; Bradbury *et al.*, 2018). A way to prevent the over-formulation of Ca is being developed by researchers working towards formulation based on a digestible calcium (dCa) system to ensure an optimal supply of Ca in the diet and, subsequently, optimise P nutrition (Kim *et al.*, 2019; Walk *et al.*, 2021; Angel *et al.*, 2022; Venter *et al.*, 2022).


Jamie Fourie.

Variable availability of P

Commonly used P_i sources differ in their availability and/or digestibility of P based on several characteristics. Even between the same ingredients, the presentation of the anhydrous or hydrous form can largely impact digP values. For this reason, a globally accepted accurate assessment of digP is necessary to accurately assess the P that is available to the bird. When selecting a rock phosphate source, one needs to evaluate it on a cost/dP unit to ensure one is optimising cost/kg broiler.

Conclusion

The importance of understanding and evaluating P digestibility has been highlighted, along with elaboration on its impact on feed affordability, as well as welfare and environmental concerns. Currently, methods to ensure optimal utilisation of P in the diet include adding phytase to the feed and ensuring optimal Ca levels in the diet to maximise P utilisation.

Research requires further development and implementation of a digP and dCa system, allowing nutritionists to formulate diets to the birds' exact requirements, mitigating the detrimental effects of excessive P in poultry diets.

References available on request. For more information, send an email to Jamie Fourie at jamie@chemunique.co.za or phone 079 796 6453.

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AFMA INTERVARSITY WRITER'S CUP 2024: WINNER ROUND 2 / OWN RESEARCH

The effect of *Ulva lactuca* in feedlot lamb diets on methane emissions and animal performance

By Soné Nell, Stellenbosch University

In pursuit of sustainable agriculture, research into mitigating methane emissions from ruminant livestock has gained considerable momentum. The Food and Agriculture Organisation (FAO) of the United Nations reports ruminant livestock to be responsible for 10% of all anthropogenic greenhouse gas emissions (Gerber *et al*., 2013).

Utilisation of marine algae as a feed source for animals and humans alike has been practised for centuries, but in recent years seaweed has been noted for its potential to reduce methane emissions when included in the diets of ruminants. The exploration of innovative approaches such as incorporating seaweed into animal diets is crucial for enhancing the sustainability of animal product production and mitigating environmental impact.

This study aimed to determine whether *Ulva lactuca*, a green seaweed found along the coasts of South Africa, could reduce methane emissions from sheep while yielding similar growth patterns.

Background

In addition to the volatile fatty acids produced when feed is anaerobically digested in the rumen, methanogenic archaea produce carbon dioxide (CO_2) and methane (CH_4), which are eliminated by means of eructation (Weiss *et al*., 1953; Liu and Whitman, 2008; Janssen and Kirs, 2008; Martin *et al*., 2010; Oertel *et al*., 2018).

The process of fermentation is oxidative, and the reduced cofactors (NADH, NADPH and FADH) undergo dehydrogenation reactions to be re-oxidised (NAD^+, $NADP^+$ and FAD^+), subsequently releasing dissolved dihydrogen molecules (dH_2) in the rumen when monosaccharides are hydrolysed to glucose. As soon as it is produced, dH_2 is utilised by methanogenic archaea in the rumen to reduce CO_2 into CH_4 (Wang *et al*., 2014).

Methane production not only has a major impact on the environment but also on livestock production, as 2 to 12% of dietary energy is lost to the production of methane (McDonald *et al*., 1988; Johnson and Johnson, 1995; IPCC, 2007). This is equivalent to 28ℓ of methane (or 15g) produced per kilogram of dry matter intake (DMI) (Mathison *et al*., 1998).

The reduction in the amount of methane produced by ruminants when fed diets containing seaweed is mainly attributed to the chemical compound, bromoform (Machado *et al*., 2016).

Bromoform disrupts the methane biosynthetic pathway (also known as the Wolfe cycle) within methanogenic microbes (Machado *et al*., 2015), without affecting other microbes in the rumen (Kinley *et al*., 2020). Bromoform is part of a group of halogenated methane analogue (HMA) components that inhibit key steps of methanogenesis (Bauchop, 1967; Wood *et al*., 1968; Machado *et al*., 2015).

Halogenated methane analogues, such as bromoform, inhibit methanogenesis by impeding the action of the Wolfe cycle's essential metalloenzymes (Bauchop, 1967; Wood *et al*., 1968; Gunsalus and Wolfe, 1978; Wolfe, 1985; Belay and Daniels, 1987; Oremland and Capone, 1988; Krone *et al*., 1989a, b; Thauer, 2012). The HMAs disrupt the Wolfe cycle by competitively reacting with the substrates of the catalyst enzymes, co-enzyme M methyltransferase and methyl co-enzyme M reductase (Brot and Weissbach, 1965; Wood *et al*., 1968; Oremland and Capone, 1988; Krone *et al*., 1989a, b).

The red seaweed, *Asparagopsis taxiformis*, has achieved great success in mitigating methane emissions from cattle, reducing it by as much as 98% when included in the diet of steers at a rate of


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2g kg^{-1} organic matter (Kinley *et al*., 2020). However, this species is found in tropical to temperate waters along the Australian coast and is not cultivated in South Africa (Msuya *et al*., 2022). *Ulva lactuca*, however, is commercially produced in South Africa in excess of 2 000 tonnes per year (Rothman *et al*., 2020), making it an ideal candidate for study.

Materials and methods

U. lactuca was harvested from four-paddlewheel raceways on a commercial abalone farm and then rinsed with fresh water to remove sand, debris and epiphytes. It was dried in greenhouse tunnels for seven days, after which the trial was conducted in individual pens on the Welgevallen experimental farm in Stellenbosch.

Dohne Merino ewes (N=40) were randomly allocated to one of four experimental diets containing varying amounts of *U. lactuca* in the diet: (1) 0U: 0g *Ulva* kg^{-1} dry matter (DM); (2) 25U: 25g *Ulva* kg^{-1} DM; (3) 50U: 50g *Ulva* kg^{-1} DM; and (4) 100U: 100g *Ulva* kg-1 DM, at the cost of the lucerne inclusion.

Feed intake and bodyweight were determined weekly, and methane measurements were taken during three blocks of the trial (early trial: 0 to 7 days after adaptation; mid-trial: 15 to 21 days after adaptation; late trial: 28 to 35 days after adaptation), at three different times of the day (06:30 to 08:00; 11:30 to 13:00 and 17:00 to 18:30), resulting in nine measurements per ewe. A handheld laser methane detector (LMD) was used to determine the spot-sampled methane emissions of ewes.

The LMD automatically identifies and removes errors in laser beam reflectance from the data set. Data from the LMD that was recorded when the LMD moved from the mouth and nose of the animal or when the sheep moved, was manually removed. Background methane measurements were taken in each pen two days after the ewes left the housing unit.

An average background methane value was calculated and subtracted from the data recorded to calculate the intrinsic methane emissions from each animal (Ricci *et al*., 2014). The spot-sampled methane concentrations observed for each sheep were converted to g CH_4 kg^{-1} DMI d^{-1} to determine the daily methane production per kilogram DM intake per sheep.

This was done using the equation (Van Wyngaard, 2018)

$$Y = \frac{d \times (5{,}76 \times m)}{DMI}$$

where Y is the methane production (g kg^{-1} DMI d^{-1}), d is 0,38 (if the animal is standing) or 0,31 (if the animal is lying down), m is the average methane concentration (ppm-m) and DMI is given in kilogram per day.

Table 1: Average intake and growth parameters (mean ± SEM) of Dohne Merino ewes for each treatment group.

Item[1]	Treatment[2]				P-value
	0U	25U	50U	100U	
Weight gain (kg)	9,644a ± 0,576	8,320a ± 0,546	8,920a ± 0,546	6,480b ± 0,546	0,002
ADMI (kg d^{-1})	1,632a ± 0,080	1,545a ± 0,076	1,496a ± 0,076	1,248b ± 0,076	0,009
ADMI (% LW)	4,537a ± 0,159	4,386a ± 0,151	4,187a ± 0,151	3,610b ± 0,151	0,001
ADG (kg d^{-1})	0,276a ± 0,016	0,238a ± 0,016	0,255a ± 0,016	0,185b ± 0,016	0,002
FCR	5,340 ± 0,445	5,685 ± 0,422	4,889 ± 0,422	6,026 ± 0,422	0,283

Means with superscripts (a-b) in the same row differ significantly (P ≤ 0,05). [1]ADMI: average dry matter intake; LW: live weight; ADG: average daily gain; FCR: feed conversion ratio. [2]Treatments: 0U (0g Ulva kg^{-1} DM), 25U (25g Ulva kg^{-1} DM), 50U (50g Ulva kg^{-1} DM), 100U (100g Ulva kg^{-1} DM).

Discussion

Performance parameters

Results for average feed intake and growth parameters over the total course of the trial are given in *Table 1*. No significant differences were found for total weight gain, daily DMI or average daily gain between 0U, 25U and 50U treatment groups. However, weight gain, feed intake and average daily gain were significantly lower for 100U ewes (*Table 1*; P < 0,05) compared to the other treatment groups. No significant difference in overall feed conversion ratio was observed between the treatment groups.

Intrinsic methane production

Table 2 shows the effect of *U. lactuca* inclusion on methane emissions of the animals. Overall methane production did not differ between 0U, 25U, 50U and 100U ewes for methane production throughout the entire trial (*Table 2*; *P* = 0,176).

This is in accordance with an *in vitro* study by El-Waziry *et al*. (2015) who found no differences in methane production with the inclusion of *U. lactuca* in the diet of up to 50g kg^{-1}. However, these

Table 2: Methane production (mean ± SEM) in g kg^{-1} DMI d^{-1} of Dohne Merino ewes for each treatment group for entire-, early-, mid- and late-trial.

Item[1]	Treatment[2]				P-value
	0U	25U	50U	100U	
Entire trial	62,797 ± 10,299	56,987 ± 9,668	58,938 ± 9,668	71,692 ± 9,668	0,176
ET	101,822 ± 15,219	86,174 ± 14,438	88,544 ± 14,438	108,463 ± 14,438	0,561
MT	58,453 ± 15,219	53,451 ± 14,438	56,149 ± 14,438	85,170 ± 14,438	0,097
LT	53,346b ± 15,219	57,830b ± 14,438	65,630b ± 14,438	123,471a ± 14,438	0,005

Means with superscripts (a-b) in the same row differ significantly (P ≤ 0,05).[1]Time of trial: ET: early trial (0 to 7 days); MT: mid-trial (15 to 21 days); LT: late trial (28 to 35 days). [2]Treatments: 0U (0g Ulva kg^{-1} DM), 25U (25g Ulva kg^{-1} DM), 50U (50g Ulva kg^{-1} DM), 100U (100g Ulva kg^{-1} DM).

results were in contrast to a study by Machado *et al*. (2014b), who reported *in vitro* reductions in methane emissions by up to 22% when *U. lactuca* was included in the diet at a rate of 170g kg^{-1}. To the knowledge of the author, there are no published results on *in vivo* methane emissions from sheep fed a diet containing *U. lactuca*.

Williams *et al*. (2009) reported indications that the methane-producing microbial population require an adaptation period of 30 days after switching to a new feed. No differences were observed within the ET and MT time periods. During LT, 100U ewes produced more methane compared to all other treatment groups (*Table 2*, P = 0,005).

The 100U treatment group had the lowest intake throughout the trial, but more so in the LT time period. This led to increased rumen retention time, more fermentation and thus more methane being produced per unit of DMI, agreeing with the findings of Galyean and Owens, 1991. No evidence for rumen microbiome adaptation with regard to methane production was observed.

The reduction of methane emissions by seaweeds is largely attributed to bromoform (Machado *et al*., 2015). Previous studies reported *U. lactuca* to contain 150ng bromoform g^{-1} of fresh weight (Gschwend *et al*., 1985; Manley *et al*., 1992; Collén *et al*., 1994; Carpenter and Liss, 2000). However, no bromoform was detected in the dried *Ulva* samples used in this study.

The bromoform content of *Ulva* spp is also significantly lower than in some other seaweeds. A review by Min *et al*. (2021) reported the bromoform content to be 1 723µg g^{-1} DM in *Asparagopsis taxiformis*, 1 320µg g^{-1} in *A. armata*, compared to the 150ng g^{-1} fresh weight reported by the aforementioned authors.

The drying method in the current study was chosen based on its practicality for drying the large amounts of raw material required, and its general availability and acceptance in the commercial sector. In saying this, however, it is possible that the drying technique resulted in no bromoform being detectable in the sample. Early work by Simnikov (1941) indicated that the evaporation of bromoform from samples that are processed is largely influenced by vapour pressure. Vapour pressure is a function of temperature (Felder and Rousseau, 2004), and would be approximately 5kPa for bromoform at 60°C.

Bromoform in a free form readily evaporates into the air at 20°C and it is safe to assume that at 60°C, with a vapour pressure of 5kPa and under conditions of ventilated drying, a significant proportion of the bromoform originally in *U. lactuca* could have been lost. From the lack of detection of bromoform in the trial diets and the absence of animal response (*Table 2*), the little bromoform to be found in *Ulva* spp would likely have been lost under drying conditions utilised in the present study.

Photograph by W Carter, commons.wikimedia.org/w/index.php?curid=90089793.

Conclusion

U. lactuca cannot be ruled out as a potential anti-methanogenic, as the active compound responsible for reductions in methane emissions from ruminants was absent in the sample due to the drying technique used. While no conclusion can be drawn with regard to its potential to reduce methane emissions from ruminants, *U. lactuca* can successfully replace lucerne at a rate of up to 50g kg^{-1} without any negative influence on the production performance of Dohne Merino ewes.

References available on request. For more information, send an email to Soné Nell at 20791925@sun.ac.za or phone 060 984 2867.

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