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Matrix

AFMA
SAFE FEED FOR SAFE FOOD
Animal Feed Manufacturers Association

Quarterly magazine of the Animal Feed Manufacturers Association

Carryover regulations • Feed Miller course feedback • Biofuels • Methionine
Hammermill screen selection • Amino acids • Salmonella in poultry production

A sense of optimism amid challenges

By Michael Schmitz, vice chairperson, AFMA

These are certainly trying and uncertain times we are currently living in, both for the business community as well as the fatigued consumer, and to not acknowledge the existence of these uncertainties is akin to either living in a fool's paradise or being blatantly irresponsible.

At the time of writing this preface, Statistics SA's April 2023 publication reported that annual inflation had increased to 6,8% compared to 5,9% in March 2022, with the consumer basket of milk, eggs and cheese recording an annual price increase of 14,5% and meat 9,5%.

These rising costs are further compounded by the increase in the prime lending rate, which the Reserve Bank has increased by 475 basis points since November 2021 to curb rampant inflation to the midpoint of its inflation target of 4,5%. Using an online tool from one of the leading banks to demonstrate the impact this has had, a consumer with a R1 million housing bond and R200 000 in vehicle finance will pay R3 500 more per month to repay his or her debt.

This additional financial burden is one many households can ill afford and has forced consumers to change their spending patterns, manifesting itself in many different ways, depending on the industry. The animal feed industry will no doubt also continue to feel the ripple effect of the changes animal protein producers are implementing.

Major impacting factors

So, what are the causes of this dire situation? The answer, it would regrettably appear, is that South Africa has an ongoing propensity to score 'own goals'. A list of factors viewed by economic analysts as continuing to have a negative impact on the economy includes load shedding, which will be exacerbated by the increased likelihood of more hours without electricity – Eskom recently announced an extremely high chance of Stage 8 load shedding in July and August this year, which equates to 16 hours without electricity in a 32-hour cycle.

Added to the cost impact of load shedding is the failure of logistics networks to function effectively, which will have a long-term impact on job creation and government revenue. A loss in revenue means that less money is available for social and infrastructure spending.

As if that is not enough, a number of diplomatic clashes with the United States (US) have placed our access to US markets under the African Growth and Opportunity Act (AGOA), representing approximately R400 billion in trade on an annual basis, at risk.

Grain and oilseeds lead the way

Fortunately, there are still some positives amid the doom and gloom, the most critical being the recent decline in soft commodity prices, despite the weakness of the Rand. Since December 2022, the international prices of grain and oilseeds have declined somewhat following the extension of the Ukraine:Russia grain export agreement, the large combined maize and soya bean crops out of South America, and optimal planting conditions in the US.

Based on current weather conditions, the US is set for a record maize crop which could result in a significant increase in the maize stock-to-use ratio at the end of the 2024 season. This will support a drop in maize prices on the Chicago Board of Trade and could potentially assist in keeping the Safex maize price at export parity levels.

Harvesting of maize and soya beans is underway locally and we are currently set for another large maize crop, as well as a record soya bean crop. There should be ample maize and soya beans to satisfy local feed, food and export demand.

Hot, dry weather forecasted

There is, however, a strong possibility that South Africa will transition from a La Niña to an El Niño weather phenomenon, which historically means above-normal temperatures and below-average rainfall. If this materialises, the market could react and cause maize to trade off export parity once planting of the next season's crops commences. Producers should have sufficient soil moisture to start planting in October, but follow-up rain will be needed to ensure another decent-sized crop.

These weather conditions potentially bode well for the industry in the short term, as the reduction in soft commodity prices will continue to assist in reducing the input costs of feed and offset some of the additional expenses incurred during load shedding, until such time as private investment into alternative energy comes online in a significant way.

These factors have certainly forced us to reconsider how we do business, and influence the decisions we make in our private lives. I certainly hope that we experience some sense of increased optimism as we navigate these uncharted waters and apply innovative solutions to the challenges we face. ❖

For enquiries, send an email to Michael Schmitz at Michael.Schmitz@meadowfeeds.co.za.


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Editorial Committee

Published by: Plaas Media (Pty) Ltd
217 Clifton Ave, Lyttelton, Centurion, RSA
Private Bag X2010, Lyttelton, 0140, RSA
Tel: +27 12 664 4793 • *www.agriorbit.com*

Associate editor: Liesl Breytenbach
+27 12 663 9097 • liesl@afma.co.za

Chief editor: Lynette Louw
+27 84 580 5120 • lynette@plaasmedia.co.za

Deputy editor: Jayne du Plooy
+27 82 414 0151 • jayne@plaasmedia.co.za

News editor: Elmarie Helberg
+27 73 339 2920 • elmarie@plaasmedia.co.za

Design & layout: Inge Gieros
+27 82 959 9607 • inge@plaasmedia.co.za

Advertising:
Karin Changuion-Duffy
+27 82 376 6396 • karin@plaasmedia.co.za
Susan Steyn
+27 82 657 1262 • susan@plaasmedia.co.za
Zona Haasbroek
+27 82 960 7988 • zona@plaasmedia.co.za

Sales manager: Marné Anderson
+27 72 639 1805 • marne@plaasmedia.co.za

Subscriptions: Beauty Mthombeni
+27 64 890 6941 • beauty@plaasmedia.co.za

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Published on behalf of AFMA
Agri-Hub Office Park, Block B, 477 Witherite Str,
The Willows, Pretoria
+27 12 663 9097
www.afma.co.za

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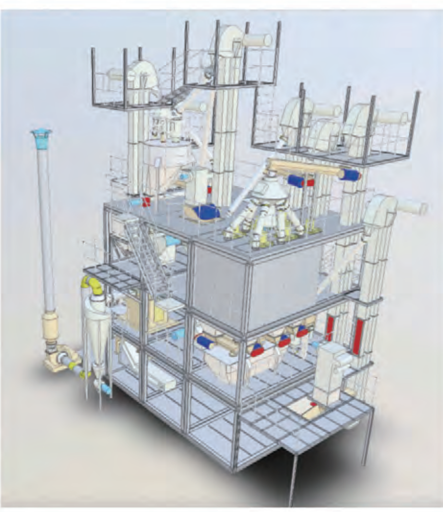
Development Background

Dumping	10-15 t/h
Grinding	5-6 t/h (3mm or 3.5mm)
Batching	Automatic batching system
Mixing	5-6 t/h, 500 kg/bat,10-12 bat/h (0.60t/m³)
Pelleting	3-5 t/h , Ø3 mm, 1:12, Conditioning 15-20s, 70-80℃
Packaging	6 -10t/h
Oil adding	4-100L/min
Control system	Automatic control （Computer system）

Containerised Feed Mill advantage



- Key equipment and main components of the feed mill are pre-assembled in the workshop thus reducing on-site installation time up to 75%;
- Pre-assembled and tested before delivery to ensure the installation quality thus improving commissioning efficiency up to 50%
- Civil works are kept to a minimum since the separate support is not needed thus reducing construction cost and customer TCO up to 95% and 25% respectively;
- Reduce installing skill requirements to avoid the shortage of local skilled labour;

- No pit design facilitates future maintenance
- Suitable for various transportation modes including road, rail and sea, etc.
- Able to be relocated and rebuilt on a different location in one piece
- Modular design and plant extension to customer needs (automatic or manual batching, control room, compressed air station, changing room, etc.).

NEWS & views

AFMA Forum 2023 programme now available

'Feed & Food – The 4th Agricultural Revolution' is the theme for the 2023 AFMA Forum to be held from 5 to 7 September at Sun City. Be sure to block out the date in your diary to be part of this highlight on the annual agricultural calendar.

Take a sneak peek at the incredible line-up of sessions, expert speakers and thought-provoking discussions that await you at the 11th AFMA Forum. From sustainable production and health to precision nutrition and cutting-edge technologies, AFMA Forum 2023 promises to be an unforgettable experience.

AFMA FORUM
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Expensive animal feed hurting livestock farming

The big jump in the price of animal feed – a global problem resulting from the war in Ukraine – has led Greek livestock farmers to feed their animals as little as possible or having to send their herds for slaughter, while a big drop in the production of sheep's milk in Crete means that locally produced cheese such as graviera and anthotyro could be in short supply in the market.

In regions like Thessaly in Central Greece, where land is abundant, it is possible for cattle owners to produce their own feed and limit expenses, but in the islands of the Aegean and Crete, livestock farming is facing a problem of survival.

"Due to the dry climate of the islands, no fodder crops can be grown and the farmers need to buy all the feed. If we add the cost of transportation, the production of sheep's milk is now unprofitable," said Lefteris Gitsas, president of the National Interprofessional Meat Organization.

At the same time, "many herds of productive animals (animals at the age of giving birth and milk), which had another five years of opportunity to produce products, are slaughtered by the breeders because they cannot face the costs of animal feed," explained Yannis Glentzakis, president of the Agricultural Cooperative of Rethymnon, in Crete. – *ekathimerini.com*

Poultry litter as source of feed protein

A group of scientists from Russia's Ufa Science and Technology University said it developed a promising scheme for extracting protein from poultry litter. The authors of the study say that they developed a special solution, allowing them to dissolve poultry litter and obtain protein, among other substances. If the technology is proven effective, it will save poultry farmers a lot of money.

The scientists have already launched a pilot laboratory installation, while the first industrial line producing protein from poultry litter is in the pipeline, said Vadim Zakharov, acting rector of Ufa University. The authors of the study believe that this protein could be swiftly absorbed by chickens when passing through their organism the second time, though additional studies are required to prove that assumption.

The feed protein sourced from the chicken litter has already been successfully tried in manufacturing cattle feed, local press reported, not providing additional details. Currently, the authors are preparing for the first field trials at a poultry farm, stating that "the chance for success is great". – *All About Feed*

Ketels to step down as CEO of Orffa

Eddy Ketels will step down as CEO of Orffa at the end of 2023. He will be succeeded by Haiko Zuidhoff, who will start in his new role as of 1 October 2023.

Ketels has been working for 35 years at Orffa. He became CEO in 2001. Under his leadership, Orffa has developed from a local player, mainly focussed on ingredients in Belgium and the Netherlands, into a global, innovative, value-adding company having a presence in the main feed producing countries, offering a balanced portfolio of feed additives.

Zuidhoff has worked in Asia for 25 years and moved back to the Netherlands in 2022. He most recently served IMCD as president for the Asia region, overseeing IMCD's vision, strategy and accelerated growth through both organic growth and M&A. Prior to IMCD he served in various senior leadership roles at Trouw Nutrition, Nutreco and Corbion. Zuidhoff will be based at Orffa's headquarter in Breda, the Netherlands. – *Press release, Orffa*

Haiko Zuidhoff will start in his new role as CEO of Orffa in October.

Third largest maize harvest on the cards

South Africa's Crop Estimates Committee (CEC) lifted the country's 2022/23 maize production estimate by 2% to 16,1 million tons. This is 5% more than the 2021/22 season and the third-largest harvest on record. The expected large harvest is primarily on the back of large yields, as the area planted is slightly down from the 2021/22 season.

The harvest comprises of 8,5 million tons of white maize and 7,6 million tons of yellow maize. A crop of 16,1 million tons implies South Africa will have sufficient supplies to meet domestic maize needs of roughly 11,4 million tons and have over three million tons available for export markets in the 2023/24 marketing year.

The soya bean harvest remained unchanged from April's record estimate of 2,8 million tons (up 24% year-on-year). The crop improvement is due to an expansion in the planted area and the expected higher yields. Thanks to the ample soya bean harvest, South Africa could meet its domestic demand and retain over 300 000 tons of soya beans for export markets. This soya bean export expansion is new territory for South Africa which, until recently, was a net importer of soya beans and soya bean products – this is beneficial to the agricultural trade balance. – *Wandile Sihlobo, Agbiz*

Merger between VKB and GWK approved

The merger between VKB and GWK received final approval by the relevant regulatory authorities, including competition authorities, after the final suspensive conditions required for the transaction were fulfilled. This means that these two agricultural groups will now operate as a new combined group. A joint notice and outline of the finalisation of the scheme is available on the two companies' respective websites.

The announcement follows the approval of the merger on 21 December 2022 by the Competition Tribunal, subject to certain conditions that were approved by VKB and GWK, shortly after a recommendation made by the Competition Commission in favour of the merger. Discussions between the two companies started in July 2020, after which GWK's shareholders voted in favour of the merger on 11 August 2022, and VKB's board of directors approved the transaction within their mandate earlier in 2022. – *Press release, GWK & VKB*

Mycotoxin risk management strategy is necessary

Decreased gastrointestinal integrity, immunity and performance in broilers leading to economic losses caused by mycotoxins mean poultry producers should look to draw up risk management strategies.

Animal health and nutrition company, DSM-Firmenich, says producers need to look at three considerations to evaluate the impact of mycotoxins on poultry farms: measure the contamination levels in ingredients and finished feed; include mycotoxins as potential predisposing factors to health challenges; assess low-level mycotoxin contamination on performance and profitability.

Mycotoxins are frequently found in poultry feed, with the DSM-Firmenich *World Mycotoxin Survey* showing that more than 33 000 analyses of finished poultry feed tested positive for mycotoxins in the last ten years (2013-2023). And of these, more than four-fifths of samples (83%) are contaminated with more than one mycotoxin and as many as 50 mycotoxins were found in a single sample.

Exposure to multiple mycotoxins simultaneously can lead to more severe effects on health and bird performance, so the company suggests testing the finished feed and/or their ingredients to evaluate the risk for the bird's health. – *Poultry World*

Astral finances bleed amid load shedding

The cost of load shedding has wreaked havoc on the profit margins of South Africa's largest integrated poultry company, Astral Foods Ltd, for the six-month period ending 31 March.

Its operating profit declined by 88% to R98 million (March 2022: R785 million) over the same period. This decline was mainly due to the impact of load shedding and failing municipal infrastructure. The company's load shedding costs alone amounted to R741 million.

The group's revenue grew to R10 billion (March 2022: R9,4 billion). The increase in revenue was due to higher feed prices, which rose due to soaring raw material costs (high international grain prices), said Chris Schutte, CEO of Astral Foods.

"Load shedding forced us to feed the broilers for longer, which meant that additional feed costs were incurred," Schutte said during the results presentation. "Furthermore, workers worked additional shifts, which led to increased labour and other related costs. We also had to spend more money on generator operating costs."

Schutte said ironically enough, the group would have reported one of its strongest half year results if it was not burdened by load shedding. "At any given time, Astral has to take care of 40 million live chicks that are either being fed, reared or processed. It is logical that load shedding will have a massive impact on our business." – *Susan Marais, Plaas Media*

Morocco offers help with access to feed

Morocco will spend $0,5 billion to help farmers access animal feed at a subsidised price as the country's grains agency tenders for 500 000 feed barley. The aid is part of a $1 billion package announced last month in aid of farmers to help mitigate drought impact on crops and cattle.

Recurrent droughts slashed Morocco's cereals harvest in recent years and drained the finances of small farmers, leaving many struggling with high agricultural inputs.

The government has also cancelled the value-added tax on agricultural inputs after prices of fertilisers and seeds surged in the international market.

Although the country's annual inflation slowed in April to 7,8%, its food component is still high at 16,3%. – *The North Africa Post* ❖

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FORMAT AND PARTICIPATION OPTIONS

- Agrijob liaises with universities to identify the top students in animal science at each institution.
- Agrijob organises transport and accommodation to the AFMA Forum for the elected students.
- The Universities include UP, UFS, SU, UKZN, UL, NWU, UMP, UZ and Fort Hare (budget permitting).
- Companies engage with top students at organised breakaway sessions as part of the student programme.
- Student programme gatherings and company interactions are video recorded for post-event benefit.
- Cost to participate: **R10 000 + VAT per student**.

PROVISIONAL PROGRAMME

Tuesday, 5 September 2023 • 13:30
Student welcome & briefing

Wednesday, 6 September 2023 • 12:45–13:25
Lunch gathering in Seers

Thursday, 7 September 2023 • 07:45–08:25
Coffee gathering in Seers

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ENQUIRIES:
Marianne van der Laarse | marianne@agrijob.co.za | 082 388 1000
Majella van der Arend | majella@agrijob.co.za | 064 757 1140
Tanja Werle | tanja@agrijob.co.za | 066 479 0951

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AGRIJOB
YOUR CAREER CONNECTION

New AFMA CEO to focus on stronger working relations

By Elmarie Helberg

Dr Sifiso Ntombela joined the Animal Feed Manufacturers' Association (AFMA) team as its new executive director on 1 July, following a thorough recruiting process. Liesl Breytenbach served as interim executive director from January 2023.

Dr Ntombela has vast experience in the agricultural sector, and the AFMA board is confident that he will add tremendous value to AFMA and the greater animal feed and agricultural value chain.

Dr Ntombela holds a PhD in agricultural economics from the University of Pretoria and is an alumnus of Stellenbosch University where he obtained his bachelors, honours and master's degrees. He is an agricultural economist with more than a decade's experience in strategic planning, agricultural policy and economic modelling. In addition, he was the National Agricultural Marketing Council's (NAMC) chief economist responsible for trade research and policy advisory, and served as the head of trade and intelligence at Agbiz.

Dr Sifiso Ntombela.

Relationships are key

According to Dr Ntombela, he would like to see stronger working relations between the animal feed industry role-players and government in all spheres, and intends to create policies and regulations that promote competitiveness, inclusive growth and profitability in the animal feed industry.

"Focussing on expanding export opportunities to drive demand-led investments in the animal feed industry will be one of my areas of priority. This includes fostering close relationships with different stakeholders and academia to constantly improve skills, innovation and research so the animal feed industry can enhance its global position as a producer of safe high-quality products. Even more important is improving relations, especially in terms of complementary functions, with other industries that either provide raw material or are the users of animal feed," he says.

Over the past three years, Dr Ntombela was the national co-ordinator of the Agriculture and Agro-processing Master Plan. He served in government's economic cluster and was part of the team of technical specialists who drafted the *Reconstruction and Economic Recovery Plan of South Africa* in 2020. He still serves on the agricultural advisory body for the National Planning Commission and acts as vice-president of the Agricultural Economics Association of South Africa. Dr Ntombela is a former director for strategic partnerships at the Department of Public Enterprises.

Building a strong industry

AFMA continuously strives to support its members by ensuring that its strategic focus areas remain relevant to the challenges faced by the animal feed industry. Conducting business in a stable environment is not something the association had the privilege of doing over the last couple of years, and therefore AFMA needs to foresee and mitigate the risks the industry experiences daily.

"One of the most exciting areas is bridging the working gap between the animal feed industry and relevant government institutions to build a viable and competitive industry," says Dr Ntombela.

For more information, phone AFMA on 012 663 9097 or visit *www.afma.co.za.*

Upcoming carryover regulations and their impact on the animal feed industry

By Bonita Cilliers, technical and regulatory advisor, AFMA

Feed manufacturers are responsible for ensuring the safety, quality and integrity of the feed they produce. One potential challenge in this regard is carryover, which occurs when traces of a substance are inadvertently transferred (carried) from an 'acceptable' location or feed to an 'unacceptable' one. This can occur during various stages of feed production, handling, storage and delivery, as well as at farm level during feed storage and distribution.

While carryover typically poses minimal risk to animal health and food safety, certain ingredients can increase the potential for contamination, leading to unsafe carryover in subsequent feeds. This is especially concerning when tailored feeds contain unsafe concentrations of minerals, vitamins or medications for non-target species or classes. Supplements or premixtures with high ingredient concentrations also contribute to this issue.

Unsafe carryover compromises product purity, posing risks to animal health and food safety. It results in excessive levels of undesired substances in animal feed, as well as veterinary medicine (drug) residues in meat, milk or eggs, rendering them unsafe for human consumption. Furthermore, carryover exacerbates the pressing concern of antimicrobial resistance (AMR).

Guidance to new legislation

In 2018, to address the issue of carryover, the Department of Agriculture, Land Reform and Rural Development (DALRRD) prioritised the safety and quality of animal feed for non-target species. As part of this initiative, they informed the animal feed industry of its intentions to regulate the maximum allowable level of carryover for authorised feed additives in non-target species.

In response, the Animal Feed Manufacturers' Association (AFMA) established a medicated feed subcommittee to develop a guideline for managing carryover. This guideline aims to assist the animal feed industry in assessing and minimising carryover risks, verifying protocol effectiveness and ensuring compliance with upcoming regulations. By following these guidelines, the industry can enhance its ability to prevent and manage carryover, and maintain high standards of quality, safety and compliance.

Adequate preparation, including a proactive approach, necessary arrangements and stakeholder involvement, is essential for a smooth transition to compliance and successful implementation of the forthcoming legislation.

Importance of risk evaluation

Facilities should regularly evaluate feed production risks, taking into consideration food safety, animal and human well-being, and trade considerations. Each facility is responsible to assess its carryover risk based on its operations and implement the necessary procedures to mitigate unsafe carryover.

Facilities producing multiple species or different feed types, medicated feed, or concentrated mineral supplements or premixtures pose a higher risk (*Table 1*). Facilities focussing on a single species with medications not requiring withdrawal periods pose a lower risk. Separate delivery systems and implementing sequencing, flushing and cleaning procedures can reduce the risk for facilities using medications with withdrawal periods. Multi-species facilities face a higher risk of cross-contamination and should follow mitigation procedures regardless of the type of medication used.

A plan to minimise carryover

A comprehensive assessment of ingredients, processes and equipment is necessary to identify and minimise carryover risks. Subsequently, procedures should be developed to address mixers, processing lines, storage bins and feed delivery systems. Adhering to good manufacturing practices is crucial for maintaining quality and feed safety standards.

Regular equipment maintenance helps reduce carryover risk; however, physical cleanout is necessary for the complete elimination of unwanted residues. Flushing and sequencing remain commonly used practices in the animal feed industry to reduce carryover.

Prevention practices will vary based on each facility's design, operation and types of feed produced. Furthermore, procedures implemented may differ for each ingredient based on carryover risk, with some requiring flushing and sequencing while others require only sequencing.

Risk mitigation procedures may also vary between manufacturing lines due to equipment limitations. Some facilities may designate specific equipment for high-risk ingredients to prevent cross-contamination.

Table 2: Risk matrix for single versus multiple species facilities and risk mitigation strategies.

Number of species	Type of medication	Risk	Risk mitigation
Single	Medication without withdrawal	Low	• Sequencing
Single	Medication without withdrawal	High	• Separate feed delivery systems • Sequencing • Flushing of feed processing equipment • Cleaning of processing equipment
Multiple	Medication without withdrawal	High	• Manufacturing sequencing • Flushing of processing equipment • Equipment clean-out of feed processing and delivery equipment
Multiple	Medication without withdrawal	High	• Manufacturing sequencing • Flushing of processing equipment • Equipment clean-out of feed processing and delivery equipment

Flushing: Flushing is a crucial step in manufacturing, and is aimed at removing residues after having processed a batch of feed. It involves passing a large quantity of a known ingredient through the system to eliminate unsafe contaminants and prevent their presence in subsequent batches.

Each facility must determine the suitable type and quantity of flush material used based on its operations. Consulting the mixer manufacturer also helps determine the residue percentage remaining after discharge and the recommended volume and type of flush material for sufficient cleaning.

For self-cleaning systems, a minimum flushing volume of 5% of the mixer's capacity is recommended, while non-self-cleaning systems should use at least 25% of the mixer's volume. Manufacturers should document their flushing procedures and maintain production sequencing and batching records.

During flushing, the flush material should be added at the stage where undesired substances enter the process. The mixer should run for at least one minute to allow the flush material to flow throughout the entire system. Afterwards, the material can be bagged or stored separately.

The entire manufacturing system should be thoroughly flushed, and the flush material inspected for extraneous substances. It is crucial that the same amount of material initially placed into the system is recovered. Proper identification, storage and disposal of the flush material are also essential.

To maintain feed safety standards and keep undesirable substances below allowable limits for non-target species, flush material should be used in lower-risk products. Truck drivers and operators should ensure that bulk trucks are properly flushed before loading another feed batch into the bins. These measures help prevent carryover and maintain feed safety standards.

Sequencing: Proper sequencing practices help prevent carryover and ensure feed safety. Sequencing is a strategic practice that involves carefully planning the order of production, storage and distribution of different animal feeds to prevent unsafe contamination through carryover.

It is preferred over flushing and equipment cleaning as it reduces downtime and minimises the loss of usable feed, resulting in cost savings. However, equipment cleanout may be necessary in cases of maintenance or design issues when scheduling alone cannot reduce carryover to acceptable levels.

When implementing sequencing, feed manufacturers should consider factors such as species, sensitivity to previous medications, age, class type and drug purpose. Here are key factors to bear in mind for effective sequencing:

- **Feed prioritisation:** Prioritise feeds for animals nearing slaughter, lactating dairy animals and laying hens before handling medicated feeds or feeds with withdrawal periods. Group products containing medications together and produce 'lower' risk non-ruminant feeds next. In general, non-medicated feeds should be produced before medicated feeds.
- **Sequencing of medicated feed:** When producing medicated feeds with withdrawal periods, follow it with feeds for growing animals of the same species. For swine, the recommended production order is pig creep/weaner ration with withdrawal medication, sow ration, grower ration and finishing ration. Follow this sequence with a non-medicated feed for the same target species, allowing sufficient time for drug clearance.
- **Careful sequencing of different drugs:** Sequence batches carefully to avoid unsafe drug carryover. Start with the highest drug concentration, progressing to lower concentrations for a specific species. Non-medicated feeds for the same species, or medicated feeds with the same drug for other species, should follow. The feed with the lowest potential for carryover should be produced last, followed by a thorough system cleanout before restarting the sequence. Precautions should be taken to avoid contamination and transfer of residues in food-producing animals.

- **Sensitivity to specific drugs:** Avoid manufacturing feeds immediately after medicated feeds that contain substances to which animals are sensitive. Implement specific risk mitigation measures for species with sensitivity to medications, such as horses and the potential toxicity of monensin.
- **Approval by a qualified individual:** To ensure adherence to sequencing protocols, the sequencing schedule should be approved by a qualified individual who is a professionally registered animal scientist with the South African Council for Natural Scientific Professions (SACNASP).

Clean-out and other procedures

Equipment clean-out is considered the most effective way to prevent carryover during animal feed production and delivery. However, physical cleanout is not commonly practised due to process shutdown requirements and associated risks to employee safety. However, it becomes necessary when there is evidence of material build-up in equipment, which can impact performance and invalidate previous flushing efforts.

Regular physical cleanouts, with appropriate safety measures, should be included in the carryover mitigation plan to prevent contamination. To reduce cross-contamination risks, designating specific equipment for different feed types and implementing automated systems can be effective. This applies to mixers, conveyance systems, storage bins and trucks.

However, even with these measures, additional steps such as flushing and sequencing may still be needed when producing multiple feed types to ensure accurate formulations, animal health and compliance with regulations.

Evaluating procedure effectiveness

To comply with new carryover regulations, feed manufacturers will need to demonstrate the effectiveness of control measures such as sequencing, flushing and equipment clean-out in ensuring feed safety. However, there is no universally accepted validation method due to the diverse manufacturing practices and equipment used worldwide. Each manufacturer must establish its own procedures backed by technical evidence.

'Flush verification' is a crucial process to validate the effectiveness of flushing. Sampling and testing are essential for verification, including assessing sequencing and other cleanout methods. The verification process should specifically identify carryover by testing the most concentrated formula and sampling the initial portion of the subsequent batch.

Capturing the 'worst-case scenario' for carryover provides a reliable assessment of the cleanout procedure's effectiveness. Conducting mixer efficiency studies is also important to prevent toxicity concerns in each feed batch.

Value of AFMA membership

AFMA membership provides feed manufacturers with significant advantages, particularly regarding carryover prevention and mitigation. As a respected industry organisation in South Africa, AFMA sets stringent standards through its Code of Conduct and offers access to a network of professionals and resources.

By joining AFMA and showing compliance with its Code of Conduct, manufacturers demonstrate their commitment to following good manufacturing practices and producing safe animal feed free from carryover and residues.

> As a respected industry organisation in South Africa, AFMA sets stringent standards through its Code of Conduct and offers access to a network of professionals and resources.

The association keeps members informed about the latest advancements, research and regulations in animal feed manufacturing. Additionally, AFMA advocates for members interests and shapes policies and legislation while considering manufacturers' specific needs.

In summary, AFMA membership supports feed manufacturers by providing knowledge, guidance and industry collaboration while measuring regulatory compliance.

For enquiries, send an email to Bonita Cilliers at technical@afma.co.za or visit *www.afma.co.za*.

German biofuel industry in jeopardy

By Vladislav Vorotnikov, All About Feed

Germany mulls phasing out crop-based bioethanol production in the coming decade, chasing a declared goal to fortify European and world food security. However, as market players reveal, the effect of the widely discussed reform is likely to be quite the opposite.

In early 2023, German environment minister, Steffi Lemke, prepared a draft bill proposing an end to the production of crop-based biofuels in stages by 2030.

"Biofuels stand for land consumption and loss of biological diversity," Lemke said in a statement published on the environment ministry's website in January 2023. "To replace only around 4% of fossil fuel use in German road transport, a land space in Germany and abroad is needed, equal to about 20% of the German agricultural area. That is not future-orientated."

Rather produce 'real bioethanol'

As Lemke explained, it would be better to encourage the production of 'real bioethanol' from garbage, waste and used edible oil instead of agricultural commodities.

The German bioethanol industry association calculated that only 4% of the grain harvest in Germany was used for bioethanol. "Only 2% of the arable land is required for bioethanol production," said Stefan Walter, the association's managing director.

The feed industry to suffer

Fabian Preuss, communications manager of the German Association for Animal Nutrition, declined to comment on how the proposed restrictions could impact the German feed industry. He explained that if the measure is enforced, the impact will be felt not just in Germany but in the entire European feedstuff market, which means it should be better handled by the European feed industry associations.

FEDIOL, in general, agreed with the calculations of the German biodiesel industry organisations suggesting that the ban could end the production of three million tons of feedstuff per year. "Germany used 2,516 million tons of biodiesel in 2022. If all that biodiesel would come from rapeseed oil, that would represent close to 3,5 million tons of rapeseed meal, which would not be produced if there is no more demand for rapeseed oil-based biodiesel," commented Geert Vanmarcke, international market adviser of FEDIOL.

The German environmental ministry believes that the ban on crop-based biofuel would drive farmers to grow other edible products, meaning that the food and feed sector will eventually benefit from the decision. However, market players pointed out that this is not how the industry works.

"They seem to forget that growing rapeseed, whereby part of the resulting rapeseed oil goes to biodiesel, also results in a substantial production of rapeseed meal as protein for feed," Vanmarcke said.

"If the production of rapeseed is abandoned because of its use in biodiesel – and I do not see why there would be any environmental concerns around this – there would be no possibility of growing similar amounts of protein crops and the EU and German protein balance would be endangered," Vanmarcke added.

An acceptable compromise

Asbjørn Børsting, FEFAC president, commented that the sector which processes crop-based biofuels for renewable energy purposes, such as biodiesel and bioethanol production, is an important supplier of key protein-rich co-products, in particular rapeseed and sunflower meal and DDGS.

"These resources are essential to the EU feed sector to help reduce EU import dependency on imported soya bean meal. The production of bioethanol competes

directly for certain cereals (mainly maize), which in poor crop years (drought impacts) may increase pressure for access to competitive feed grains for the feed industry, as happened in 2022 in South-Eastern Europe," Børsting said.

FEFAC therefore considers that the current maximum EU threshold of 7% for the contribution of crop-based biofuels towards renewable energy production targets, is an acceptable compromise in terms of balancing feed industry access to both protein-rich feed ingredients and feed grains, Børsting said, adding that FEFAC recommends the EU to include a safeguard based on the EU food waste hierarchy to ensure feed security and functioning of the Single Market in case of crop shortages.

Better use of waste-based streams

According to Børsting, EU tax subsidies for the growing use of biomass for anaerobic digestion provide new opportunities to convert waste-based streams into renewable energy. However, he added, FEFAC members are deeply concerned that diverting co-products traditionally used in animal feed, such as wheat bran, sugar beet pulp and former foodstuffs, to anaerobic digestion will reduce the EU's feed autonomy and the feed sector capacity to reduce GHG emissions linked to feed production, which heavily relies on raising the share of co-products in feed formulation. FEFAC therefore remains opposed to the classification of co-products as advanced biofuel.

Biogas production typically does not deliver any co-product that is used in animal feed production, meaning that this non-human edible nutrient source is lost from the feed and food production systems, Børsting said. However, FEFAC fully acknowledges that an increase in biomass production and better use of food waste streams for biomass production – excluding co-products traditionally destined for animal feed – can help meet the increasing demand for both animal protein and renewable energy.

"There are clear synergies to be obtained from policies stimulating sustainable, integrated bioeconomy activities, such as the bio-refining of grass. FEFAC therefore supports the establishment of a comprehensive EU biomass factsheet in partnership with the EU farming, food processing and bioenergy sector," Børsting said.

The legal battle is far from over

The idea to decrease crop-based biofuel use has been brewing in the German environmental ministry for several years. However, it fully materialised with the beginning of the Ukrainian military conflict, commented Frank Brühning, spokesperson for the German Biofuel Industry. As Ukraine started experiencing difficulties with exporting food, it was assumed that manufacturing biofuels from edible feedstock should be curtailed to maintain the market's stability, Brühning explained.

"This proved to be wrong. On the one hand, because there is still a lot of oil, grain and oilseeds coming from Ukraine and on the other hand, because there is enough feedstock available on the global market, supply is not the problem," Brühning said.

The discussions about the future of biofuels in Europe are linked to their performance in reducing CO_2. This leads to the theory of indirect land use change (ILUC): land use for the production of biofuels feedstock could displace food production and lead to expanding agricultural land elsewhere, for instance in high-carbon forests and peatland.

According to the ILUC theory, the emissions caused by this effect undo the savings achieved by biofuels replacing fossil fuels. Brühning noted that the world's top scientific organisation, the Intergovernmental Panel on Climate Change (IPCC), clearly states that due to a great number of shortcomings of the theory, there is low confidence in the attribution of emissions from ILUC to bioenergy.

However, for the German Green Party, which is currently controlling the environmental ministry, these arguments don't sound convincing enough.

The proposal is still the subject of debate among German government officials and lawmakers. Steffen Bilger, a member of the German parliament, has recently accused the Green Party members of chasing "policy with simple enemy images, clumsy black-and-white painting and under-complex arguments". Biofuels from food are already capped in Germany more strictly than required by the EU. The use of waste and residual materials "that nobody wants on their plate" is particularly encouraged, explained Bilger.

What will farmers do?

"If crop-based biofuels like bioethanol and biodiesel are not produced anymore, demand for agricultural products goes down, leading to the question: what will farmers do?" Brühning said.

"There is not enough demand for rape oil because humans do not need such quantities of rape oil. And you won't produce rape only to put it in the feed market since the higher value comes from the oil. It would put a completely new framework to the market, and the farmers would have to figure out how to proceed with their business," Brühning added.

German Green Party members believe the farmers should quit growing food and rather capture CO_2 by leaving their land to restoration, but financing this and stabilising the captured carbon in the long term is questionable, Brühning claimed.

Market players believe there is room for a bad scenario where the European feed industry loses three million tons of feedstuff coming from rapeseed processing in the next few years. There are no guarantees that it will happen, even if the German restrictions on crop-based biofuels are enforced, because there are many other factors in play. However, this scenario seems likely.

"What's clear is that we have a gap in protein feed, especially in Germany," Brühning said, citing OVID data indicating that 70% of protein feed on the German market has to be imported. "That gap would widen."

In addition, the German government set a target to become more independent of feed protein in the coming years. "So, in light of this, phasing out crop-based biofuels would be a move in the wrong direction," Brühning warned.

The green agenda

Another part of the problem is that European green parties have similar views about crop-based biofuel production, which means that in the long run, biofuel production could be endangered in other parts of the continent. In addition to Germany, greens are now presented in the governments of Austria, Belgium, Finland, the Republic of Ireland, Luxembourg, Montenegro and Scotland.

Brühning said that the challenges the feed industry may face due to a ban on crop-based biofuels will not convince environmentalists. "When we say that protein feed production will decline, they answer that we should not produce that much feed anyway because we should reduce our meat consumption in the first place. We are kind of living in different worlds," he added.

The European bioethanol sector has been steadily growing during the past decade, and the trend is expected to continue as the EU aims to increase the share of renewable energy in transport to at least 14% by 2030. It is yet to be seen whether the German environmental ministry's proposal can prevent this from happening.

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Swiss feed milling experience shared on local soil

By Phillip Crafford, Plaas Media

Feed milling refers to the combination of processes utilised to produce feed suitable for animal consumption. As it is an important part of the South African agro-processing landscape, the Animal Feed Manufacturers' Association (AFMA) has been hosting a biennial two-week short course on feed milling since 2010. The course came into being after a gap was identified in the knowledge chain of local feed milling operations.

The course is presented by Ernst Nef of Nef Feed Milling Consulting. He has more than 25 years' experience in the feed milling industry. Nef started his career as a millwright at the Bühler Group in his native Switzerland and has 18 years' experience in the installation of feed and flour mills.

This year's top course participants were, from the left, Waldo Macdonald of Nutri Feeds, Ashley Ndaba of National Foods Stockfeeds, Jonathan Miles of Nutroteq, Willem Steyn of SwiNE Nutrition Management, and Willem Augustyn of Nutri Feeds. With them are the course presenter, Ernst Nef (in the middle), and Liesl Breytenbach, interim executive director of AFMA.

A need for knowledge

The popularity of the course is proof of its value and emphasises the importance of education and training in the sector.

Nef says knowledge exchange is necessary for feed millers to understand the entire process. Without an understanding of each individual link in the chain, operators could end up not being aware of the challenges and/or problems that may arise during each step.

He added that the course will add value for feed mill operators, allowing for more than just starting and stopping a process with the push of a button.

Vital topics in the feed milling industry such as the legal requirements pertaining to animal feed, transport logistics and ways of addressing the effects of load shedding, were also discussed.

Record participation

Liesl Breytenbach, interim executive director of AFMA, said this year's course drew a record 57 participants from 25 feed milling companies across Southern Africa and Mauritius. The increased level of participation over the last 13 years highlights the importance and value of training and skills development opportunities such as this.

She said AFMA is looking forward to continuing their partnership with Nef Feed Milling Consulting and thanked the course partner, Automill, for their financial assistance in hosting the course. AFMA is also aiming to further expand the course to include a practical component at a local research and training feed mill in future.

A social dinner was part of the course programme. The event, which was co-hosted by the Bühler Group, offered participants the opportunity to network. Breytenbach and Alec Audie, area sales manager at Bühler, welcomed participants to the event.

A total of 57 participants completed this year's course. The top five participants were Waldo Macdonald of Nutri Feeds, Ashley Ndaba of National Foods Stockfeeds, Jonathan Miles of Nutroteq, Willem Steyn of SwiNE Nutrition Management, and Willem Augustyn, also of Nutri Feeds.

The course was presented by AFMA with Automill as one of the course sponsors. From the left are Olivia Botha and Bee Oelofsen of AFMA, Jacques Prinsloo of Automill, and Liesl Breytenbach of AFMA.

For enquiries, visit AFMA's website at *www.afma.co.za*.


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Hammermill screen selection for soya bean processing: Effects of soya bean meal particle size on amino acid digestibility, feed milling efficiency, and D1-42 broiler performance

By AM Lyons, CM Poholsky, LS Erb, PH Patterson and JW Boney

Maize particle size (PS) affects feed milling parameters, amino acid (AA) digestibility and broiler performance. However, there is limited work on soya bean meal (SBM) PS. Three experiments were conducted to determine the effects of SBM PS on d21 apparent ideal AA digestibility (AIAAD), feed milling efficiency, pellet quality (PQ) and d1 to 42 broiler performance.

A hammermill fitted with one of three screens (2,4mm, 5,6mm, 7,9mm) was used to process the SBM used in all experiments. In experiment 1, an indigestible marker, titanium dioxide, was added to the diet and its concentration in digesta was used to calculate AIAAD.

D21 AIAAD was influenced by SBM PS where fine SBM (2,4mm screen) reduced AA digestibility for all AA compared to other treatments (P < 0,05). In experiment 2, SBM PS did not affect hot pellet temperature or production rate (P > 0,05). However, PQ improvements were apparent when the fine SBM was used (P < 0,05).

In experiment 3, SBM PS impacted broiler performance in all feeding phases (P < 0,05). Overall (d1 to 42) FI, BWG and FCR increased when fine SBM was fed compared to when medium (5,6mm screen) and coarse (7,9mm screen) SBM was fed (P < 0,05). Finally, SBM PS did not affect gizzard size (P > 0,05). These experiments suggest that although finely ground SBM improves PQ between 5 and 8%, the fine SBM PS reduces AIAAD and worsens broiler FCR.

Problem investigation

Feed ingredients and feed manufacturing represent considerable costs to an integrated broiler operation. Within these costs, ingredient particle size (PS) reduction is the second largest expenditure after pelleting (Reece *et al*., 1985). Generally, the cost of grinding feed ingredients increases as the desired PS of the ingredient decreases (Reece *et al*., 1986; Wondra *et al*., 1995).

Lyons *et al*. (2022) demonstrated that reducing the hammermill screen size from 7,9mm to 5,6mm and 2,4mm increased energy consumption by 0,72 kW/ton and 1,67 kW/ton, respectively. Coarse grinding of ingredients can reduce costs associated with milling. Despite this, the relationship between ingredient PS and bird performance should be considered before using feed production methods that reduce production costs.

Ingredient PS has been reported to impact various aspects of poultry production, including digestive tract development, feed passage rate, nutrient digestibility and bird performance (Gabriel *et al*., 2003; Amerah *et al*., 2007; Lyu *et al*., 2021; Marx *et al*., 2021). These PS effects are more prominent in mash diets compared to pelleted diets (Amerah *et al*., 2007). However, ingredient PS can impact bird performance after pelleting (Peron *et al*., 2005; Lentle *et al*., 2006).

Pellet quality (PQ) may also be influenced by ingredient PS. Improvements in pellet durability when maize PS was reduced have been reported (Wondra *et al*., 1995; Chewning *et al*., 2012), and are known to improve BW and FCR in broilers (McKinney and Teeter 2004; Corzo *et al*., 2011; Lilly *et al*., 2011; Glover *et al*., 2016). Although, the PS of feed ingredients for optimal pelleting efficiency is unclear.

A majority of PS research has focussed on cereal grains, while only a few researchers have studied the PS of soya bean meal (SBM). Considering the importance of ingredient PS on both feed and poultry production, three experiments were conducted to determine the effects of SBM PS on apparent ileal amino acid digestibility (AIAAD), feed milling efficiency, PQ parameters, and d1 to 42 broiler performance.

Table 1: Analysed nutrient values and particle size of diets fed to Ross x Ross 708 male broilers from d11 to 21 (experiment 1).

SBM particle size	Crude protein[1] (%)	Crude fat[2] (%)	Crude fibre[3] (%)	Ash[4] (%)	Moisture[5] (%)	Gross energy[6] (kcal/kg)	Particle size[7] (µm)
Fine	20,38	6,08	3,10	5,80	9,38	4,123	571,11 ± 2,22
Medium	20,31	6,35	3,70	5,90	9,05	4,145	602,77 ± 2,36
Coarse	20,75	5,92	3,10	6,42	9,66	4,123	668,85 ± 2,38

[1]Crude protein determined using AOAC 992,15; AOAC 990,03; AOCS Ba 4e-93. [2]Crude fat determined using AOAC 920,39. [3]Crude fibre determined using AOAC 962,09; AOCS Ba 6-84. [4]Ash determined using AOAC 942,05. [5]Moisture determined using AOAC 930,15. [6]Gross energy was analysed using a Parr 6200 isoperibol bomb calorimeter (Parr Instrument Co, Moline, IL). [7]Particle size determined using ASAE S319,4. The means presented are the average of duplicate samples ± SD.

Experiment 1: Apparent ileal amino acid digestibility

Particle size analysis: Results from the PS analysis of the diets are located in *Table 1*. The diet containing fine (2,4mm) SBM generally had a smaller PS compared


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to the diets with medium (5,6mm) and coarse (7,9mm) SBM. The largest difference in PS (97µm) was between diets containing either fine or coarse SBM.

Indispensable amino acid digestibility: Hammermill screen size and resulting SBM PS influenced the digestibility of all indispensable AA. The diet containing fine SBM had the lowest digestibility coefficient for all indispensable AA (P < 0,05). The coarse SBM improved threonine digestibility by 5,73% compared to fine SBM (P < 0,001). Fine SBM reduced methionine digestibility by 1,89% relative to those consuming coarse SBM (P < 0,001). Lysine digestibility was improved by 3,30% when birds consumed a diet containing coarse SBM compared to those consuming fine SBM (P ≤ 0,001).

Pacheco *et al*. (2013) found that coarse SBM (971µm) inclusion in the diet improved protein digestibility by 1,1% at d21 (P < 0,05). Similarly, Selle *et al*. (2019) reported improved apparent protein digestibility in the distal ileum when broilers were fed coarse sorghum compared to fine sorghum (P < 0,05). These results support improved AIAAD for birds consuming medium and coarse SBM.

Dispensable amino acid digestibility: All dispensable AA followed a pattern similar to that of the indispensable AA with the fine SBM having the lowest digestibility coefficients (P < 0,05). The coarse SBM consistently had the highest digestibility coefficient, except for proline and alanine where digestibility was similar to the medium SBM. Coarse SBM inclusion improved cysteine digestibility by 10,39% compared to fine SBM (P < 0,001).

Improvements in AIAAD observed with medium and coarse SBM inclusion may be attributed to the feeding passage rate through the intestines. Nir *et al*. (1994) demonstrated that coarse particles are retained in the gizzard longer than fine particles and have a slower passage rate through the intestines.

Increased retention time in the gizzard and intestines increases exposure time with digestive enzymes, resulting in improved nutrient digestibility (Xu *et al*., 2015). In support, Parsons *et al*. (2006) found that including coarse maize in the diet generally led to an increase in lysine and nitrogen retention in broilers. Previous research and data from the current experiment support the idea that coarse particles improve nutrient digestibility in broilers fed mash diets.

Experiment 2: Feed milling efficiency and pellet quality

Feed milling efficiency: Neither HPT nor production rate was affected by SBM PS (P > 0,05). The subtle changes to diet PS were not expected to affect HPT or production rate. Similarly, Martin (1985) found no differences in feed mill production rate when poultry diets were pelleted with maize or sorghum passed through 3,2mm or 6,4mm hammermill screens (P > 0,05). Wondra and colleagues (1995) also reported no differences in pelleting production rate when manufacturing swine diets varying in maize PS (400 to 1 000µm).

Although no measurable differences in production rate were observed in the current experiment, feed flowability at the mill was poor when pelleting diets were made with fine SBM. The diet containing fine SBM slowed feed transport from the mixer to the surge bin above the pellet mill. In addition, the fine SBM diet plugged the pellet die during each of the manufacturing runs. This required feed manufacturing to halt and ultimately reduced throughput. Unfortunately, this was not measurable but should be considered when selecting SBM PS.

Pellet quality analyses: Fine SBM improved pellet durability (P < 0,05), likely attributed to an increase in surface area with fine particles, allowing better agglomeration and binding of the pellet (Corzo *et al*., 2011). When considering pellets made with 0,5% mixer added fat, fine SBM improved PDI and MPDI while coarse SBM decreased pellet durability (P < 0,05). Diets containing medium SBM were intermediate in PDI and MPDI.

Pellet quality patterns were similar when considering pellets collected after PP fat application. Fine SBM improved NHPT, PDI and MPDI while diets manufactured with medium and coarse SBM had reduced pellet durability (P < 0,05). In the current experiment, fine SBM improved PDI by 3,92% compared to coarse SBM. When Amerah *et al*. (2008) pelleted broiler rations containing fine maize or wheat, pellet durability improved by 2,13% compared to coarse cereal grains (P < 0,05).

Svihus *et al*. (2004) suggested that coarse particles may cause pellets to fracture more easily and reduce the PQ. Improved pellet durability resulted in an increased percentage of pellets. Fine SBM increased the percentage of pellets by 30% compared to the coarse SBM (P = 0,003). Although PQ improved with the fine SBM, poor feed flowability through the mill and plugging of the pellet die should be considered before using fine SBM as a PQ improvement strategy.

Experiment 3: Broiler performance

Soya bean meal PS influenced broiler performance throughout all three feeding phases (*Table 3*). Birds consuming feed

Table 2: Analysed nutrient values of diets fed to Hubbard x Ross 708 male broilers from d1 to 42 (experiment 3).

Diet phase	SBM particle size	Crude protein[1] (%)	Crude fat[2] (%)	Crude fibre[3] (%)	Ash[4] (%)	Moisture[5] (%)	Gross energy[6] (kcal/kg)
Starter (d1 to 14)	Fine Medium Coarse	18,38 22,69 23,06	6,04 6,49 6,29	2,65 2,50 2,50	5,24 5,88 5,45	12,33 12,47 12,47	3,990 4,101 4,079
Grower (d15 to 28)	Fine Medium Coarse	21,25 23,38 21,94	7,87 7,82 7,71	2,30 2,50 2,20	4,97 4,73 4,86	13,84 11,90 12,61	4,167 4,233 4,145
Finisher (d29 to 42)	Fine Medium Coarse	19,75 19,75 19,56	8,48 7,89 8,31	2,20 2,30 2,20	4,93 4,92 4,60	12,45 12,28 12,24	4,167 4,145 4,189

[1]Crude protein determined using AOAC 992,15; AOAC 990,03; AOCS Ba 4e-93. [2]Crude fat determined using AOAC 920,39. [3]Crude fibre determined using AOAC 962,09; AOCS Ba 6-84. [4]Ash determined using AOAC 942,05. [5]Moisture determined using AOAC 930,15. [6]Gross energy was analysed using a Parr 6200 isoperibol bomb calorimeter (Parr Instrument Co., Moline, IL).

Table 3: Effects of soya bean meal particle size on Hubbard x Ross 708 male broiler performance from d1 to 42 (experiment 3).

	Starter (d1 to 14)			Grower (d15 to 28)			Finisher (d29 to 42)			Overall (d1 to 42)			Gizzard (d42)	
SBM particle size	FI (kg/bd)	BWG (kg/bd)	FCR[1](kg: kg)	FI (kg/bd)	BWG (kg/bd)	FCR (kg:kg)	FI (kg/bd)	BWG (kg/bd)	FCR (kg: kg)	FI (kg/bd)	BWG (kg/bd)	FCR (kg: kg)	Weight (g)	Yield (%)
Fine	0,474[a]	0,352	1,389[a]	1,550[a]	1,103[a]	1,409[b]	2,647[a]	1,565[a]	1,692[a]	4,700[a]	3,038[a]	1,547[a]	31,8	1,06
Medium	0,454[b]	0,362	1,256[b]	1,175[c]	0,814[c]	1,473[a]	2,401[b]	1,479[b]	1,624[b]	4,107[b]	2,697[c]	1,523[b]	29,6	1,11
Coarse	0,464[ab]	0,374	1,241[b]	1,286[b]	0,886[b]	1,466[a]	2,417[b]	1,477[b]	1,637[b]	4,237[b]	2,784[b]	1,522[b]	30,0	1,10
P-value	0,003	0,317	0,046	<0,001	<0,001	<0,001	<0,001	0,002	<0,001	<0,001	<0,001	0,002	0,193	0,660
LSD	0,011	0,030	0,127	0,059	0,048	0,027	0,071	0,040	0,027	0,131	0,086	0,012	2,6	0,10
SEM	0,004	0,010	0,043	0,020	0,016	0,009	0,024	0,014	0,009	0,045	0,029	0,004	0,9	0,04

[a-c] *Means within a column with different superscripts differ (P < 0,05).* [1]*Mortality corrected FCR: mcFCR = FI/(LWG + Wt of mortality).*

with fine SBM consumed more feed in all three feeding phases compared to other treatments (P < 0,05). The analysed crude protein and gross energy of the starter diet were lower for the fine SBM treatment compared to the medium and coarse SBM treatments (*Table 2*).

It is possible that a feed sampling error occurred. Mash feed was mixed before pelleting and these mash feed nutrient values closely align with the diet formulation and other starter period treatments. Regardless, FI was only 20g per bird less when comparing the fine SBM and medium SBM treatments during the starter phase.

The magnitude of FI differences increases in the grower and finisher phases where analysed nutrient values were similar across dietary treatments. In the grower and finisher phases, BWG was highest for birds fed diets with fine SBM (P < 0,05). Feed conversion ratio was improved for birds fed the diets containing medium and coarse SBM in the starter and finisher phases (P < 0,05).

Overall (d1 to 42), SBM PS influenced all performance parameters (*Table 3*). Broilers consuming diets with fine SBM increased FI by 463g/bird compared to those fed diets with coarse SBM (P < 0,001). Feed intake may have been influenced by the reduced AA digestibility, as explained in Experiment 1.

Further, steam conditioning and pelleting are known to impact nutrient digestibility. Barua *et al*. (2020) reported that broilers consuming pelleted diets had reduced AA digestibility for all dispensable AA compared to birds consuming mash diets (P < 0,05). Perhaps, the increased surface area of feed with fine SBM PS exposed the proteins to harsh temperatures during steam conditioning, further reducing AA digestibility.

To compensate and meet their AA requirements, broilers increased their FI. This speculation is further supported by Glover *et al*. (2016) where similar, marginal improvements to the percentage of pellets in the finisher phase had no impact on broiler FI.

In the current experiment, broilers consuming feed made with fine SBM had increased BWG by 254g/bird compared to those fed diets containing coarse SBM (P < 0,001). Although BWG was greater for birds consuming the fine SBM diet, they converted feed to gain less efficiently, which is reflected in the FCR. Birds consuming diets containing coarse SBM demonstrated a 0,025 improvement in FCR compared to those fed diets containing fine SBM (P = 0,002).

Abdollahi *et al*. (2018) suggested that pelleted diets may increase the passage rate of feed and when paired with an increase in FI, nutrient digestibility may be compromised. Similar to the current experiment, Amerah *et al*. (2008) found that broilers consuming a pelleted diet with fine cereal grains demonstrated higher FI (P < 0,05), whereas birds consuming a diet with coarse cereal grains consumed less feed and exhibited a 0,074 FCR improvement. Xu *et al*. (2015) reported significant improvements in FCR when birds were fed a pelleted diet containing 50% coarse maize relative to a 0% inclusion, however, FI was not different (P > 0,05).

Feed conversion ratio improvements may be associated with increased retention time in response to the coarse particles, leading to improved nutrient digestibility. Overall, when provided in a pelleted diet, medium and coarse SBM improve feed efficiency.

Gizzard weights: Neither gizzard weight nor yield was influenced by SBM PS (*Table 3*; P > 0,05). Previous work indicates that maize PS has a greater effect on gizzard weight than SBM or wheat (Amerah *et al*., 2008; Pacheco *et al*., 2013). The PS of feed ingredients is reduced during conditioning and pelleting. Higher proportions of pelleted feed particles occur on sieves with smaller openings when compared to mash feed particles.

This significant level of grinding and particle distribution resulted in a smaller post-pellet PS for dietary treatments containing medium and coarse SBM. Therefore, similar gizzard weights and yields were expected.

Conclusions and applications

Finely ground SBM (2,4mm hammermill screen size) reduced AIAAD. Fine SBM inclusion improved pellet durability, feed intake and weight gain but reduced feed flow and plugged the pellet die in all three feed manufacturing replicates. In addition, including fine SBM in pelleted diets worsened FCR, likely associated with further reduction in AIAAD from the pelleting process. Using either the 5,6mm or 7,9mm hammermill screen to grind SBM improved the FCR of broilers fed a pelleted diet from d1 to 42.

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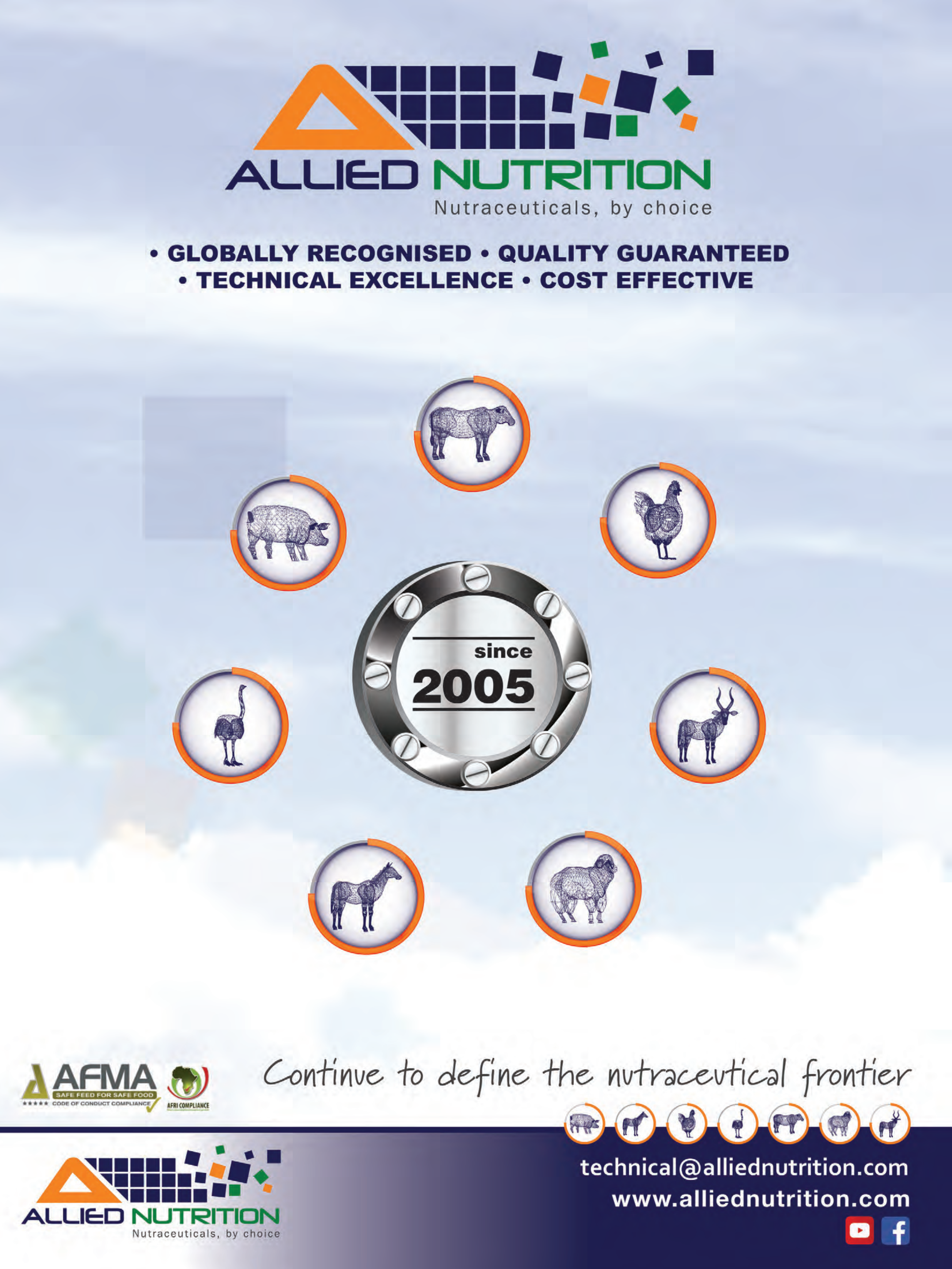
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Poultry industry paradigms: Connecting the dots

By FLS Castro, L Chai, J Arango, CM Owens, PA Smith, S Reichelt, C DuBois and A Menconi

Providing high-quality food for the increasing world population with limited natural resources is a challenge for animal agriculture. Over the past decades, poultry production has undergone remarkable advancements to adapt to challenges and changes in consumer expectations.

Among these changes, the need for an animal protein production system that considers the social, economic, and environmental aspects of sustainability has increased. With that in mind, efforts were and will continue to be made toward improving various aspects of the poultry production chain.

It has been predicted that the global population will reach over 9,2 billion in 2050 (FAO, 2012) and that the total global food demand will increase by 35 to 56% between 2010 and 2050 (Van Dijk *et al*., 2021). For this reason, it is crucial to improve the efficiency of protein production in a sustainable way.

The objective of this overview is to describe the evolution and provide insight into possible future directions of different components of the poultry industry.

The expectation of consumers

Consumer expectations regarding food consumption have changed over the years. These changes have been associated with increased health consciousness, concern with product quality and safety, ethical perceptions, environmental friendliness, animal welfare, social consciousness and price (IRI, 2020a, 2021a, b). External factors are also able to change consumer behaviour and an example of that was the 2020 COVID-19 global pandemic (IRI, 2020b; Tao *et al*., 2022).

Before 2020, retail food sales would increase between 1 and 3% a year, with some variation by department and category. Back in the mid-2010s, the fresh food categories grew faster than total store, but just before 2020, fresh food growth slowed down (IRI, internal reports). One of the hallmarks of that decade was the continued faster growth of out-of-home food vs in-home food. The Covid-19 pandemic, however, altered long standing trends and established new ones (Tao *et al*., 2022).

During the pandemic, in-home cooking significantly increased in the United States (US), with grocery spending spiking by more than 50% and spending in restaurants and hotels decreasing by more than 60% during the first months of 2020 (USDA, 2021). On average, approximately ten different cuts of meat per year were consumed by the population before 2020. Now, there is an increase in 30% of households buying and rebuying additional cuts and meats, such as seafood, premium meat cuts, and different proteins (e.g., lamb and veal; IRI, internal reports).

Moreover, over the last two years, almost every category in the store saw a major share shift from value products to premium products (IRI, internal reports). This trend is seen across all income categories and it might be due to the transition to working from home, as well as improved cooking skills.

Additionally, with people spending more time in their homes, sales of appliances soared. The purchase of grills increased by 30% and a significant higher volume of sales has been reported for small appliances such as slow cookers, air fryers, pizza ovens and cookware (IRI, internal reports). The purchase of chicken wings has increased 14% over the last year, which could be related to the higher use of air fryers, that make it easy to cook and customise (IRI, internal reports).

With that in mind, companies are now creating food products for these appliances, transforming them into innovation platforms that are driving growth for products.

Welfare and sustainability

Poultry production continues to play an increasing role in providing safe, nutritious and affordable animal protein to the growing global population because of its shorter lifecycle and high feed efficiency when compared to other livestock species. Poultry production has continued to grow at a linear rate of about 2,8% per year since 2000 (FAO, 2018). From 2001 to 2020, poultry meat production grew from 14 to 20 million tons, whereas the production of eggs increased from 7,2 to 9,3 million dozen eggs in the US (FAOSTAT, 2022).

Sustainability in livestock production is often associated with economics and the environment. It considers the efficiency with which livestock animal species can best utilise the planet's resources (raw materials, energy, land and water), transforming them into high-quality animal protein while focussing on financial success, meeting consumer expectations, and minimising its impact on the planet.

From the environmental standpoint, agriculture and related land use were shown to be responsible for 17% of global greenhouse gas (GHG) emissions from all sectors in 2018, with livestock production contributing two-thirds of this total (FAOSTAT, 2018). When categorised by species, in 2017, poultry contributed to the global GHS emissions by 10,8%, whereas cattle and swine contributed by 62,2 and 10,1%, respectively (FAO, 2017).

Mitigation strategies, such as genetic selection, technical improvement (e.g., management, precision livestock farming), dietary changes and manure management, have been proposed in order to minimise the environmental impact of livestock production (Grossi *et al*., 2018; Tullo *et al*., 2019). The use of breeding showed the potential to reduce emissions from dairy cattle, for example, by selecting correlated traits, such as feed efficiency and longevity (Wall *et al*., 2010). The chicken egg-layer industry has had the greatest gains (-25%), followed by broilers (-23%) when compared to beef cattle (0%) and sheep (-1%). Other livestock (dairy cattle and pigs) showed intermediate reductions.

Additional social aspects are the attraction and retention of quality employees and the impact of livestock production on the society. Investment in well-trained and properly compensated stock people is essential to ensure good animal welfare (Daigle and Ridge, 2018). Satisfied employees are more likely to frequently inspect the animals, increasing the early detection of problems (Dawkins, 2017) and are often proud of producing healthy cared-for animals (Hemsworth *et al*., 2009).

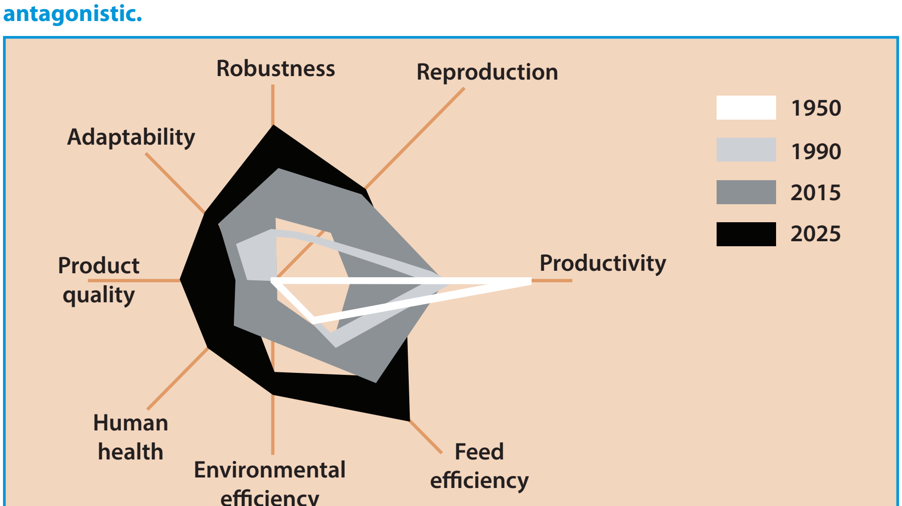
Moreover, organisations such as the National Chicken Council and Animal Agricultural Alliance have moved toward releasing annual sustainability reports and Corporate Social Responsibility is becoming an important part of the company business model (Dawkins, 2017).

Genetic revolution

Poultry production has become more efficient in the last five decades. Behind the progress in the broiler and layer industries is an increase in the genetic potential of commercial birds used for meat or egg production. A pivotal moment for the evolution of the poultry industry was the specialisation and diversification of genetic stock developed and selected for meat production vs egg production, rather than selected for dual-purpose production.

In addition, the genetic improvement of commercial poultry products relies not only on a robust evaluation programme, whose core component is the intense selection process for key economic traits. It also relies on an overall breeding programme, which includes a pyramidal gene flow that uses hybridisation along the multiplication stages from great-grandparents (GGP) to grandparents (GP) and parent stock (PS).

Figure 1: Adapted from Neeteson-van Nieuwenhoven *et al*. (2013). Radar plots showing the relative importance of its elements based on the distance from the origin for each polygon angle; elements on opposite axes are not implied to be antagonistic.



Phenotype is king

Genetic improvement is possible thanks to phenotypes recorded as quantitative or categorical values, compiled in datasets, to feed into software for predicting breeding values. Contrary to the original expectations, practical implementation of genomic selection requires the continual collection of good quality genotypes and phenotypes and model retraining to maximise its benefits (Weng *et al*., 2016).

Breeding goals evolved from simply recording body weight for broilers, and counting eggs for layers and utilising family means to more sophisticated evaluation programmes for broilers and layers, including different components of the production (PD) curve, efficiency (feed intake and feed conversion), product quality, fitness, and reproduction, behavioural and disease-related traits.

More recently, breeding goals have expanded to include welfare-related traits such as bone strength, gait, which are measured using novel technologies, such as x-ray, CT-scan, iStat, automated camera gait scores, hypobaric chambers, and specific disease trials (Wolc, 2022); as well as behaviour-observation traits, such as temperament, flightiness, and perching and nesting behaviour.

Moreover, data collection must adapt to changing housing systems to collect individual data in group housing: feeding stations for broilers, automatic nests for layers, and sensors to track behaviour, among others. The importance of sustainability for food security is reflected in the evolution of breeding goals. Neeteson-van Nieuwenhoven *et al*. (2013) presented a graphic representation of the relative importance of productivity, feed efficiency, environmental efficiency, human health, product quality, adaptability, robustness, and reproduction for a 75-year horizon (*Figure 1*).

The graph offers an interesting perspective from the evolution of breeding goals, which mainly emphasised productivity (1950), to complex and balanced set that include all the aspects mentioned (2015 to 2025). In the future, we may rely on advance technologies, such as vision-devices combined with artificial intelligence to process big data for real-time collection. These advancements would enable tracking of bird movements, navigation of housing systems, utilisation of nests, feeding and drinking spaces, and predict or notify about potential health issues.

Nutritional developments

In feed formulation, improved feed efficiency and low cost per kilogram of carcass meat yield is the major goal of commercial nutritionists. These factors drive sustainability, as nutritionists start formulating to meet the bird's daily nutrient requirements, resulting in the production of more meat with fewer inputs and reducing the carbon footprint and cost of feed.

Furthermore, precision nutrition is a way to improve animal welfare, which is an important pillar of sustainability, as previously mentioned. In order to achieve this level of precision, it is important to continuously improve the methods for evaluating the nutrient value of raw ingredients, invest in the research of different feed additives, as well as determine the animal requirements of the constantly evolving genetic lines.

With that in mind, it is expected that the formulation of diets will shift from a metabolisable energy (ME) based-system to a net energy (NE) system-based. Net energy has been successfully used in swine nutrition as it allows for a more accurate prediction of production performance. However, currently, NE has a smaller advantage over ME for poultry, which is partially attributed to the lower dietary crude fibre content and hindgut fermentation in poultry compared to swine (Van der Klis and Jansman, 2019).

It is also expected that diets will be formulated with more synthetic amino acids beyond the currently economically available methionine, lysine, threonine, valine, arginine and tryptophan that are selectively in use today. This could reduce, to some degree, the crude protein level in the diets, helping to reduce feed costs.

The usage of feed additives, such as enzymes, will continue to be refined and improved, allowing better digestion of nutrients, and computer modelling programs will be utilised more in the future to help poultry nutritionists to optimise feed formulation by focussing on profitability. Moreover, as birds are grown to larger market weights for deboning or roaster markets, separate sex feeding will become more important. This will allow the nutritionist to meet the nutrient requirements of each sex more precisely, avoiding waste of nutrients and resources.

Formulations for niche markets

It is expected that niche markets, such as 'no antibiotics ever', 'all vegetable diets', slow-growing programmes, GMO-free diets, organic, free-range, and diets for designer meats (e.g., extra omega fat content) will continue to exist, considering that they are often consumer-driven. While these marketing programmes are in place today, the nutritionist will be continuously challenged to find the ingredients to support the future growth of these businesses, maintaining profitability and sustainability compared to the conventional programmes.

Recent studies have been focussing on the nutraceutical properties of feed ingredients, such as amino acids, vitamins, minerals and fatty acids in improving health (Lee *et al*., 2019; Castro and Kim, 2020; Alagawany *et al*., 2021; Kim *et al*., 2022). However, more research is needed to understand how current feed ingredients can contribute to animal health, especially considering systems in which antibiotics cannot be used.

Feed additives and regulation

The use of different feed additives such as probiotics, prebiotics (e.g., mannan-oligosaccharides and beta-glucans), postbiotics, phytogenic compounds (e.g., oregano oil and saponins), trace elements and short and medium chain fatty acids to help with gut health and improve live performance will continue to be refined. While these products cannot replace antibiotics, they can help the birds to cope with different stressors as well as assist with their recovery.

Because of the large amount of available feed additives and their combinations in the market, nutritionists often struggle when deciding on what to include in their formulations. Furthermore, it is not rare that the results observed in the field are somewhat inconsistent with experimental set-ups, as they fail to show a benefit and some products are not cost-effective.

Thus, nutritionists will need to continue to evaluate different product options that fit their nutritional goals are safe, and are economically feasible, as there is an increasing need to feed the growing population while using fewer resources.

Processing and meat quality

Over the past several decades, the poultry industry has had an emphasis on production efficiency, health, and quantity of broiler meat, but the industry needs to focus on quality as well. There has been an unprecedented emergence of myopathies in recent years in most commercial broilers that have negative impacts on quality. These myopathies have been associated with rapid growth and high breast yield.

Three major issues that have become apparent in recent years are white striping (WS), woody (i.e., hardness) breast (WB) and spaghetti meat (SM). These myopathies are issues around the world that have major economic implications, based on 10 to 40% incidence of moderate/severe cases. Oftentimes, fillets can have more than one of the myopathies/defects present. Severe cases have resulted in unnecessary condemnations, decreased meat quality and yield, changed nutritional content, and continued reduced customer/consumer acceptance.

Various factors have been associated with woody breast in broilers, including metabolic and growth problems associated with large muscle fibres produced by fast-growing broilers, reductions in muscle stem cells, and satellite cell functions (activity and number). While there is ongoing research to determine the root causes of these myopathies, the focus has also been placed on mitigation strategies.

These mitigation strategies should involve genetic and live production factors, including nutritional strategies. However, there is also a need for strategies in the processing plant. This includes quality-based sorting of meat to provide the highest quality end product to consumers, which requires identification and detection of quality issues.

Chilling improvements

Chilling is an important step in poultry processing. It is critical to reduce carcasses temperatures promptly and it is a primary area where antimicrobials are used. With the increase in bird sizes over recent years, there has been a subsequent increase in dwell time in the chiller. This uses valuable resources such as water, energy and even space.

Researchers have developed new chilling methods that would decrease chill time, thereby saving on resources and improving food safety. The use of sub-zero saline chilling (SSC) has been studied to chill carcasses faster and also for bacterial reduction.

Innovation in processing plants

Labour is a critical need for the poultry industry, but in recent times, labour shortages have also been a major issue as a result of high turnover due to working environments (cold), repetitive and tedious tasks and the pandemic. The development of innovative technologies in completing certain tasks does only improve the use of labour (reducing the tedious tasks) but also provides a solution to labour shortages in some cases.

Scientists have developed a virtual reality (VR) based operation to assist in broiler deboning operations at ATRP/GTRI (Britton, 2021). One such operation is cone loading, where a human would operate a robotic arm using VR to load front halves onto a deboning line. The use of VR could be applied to tasks that have been previously hard to automate due to the variability of the product (chicken) size and shape. These operations are directed by humans in a remote site rather than in the plant environment.

Deboning carcasses is a task that is performed manually or through automation. Automated deboners have been used in processing plants for well over 20 years and their performance has improved over the years. However, manual debone lines typically still have better performance in terms of yield when compared to automated systems.

Researchers at ATRP/GTRI have been working to develop better automated deboning systems. They have developed an intelligent cutting system that uses 3D imaging systems that communicate tasks to a robotic arm for deboning or cutting broiler carcasses. This imaging system, combined with collecting data on how humans cut (knife trajections) when deboning has allowed researchers to optimise knife path functions via machine learning.

The combination of 3D imaging and robotics can allow for intelligent adjustment of the cutting process to account for natural variation from carcass to carcass. This allows for much-improved performance that can match human deboners.

Food safety

Food safety continues to be a priority area for poultry processors as it is critical to provide a safe food supply. Micro-organisms such as *Salmonella* spp. and *Campylobacter* spp. remain a focal point for food safety research. Evaluating these micro-organisms from a system approach (live production through processing) is important as incoming microbiological loads to the processing plant can affect the loads in final products.

In processing plants, it is necessary to develop new antimicrobials to be used on poultry products, as microbes can adapt to their environment. When choosing antimicrobials, it is also key to understand the impact on product quality. Often, antimicrobials can have negative effects on quality, so, balancing quality and effectiveness against bacteria are important.

There is much ongoing research to understand the microbiome and the impact of antimicrobial treatments in live animals or postharvest. Additionally, it is a priority to use the quantification of *Salmonella* in the processing plants as a method for assessing its risk for human health, rather than its presence or absence.

Conclusions

It is clear that the poultry industry has undergone remarkable advancements over the past decades, being able to adapt to different challenges and consumer demands dynamically, and it is expected that it will continue to do so.

Consumer expectation and demand have been constantly changing over time, influenced by social trends, ethic and food safety perceptions, economics and external aspects, such as the COVID-19 pandemic. However, some of these changes are not science-based, so it is imperative that the poultry industry should focus on educating consumers in the future.

Sustainability will remain an important topic as we move forward in finding ways to produce high quality and affordable protein, while maintaining animal welfare and minimising the impact on the planet. It also becomes important as a marketing tool, as it increases the reputation of the companies in this consumer-driven industry. Therefore, a good system to track sustainable actions by consumers and supporting agencies is needed.

Precision poultry farming technologies, such as machine vision systems and deep learning models, are promising for the next generation of poultry production and sustainable development. Focus should be placed on how to handle big data collection.

Poultry nutritionists will continue to overcome the industry challenges by adjusting feed formulations to best utilise the available ingredients, meeting the nutritional goals while focussing on improving feed and cost efficiencies. Although alternative ingredients will become more available, it is possible that same ingredients used today will still constitute the majority of the diets.

Technology and automated processes should also be applied in the processing plant with the intent of improving the welfare of workers, detection of meat defects, and continue to provide safe and good quality protein source.❖

This open source paper was condensed for publication in *AFMA Matrix*. For the full paper, visit *www.sciencedirect.com/science/article/pii/S1056617122000691*.

Alternative by-products to replace soya bean in pig nutrition

By Samaneh Azarpajouh, All About Feed

There is an increase in global demand for sustainable protein sources. This article discusses alternative byproducts to replace soya bean in pigs' diets and their effects on growth performance and carcass and meat quality traits.

Factors including physical and chemical characteristics of alternative sources, level of essential amino acids, presence of anti-nutritional factors, and the conversion method into final products are essential to evaluate the potential replacement of soya bean.

Alternative byproducts used in pigs' diets include:

- Oilseed byproducts.
- Local plant byproducts.
- Byproducts from industrial processes.
- Processed animal protein byproducts.

Oilseed byproducts

Oilseed byproducts (including meals, cakes and expellers) are derived from oil-bearing plants. Defatted by-products from linseed and sesame contain high volumes of crude protein. Rapeseed meal obtained from the pressed cake remaining after oil extraction contains 35% protein and high fibre, sulphur-containing amino acids and phosphorus.

However, rapeseed meal consists of anti-nutritional factors such as glucosinolates, tannins and phenols, which limit its application in the swine diet to up to 15%. Although, the canola meal derived from a variety of rapeseed contains a low erucic acid content and glucosinolates, making it a potential candidate for soya bean replacement.

Local plant byproducts

Minor local plants such as guar can be used as soya bean substitutes. Guar meal is a byproduct of guar gum production which contains highly viscous non-starch polysaccharides such as galactomannan polysaccharide, which enhances digesta viscosity, prevents gut enzymatic activities, and decreases nutrient digestibility.

Industrial process byproducts

Distillers' dried grains with soluble are the main byproducts from the ethanol industry produced by dry-mill ethanol plants. Distillers' dried grains with soluble are a proper source of protein (25 to 30% dry matter), fat, fibre, and energy for the swine diet. In addition, they are used significantly in swine diets because of the encouraged use of renewable energy sources for the production of biofuels.

Furthermore, distillers' dried grains with soluble have well-digested protein characteristics, a low content of anti-nutritional substances, and high nutritional values. However, they are rich in unsaturated fatty acids which can negatively affect dietary intake and the oxidative stability of the byproduct. Rice distillers' byproduct is another good source of crude protein; however, the high fibre content limits its use in pig diets.

Animal protein byproducts

The main processed animal proteins used in pig diets include:

- Meat and bonemeal.
- Blood products.
- Inedible meat.
- Waste animal tissue and fat.
- Feather meal.
- Poultry byproduct meals.
- Fishmeal byproducts.

Furthermore, liquid whey residues from the cheese industry can be used as dried ingredients. Fish silage has a high protein content (39,01%), protein digestibility (93,58%) and biological value. However, due to its high moisture content, high price and limited availability, its application in swine diets has been decreased.

Growth and carcass traits

Rapeseed meal inclusion in pig diets decreases feed intake due to the presence of glucosinolate, which reduces palatability. Different inclusion levels of rapeseed meal in the growing and finishing periods have no significant effects on average daily gain (ADG) and feed conversion ratio (FCR), demonstrating the flexibility of using this protein source alone or together with other sources.

Furthermore, rapeseed meal can be added to other legumes to replace soya bean; however, it is required to add synthetic amino acids to the mixture to balance the nutritional quality. However, there are contrasting results regarding the impact of rapeseed meal on growth performance due to differences in the glucosinolate concentrations in each variety of rapeseed.

In addition, rapeseed meal contains high amounts of anti-nutritional factors in the variety used; however, rapeseed meal inclusion has no impact on carcass traits including dressing percentage, backfat thickness, lean content, weight, carcass yield and composition of cuts.

Guar meal inclusion in pig diets decreases average daily feed intake, ADG and FCR. Defatted rice bran has negative effects on performance with an increase in feed intake and a decrease in ADG and FCR; however, it has no impact on the quality of the carcass. Supplementing fishmeal decreases daily feed intake and weight gain and FCR. In addition, fishmeal inclusion reduces backfat thickness, loin depth and fat depth.

Effects on meat quality traits

Total replacement of soya bean with rapeseed meal affects the chemical composition of different pork cuts, reducing the fat content of shoulder and steak while increasing the fat content of ham and belly; however, total soya bean replacement with rapeseed had no effect on pork stability during storage.

Corn (maize) distiller's dried grain with soluble has a high content of unsaturated fatty acids which negatively impact meat quality and decreases the protein content. Fava bean as a partial replacement for soya bean increases meat tenderness, juiciness and palatability; however, the flavour is not affected. The inclusion of yellow lupin in the diet causes lower odour, taste and juiciness scores. Corn distiller's dried grain enhances the tenderness and juiciness of meat, and fishmeal silage decreases the hardness and overall acceptability of meat.

Concluding remarks

Traditionally, soya bean is the main source of protein in pig diet formulation. However, in recent years the application of soya bean has been limited due to rising prices, ethical issues, environmental impact and competition for land use.

Various byproducts, including oilseed byproducts, local plant byproducts, byproducts from industrial processes and processed animal protein are among the potential soya bean substitutes. However, further research is required to assess cost-effectiveness and effects on meat properties. ❖

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Commercial methionine sources in poultry

By Chantelle Fryer, technical service manager, Evonik Africa (Pty) Ltd

Methionine is an essential amino acid in poultry and swine nutrition and is the first limiting amino acid in commercial poultry diets. In modern poultry diets, methionine is supplemented as dry DL-methionine (DL-met, 99% pure), liquid DL-met hydroxy analogue-free acid (liquid MHA-FA, 88% dry matter content) or the calcium salt of MHA-FA, namely MHA-Ca (84% MHA, 2% water, 12 % calcium) (*Figure 1*).

There are significant differences in the chemistry of MHA-FA and MHA-Ca compared to DL-met (*Figure 2*). DL-met is chemically a real amino acid, characterised by the presence of an amino group (H_2N-). MHA-FA is the analogue of the amino acid with a hydroxyl group (HO-) replacing the amino group. The calcium salt of MHA-FA is a solid product with MHA bound to calcium ions (*Figure 2*).

Bioavailability of methionine

Bioavailability is a relative value that compares the efficacy of a nutrient with a defined standard. In the case of methionine, the bioavailability of MHA-FA and MHA-Ca is compared to DL-met. Using the right bioavailability of methionine sources is important to determine the correct commercial rating of methionine sources and has considerable effects on the cost of methionine supplementation.

Fortunately, in poultry (and especially in broilers) it is relatively easy to measure these differences since:

- Methionine is usually the first limiting amino acid.
- Practical supplementation levels are high.
- Genetic variations between individual birds are low.
- The number of animals/replicates per treatment is relatively high.

To determine the bioavailability, dose-response trials can be conducted which focus on the parameters that are of commercial importance, such as weight gain and feed conversion ratio (FCR). The correct way to design a dose-response trial is to have a basal diet deficient in methionine or M+C and then feed a set of supplemented diets with incremental methionine levels to enough animals.

Because an inadequate supply of methionine leads to performance depression, this effect can be used to describe the dose-response relationship between methionine supply and performance criteria such as weight gain and FCR.

Following the dose-response trial, the actual bioavailability can then be determined by multi-regression analysis (Littell *et al*., 1997; Sauer *et al*., 2008). Responses to supplemental amino acids follow an exponential function. *Figure 3* shows that both the starting point of the curves (control, basal diet) and the maximum response (asymptote) are identical for both products. The only difference between the curves is their

Figure 1: Composition of methionine sources.

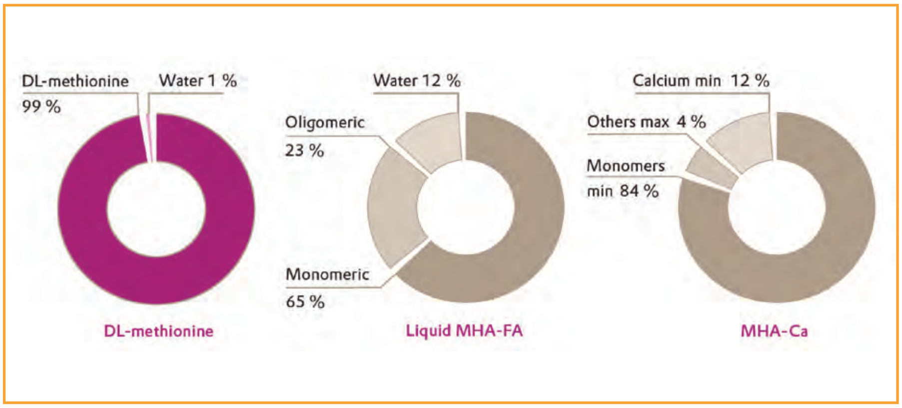
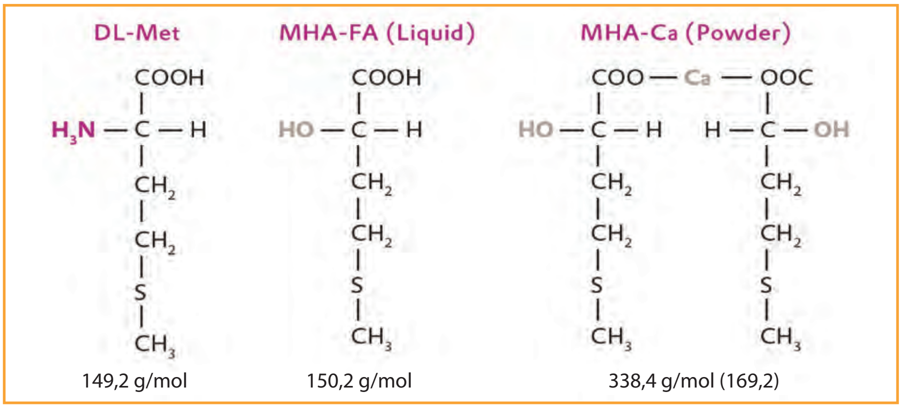


Figure 2: Chemical structure of methionine sources.



steepness, which means in this case that the curve of product 2 reaches the asymptote at a higher dosage.

Sauer *et al*. (2008) confirmed in their meta-analysis that MHA and DL-met share the same asymptote. Bioavailability is then determined by the ratio of the two regression coefficients (c_1 [product 1] and c_2 [product 2], *Figure 3*). This mathematical model is also used for many other nutrients such as phosphorous (Potter, 1998; Fernandes *et al*., 1999), iron (Boling *et al*., 1998), zinc (Swiatkiewicz *et al*., 2001 a, b) and lysine sources (Schutte and Pack, 1994).

In the last three decades, considerable research has been done, mainly in broilers, showing that the bioavailability of MHA-FA and MHA-Ca is significantly lower than DL-met (e.g. Esteves-Garcia and Austic, 1993; Hoehler *et al*., 2005b; Lemme *et al*., 2002; Rostagno and Barbarosa, 1995). For the CVB Desk Study (Jansman *et al*., 2003) an extensive literature study was done based on weight gain and FCR data from 18 broiler trials. They reported an average bioavailability of MHA-FA compared to DL-met of 68% on a weight-to-weight basis corresponding to 77% (68/0,88*0,99) on an equimolar basis.

More recently, Sauer *et al*. (2008) published a meta-analysis using 40 dose-response trials with broilers from 27 peer-reviewed publications showing an average bioavailability for weight gain and gain to feed of 70% on a weight-to-weight basis. Lemme and Petri (2003) conducted a literature review of 46 broiler trials showing an average of 63% bioavailability for weight gain, FCR and breast meat yield.

Reasons for differences

There are a few reasons for the differences in biological effectiveness. A total of 23% of the MHA-FA molecule fraction consists of dimers and oligomers which have a reduced nutritional value. MHA-FA oligomers are only 56% as efficient as DL-met on a molar basis (Van Weerden *et al*., 1992). Research by AEC/Rhone Poulenc (1983; today Adisseo) showed similar results.

Further research by Esteve-Garcia and Austic (1993), Lingens *et al*. (1996), Meanz and Engele-Shaan (1996a and 1996b), and Mitchell and Lemme (2008) indicate that:

- Di- and oligomers of liquid MHA-FA are less efficiently absorbed than DL-met.
- Monomers in MHA-FA and MHA-Ca also show slower and less efficient absorption, even under heat stress conditions.
- Since MHA-FA and MHA-Ca are absorbed slower than DL-met during passage through the digestive tract, these molecules are subject to microbial degradation.
- In biochemical terms, MHA is not an amino acid, so once absorbed it still must be actively transformed into L-methionine in the metabolism. This metabolic process is not 100% efficient.

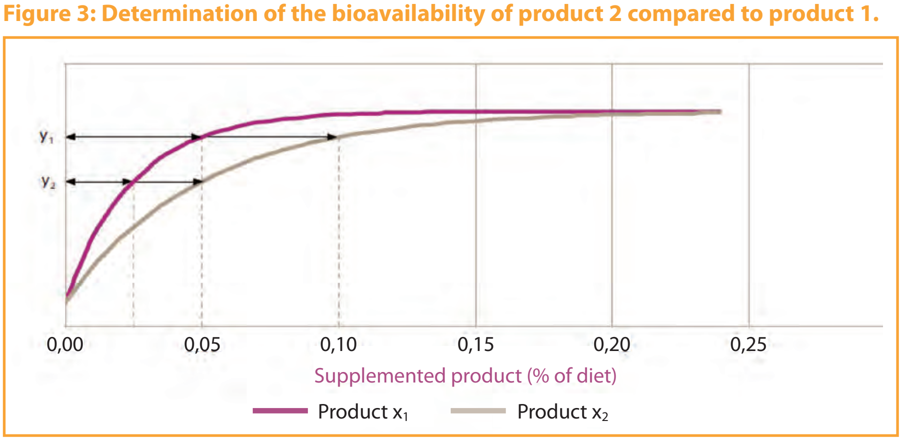
It is also important to note that the absorption of both L-met and L-MHA by simple diffusion is negligible.

In conclusion

When evaluating the relative biological efficacy of MHA compared to DL-met, the following are of importance:

- Dose-response trials should be based on a mathematical model that has been validated through numerous experiments.
- Literature provides many peer-reviewed studies confirming the relative bioavailability of 65% for MHA-Ca and MHA-FA compared to DL-met.
- Differences in the relative effectiveness of methionine sources can be explained through research into animal physiology and biochemistry.

Figure 3: Determination of the bioavailability of product 2 compared to product 1.



Relative effectiveness of product x_2

According to direct measurements: at performance level y_1: $0{,}050\ x_1/0{,}100x_2 = 50\%$

at performance level y_2: $0{,}025\ x_1/0{,}050\ x_2 = 50\%$

According to the regression equation: $y = a + b * (1 - e^{-(c_1 * x_1 + c_2 * x_2)})$

$y = 10 + 4 * (1 - e^{-(40 * x_1 + 20 * x_2)})$

at any performance level: 20/40 = 50%

For more information, send an email to Chantelle Fryer at chantelle.fryer@evonik.com.

Sources of *Salmonella* in poultry production

By J Wang, S Vaddu, S Bhumanapalli, A Mishra, T Applegate, M Singh and H Thippareddi

Salmonella and *Campylobacter* continue to be major foodborne pathogens resulting in the majority of foodborne illnesses and are reported to contribute approximately 73,4% of foodborne infections, 71,7% of hospitalisations, and 55% of the total number of deaths due to foodborne illnesses in the US (*Figure 1*; Tack *et al.*, 2019).

These micro-organisms can gain entry into the ecosystem of the bird's gut through a variety of sources, including feed, water, litter (fresh vs reused), pests (including darkling beetles), rodents, surrounding environment (wild birds) and colonise the gastrointestinal tract of the bird. Once the colonisation occurs, it is not practically possible to eliminate these micro-organisms from the bird gut, and micro-organisms eventually contaminate the poultry meat during processing. The only opportunity to reduce the risk from these foodborne pathogens is to reduce their population on the bird (surface) and in its gut.

In spite of the numerous antimicrobial interventions that poultry processors have incorporated during processing, *Salmonella* and *Campylobacter* prevalence on the whole carcass as well as chicken parts remains an issue.

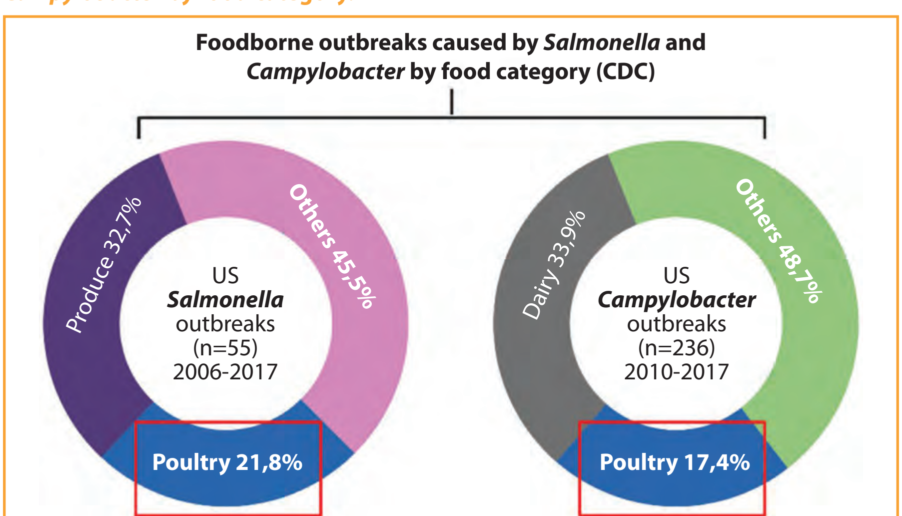
Thus, there is a need to reduce the concentrations and prevalence of these micro-organisms in the poultry gut to further reduce their prevalence in poultry meat. While numerous preharvest strategies (nutritional, immunologic [vaccines], competitive inhibition, etc.) have been evaluated and implemented, a comprehensive solution to eliminate these micro-organisms or prevent their colonisation of the gut remains elusive.

Regardless, poultry producers have incorporated numerous strategies at the preharvest stage, although these strategies are not highly effective on their own. Collectively, they have been relatively successful in reducing the prevalence of the micro-organisms.

Poultry production has gone through significant changes, for example, the increase in the no antibiotics ever (NAE) market requires producers to reduce and/or eliminate the inclusion of subtherapeutic levels of antibiotics, placing pressure on the production system to find alternative methods to control micro-organisms (such as *Eimeria* and *Clostridium perfringens*) in the ecosystem of the bird's gut during production.

These changes include improvements in management practices such as biosecurity and hygienic measures at the production farms, litter management, use of nutritional strategies to include organic acids, botanicals, bacteriocins, bacteriophages, novel compounds and feed additives, pre-and probiotics, and immunisation through oral or vaccination, etc.

Figure 1: Percentage of foodborne outbreaks caused by *Salmonella* and *Campylobacter* by food category.



Results and discussion

Several sources of *Salmonella* exist in broiler production, including the environment (exterior to the poultry house), poultry feed, hatchery for the chicks, chicks, environment in the poultry house, drinker water, faeces or bird droppings and litter in the poultry house (*Figure 2*). The prevalence of *Salmonella* from each of these sources was determined from literature. In total, there were 42 341 samples from 39 studies in the final meta-analysis. The sample source from each study was further partitioned to different contribution factors.

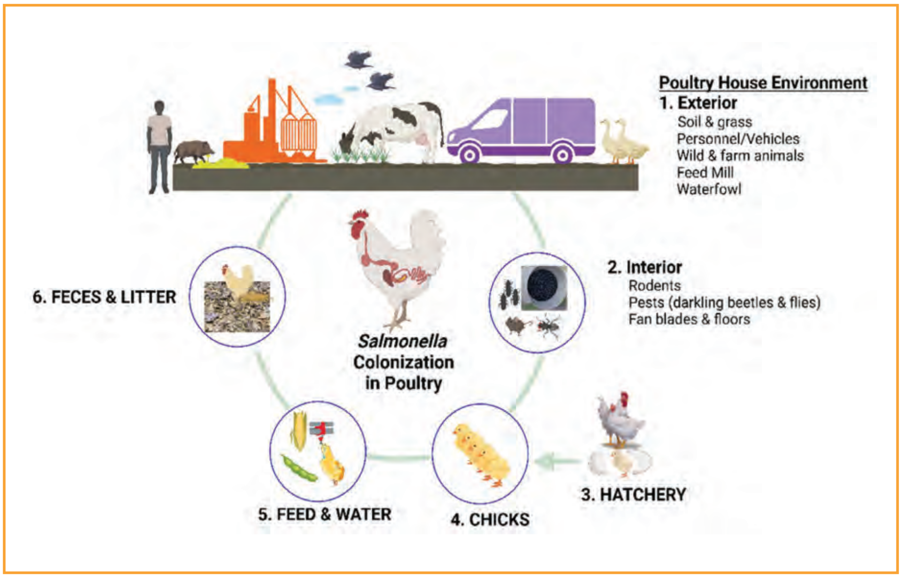
Meta-analysis model was constructed to assess each contributing factor. From the pooled forest plot, faeces (excreta), litter, feed, water, hatchery, poultry house exterior and interior environment contributed to the overall predicted *Salmonella* prevalence ($P < 0.01$). Hatchery, litter and faeces rank top three contributing factors in broiler live production.

Meta-analysis models were constructed for each contribution factor by the location of study conducted, whether in the US or other countries (non-US; *Table 1*).

Poultry house environment

Poultry house environment (external) can be a source of *Salmonella*, including the soil, standing water or puddles, presence of grass and agricultural lands in the

Figure 2: Sources of *Salmonella* colonisation of broilers in a poultry house.



vicinity of the poultry house as well as farm animals such as beef or dairy cattle, sheep, goats and other ruminants, wild birds, etc. Further, *Salmonella* can persist in soil for extended periods of time based on the soil properties, sun light, temperature fluctuations, presence of organic material, etc.

The predicted *Salmonella* prevalence in the poultry house environment (external environment, e.g., water puddles, wild birds, soil, grass, or poultry house outside surface swabs) was 4,7% (95% CI: 0,6 to 28,4%), with an overall heterogeneity of 98,4%, indicating differences in *Salmonella* prevalence between studies.

Poultry feed

Poultry feed has been implicated in several cross-continental spreads of *Salmonella* over the years. Poultry feed ingredients are sourced from various locations and if there has been an exposure to livestock or wildlife faecal material, these ingredients can be sources of *Salmonella* in the finished poultry feed. Poultry feed contains grain, milling by-products, rendered animal by-products, vitamin and mineral supplements and fats and oils (Food Drug, 2019).

While the risk of *Salmonella* from these sources can vary significantly, with vitamin and mineral supplements being free of *Salmonella* due to the manufacturing process, other ingredients can be sources of *Salmonella* to varying degrees.

Depending on the type of feed (mash vs pellet) and the processing conditions such as conditioning and pelleting temperatures, the manufacturing process can contribute to reducing the *Salmonella* populations based on the concentrations in the ingredients. *Salmonella* can persist for extended periods, over years in poultry feed processing environments such as in feed mills, grain stores, feed bins, etc. and once it gets established in such an environment, it can be difficult to eradicate (Davies and Wray, 1997).

Parker *et al*. (2022) reported a combined prevalence of *Salmonella* in raw feed components to be 18%, with finished feed at 9% and milling equipment to be 8%. This indicates that the raw materials were the main source of *Salmonella*, with the manufacturing process reducing the prevalence, although it cannot be concluded whether the *Salmonella* prevalence in the finished feed was due to survival during the manufacturing process or recontamination from the feed mill environment.

Further, the authors reported that the risk of *Salmonella* prevalence in animal-based byproducts was 3,9 times greater than plant-based raw feed components (cereal feed such as soya, maize, and dried forage and meals, etc.). The *Salmonella* prevalence in precook equipment was 1,5 times greater than the risk of detection in post-cook equipment, indicating that the post-cook equipment is more sanitary, although not free of *Salmonella* – an important risk for recontamination of the animal feed (Parker *et al*., 2022).

The *Salmonella* prevalence in poultry feed ranged from 0 to 100% across all the studies, with feed from US studies ranging from 0 to 40% and non-US studies ranging from 0 to 100% (the 100% prevalence was reported in one study with only two samples analysed). The predicted *Salmonella* prevalence in the poultry feed across the regions was 4,8% (95% CI: 1,4 to 14,7%), with an overall heterogeneity of 89,9%, indicating significant differences between the studies.

Hatchery

Hatcheries today are highly automated and have a high throughput of eggs and chicks and can contribute to the spread of *Salmonella* through the chicks. The introduction of large amounts of airborne fluff and dust generated during the hatching process was shown to be the primary source of *Salmonella* contamination of broilers (Bailey *et al*., 1992). As a consequence, the hatcher air carries *Salmonella* to the immediate environment and consequently the rest of the hatchery.

Carpenter *et al*. (1986) demonstrated the importance of dust in poultry areas as a transport mechanism for potential disease-causing organisms. They showed that reducing airborne dust in a poultry room by a factor of two reduced airborne bacteria by a factor of 100.

Salmonella has been recovered from several surfaces and the air in hatcheries, including hatchers, chick handling areas, cleaning equipment, setters, egg transfer areas, and service areas. Withenshaw *et al*. (2021) reported a *Salmonella* prevalence of 6% in all samples tested in Great Britain, with at least one sample positive for *Salmonella* in 77% of the hatcheries. The positive samples per hatchery varied from 0 to 33,3%, indicating that proper cleaning and sanitation and probably the design of the hatchery should address *Salmonella* contamination of surfaces.

In addition, the positive samples varied by hatchery area, with 0,3% positive in egg areas to 18,7% in waste areas. The hatchery characteristics and practices that contribute to a higher risk of *Salmonella* contamination include a greater number of hatchers in regular use, storing trays in the process room, drying set-up trolleys in the corridor, and skips located in an enclosed area (Withenshaw *et al*., 2021).

The prevalence of *Salmonella* in hatcheries ranged from 19 to 75%, with samples being eggshells and fluff inside the hatcher, tray liners, eggs, and setter areas.

Chicks

Whereas vertical transmission of *Salmonella* from the egg to the progeny is possible, most of the infection and subsequent colonisation probably occurs on exposure to *Salmonella* in the air during incubation and hatching or through the oral route via ingestion of *Salmonella*-contaminated membranes and other egg material. Wilding and Baxter-Jones (1985) isolated salmonellae from one out of approximately 10 000 commercial hatching eggs examined over several years and estimated one *Salmonella*-positive egg out of every 1 000 or 2 000 based on recovery from small groups of newly hatched chicks.

Contamination (experimental) of the eggshell surfaces with *Salmonella*-inoculated faeces resulted in many of the chicks being positive for *Salmonella*, indicating the respiratory system as the primary pathway for entry. Further, day-old chicks are more susceptible to *Salmonella* colonisation compared to three-day-old chicks by either oral or cloacal inoculation (Cox *et al*., 1990). Cason *et al*. (1994) reported 85% of the chicks were positive for *Salmonella* when the eggs were inoculated prior to incubation (by immersing in *Salmonella* inoculum of ca 8 log CFU/mL), and 77% of the chicks were positive for *Salmonella* that contained both inoculated eggs and non-inoculated eggs.

The *Salmonella* incidence in the chicks was 81 and 80% from the non-inoculated eggs that were placed in a tray above and below the mixed tray that contained both inoculated and non-inoculated eggs. From control eggs, 44% of digestive tracts of hatched chicks were positive, indicating that *Salmonella* in a contaminated hatcher can reach the gut of chicks hatching from *Salmonella*-free eggs before they are removed from the hatcher.

Thus, chicks can be contaminated with *Salmonella*, either through the surface, the respiratory tract, or the gastrointestinal tract. Whereas the rate of contamination of the chicks may not be as dramatic as demonstrated by Cason *et al*. (1994) resulting from lower concentrations of *Salmonella* on the eggshell surface due to natural contamination of the eggshells, the probability of chicks with *Salmonella* in their systems is relatively high. It is possible that these *Salmonella*-positive chicks further spread the micro-organism when they are placed in poultry house and act as seeder birds.

Table 1: Meta-analysis results for *Salmonella* prevalence from different sources throughout broiler live production.

Source	Region	No of reports	Population prevalence (95% CI)	τ^2	I^2
Faeces	US Non-US All regions	14 7 21	0,125(0,070 – 0,216)[a] 0,251(0,161 – 0,370)[b] 0,163 (0,106 – 0,243)	1,50 0,42 1,29	98,1 95,6 97,7
Litter	US Non-US All regions	20 6 26	0,254 (0,166 – 0,370) 0,253 (0,097 – 0,518) 0,254 (0,172 – 0,358)	1,48 1,86 1,57	97 97,9 97,1
Hatchery	US Non-US All regions	5 3 8	0,488 (0,272 – 0,708) 0,463 (0,311 – 0,622) 0,485 (0,328 – 0,645)	1,93 1,55 1,87	97,7 97,89 7,7
Chicks	US Non-US All regions	4 1 5	0,065 (0,039 – 0,105) 0,074 (0,019 – 0,252) 0,047 (0,006 – 0,284)	0,23 NA 0,21	92,3 NA 89,7
Environment – interior	US Non-US All regions	6 4 10	0,028 (0,017 – 0,157) 0,146 (0,046 – 0,379) 0,079 (0,032 – 0,181)	2,17 1,48 2,18	97 93,8 96,7
Environment – exterior	US Non-US All regions	4 1 5	0,017(0,004 – 0,071)[a] 0,530(0,007 – 0,721)[b] 0,047 (0,006 – 0,284)	1,48 NA 4,42	95,6 NA 98,4
Feed	US Non-US All regions	9 6 15	0,067 (0,007 – 0,138) 0,095 (0,020 – 0,352) 0,048 (0,014 – 0,147)	4,66 2,90 2,14	92,8 66,3 89,9
Water	US Non-US All regions	6 3 9	0,012 (0,002 – 0,056) 0,031 (0,019 – 0,049) 0,020 (0,009 – 0,043)	1,62 NA 0,58	72,8 NA 61,1

Abbreviations: CI, confidence interval; NA, not available; US, United States.
[a,b] Different superscripts mean the estimated pooled prevalence is significantly different for regions (P < 0,1).

Poultry house environment – interior

The poultry production environment is replete with vectors for *Salmonella* including rodents, pests (darkling beetles), flies and other environmental surfaces such as fan blades, floors and walls and those that are exposed to dust containing *Salmonella*. While each can be a source of *Salmonella* and its colonisation of the birds, literature on each of the specific sources and prevalence of *Salmonella* is limited.

Alphitobius diaperinus, known as the lesser mealworm beetle, darkling beetle, black beetle or litter beetle, is common in poultry production environments, including turkey and broiler operations (Axtell, 1994; Lambkin, 2001). The open floor housing and deep litter provide optimal habitat for the insects to establish, reproduce and develop (Axtell and Arends, 1990).

Salmonella-infected darkling beetles ingested by broiler chicks can colonise the gastrointestinal tract and spread *Salmonella* to other non-exposed chicks (Roche *et al*., 2009). The darkling beetles present in broiler environment can present a significant risk of colonisation by the chicks and the birds during grow-out.

Rodents are probably considered to be one of the most versatile pests. While the majority of broiler farms employ commercial pest control operations, some farms practice their own pest control measures. Literature indicates mice can be a significant source of *Salmonella* as they consume the same feed and defecate in the same environment (poultry litter) as the birds. Contaminated rodent faeces can be ingested by the birds as they are coprophagic, resulting in *Salmonella* colonisation among the birds and subsequent spread.

While other environmental surfaces in the poultry house have been positive for *Salmonella* such as fan blades, interior walls, floors and walls, these may be resulting from deposition of contaminated dust or a consequence of cross-contamination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from other sources. While these surfaces may indicate prevalence of *Salmonella*, multiplication of the micro-organism may not occur and may rather be an indicator of *Salmonella* prevalence in the poultry house and the birds.

Predicted *Salmonella* prevalence in the poultry production environment (interior) that included vectors such as mice, flies, darkling beetles and other insects and environmental surfaces such as fan blades, poultry houses inside surfaces such as floors and walls was 7,9% (95% CI: 3 to 8%).

Water

The water supply in poultry production operations is mostly through well water that is treated, and the recommendation is to provide potable water for the birds. In many of the cases, *Salmonella* contamination of well water is minimal and any treatment of the water further reduces the risk of foodborne pathogens in the water supply. However, water can get contaminated in the poultry house with birds drinking out of the drinking cups or the nipples and *Salmonella* and *Campylobacter* have been isolated from these sources.

A combination of optimal temperature (ca 25°C), low flow rates and adequate nutrients make the drinking water in poultry houses ideal for microbial contamination and the formation of biofilms, making disinfection less efficient. The prevalence of *Salmonella* in drinking water ranged from 0 to 11%, with the differences probably due to the location of sampling, whether the water was collected from the water main directly or at the drinker's nipples or cups. Also, the method of sampling could introduce variability in the prevalence, with larger volumes of collection resulting in higher prevalence.

Bailey *et al*. (2001) collected the water samples using a sterile swab or cotton tipped applicators (4 to 5), by inserting the applicators into the water line at the ends of the line or by swabbing the drinking nipples or cups. While *Salmonella* contamination of water can spread the micro-organism and consequently, colonisation of the birds, it is probably not the primary source of *Salmonella* in a poultry house.

Faeces (excreta, a mixture of faeces and urine)

Chicks are exposed to *Salmonella* at the hatchery, right after they pip from the shell (Bailey *et al*., 1994). The chicks can get colonised by *Salmonella* by respiratory (air or dust), oral (feed or water) or intracoacal routes (Cox *et al*., 1996). Once the chicks are colonised, they excrete high levels of *Salmonella* during grow-out. The excreta can be ingested by other birds and spread among the rest of the flock. Thus, excreta or faeces are a good, non-destructive indicator of colonisation of the bird and in extension, the flock.

However, it is possible that not all the birds in a flock are colonised with *Salmonella* and hence, sampling of ceca may only be related to *Salmonella* colonisation of the specific bird(s). However, the *Salmonella*-positive status of the flock can be observed by a sampling of the faeces of the birds across the poultry house using drag swabs or boot socks.

Because *Salmonella* spread in broilers is primarily through faecal-oral route, *Salmonella* colonises chicks when they are placed in the grow-out house can result in seeding or spreading *Salmonella* to other birds. The *Salmonella* positive status of chick faeces is an indication of the colonisation of the chicks at the hatchery, and even the non-colonised chicks and subsequently, the birds are exposed to *Salmonella* in the poultry house environment as discussed in other sources of *Salmonella* in this manuscript.

Litter

Broiler litter is a mixture of substrate, most often pine shavings, rice husk, cereal straw or other materials along with the faeces (excreta) of the birds from previous flocks. In the US, many of the flocks are placed into grow-out houses within a day after hatch, directly on the litter (Volkova *et al*., 2009). If *Salmonella* is present in the litter, the chicks are exposed at a time when they are highly susceptible to colonisation.

Studies have shown that the presence of *Salmonella* in the litter prior to placement of a new flock was shown to be a precursor of high *Salmonella* frequencies in the new flock at later stages of production (Rose *et al*., 1999; Rose *et al*., 2003).

In addition to the type of litter material used, the soil composition and properties were shown to affect moisture retention and indirectly the water activity of the litter and thus, *Salmonella* survival. Volkova *et al*. (2009) reported that the probability of detecting *Salmonella* in the broiler litter was dependent on the soil texture (relative proportions of particles of different sizes) and the infiltration and drainage capabilities, that is, the properties defining the pattern of water movement through the soil profile.

Conclusions

Salmonella colonisation of broilers is primarily through the faecal-oral route, although other routes of colonisation can occur such as respiratory and intracloacal. These sources of *Salmonella* in poultry production include, in the order of prevalence (higher to lower): hatchery (48,5%), litter (25,4%), faeces (16,3%), poultry house internal environment (7,9%), poultry house external environment (4,7%), feed (4,8%), chicks (4,7%), and drinker water.

Whereas all of these sources play a significant role in the colonisation of the bird and subsequent spread, the primary sources of *Salmonella* include the chicks, feed and internal environment of the poultry house (including rodents and darkling beetles). Of these three primary sources of *Salmonella*, control of the feed manufacturing process and/or additives to eliminate *Salmonella* from the feed is a more feasible and effective strategy.

Regardless, structural modifications to the poultry house need to be made to prevent infestation of the pests and rodents, and other sources of *Salmonella* to minimise the risk of *Salmonella* colonisation and further spread within the flock.

Whereas significant progress has been made at the poultry processing stage to reduce concentrations and prevalence of *Salmonella* in processed poultry (fresh), controls at production need to be incorporated to achieve further reductions in *Salmonella* in freshly processed poultry.

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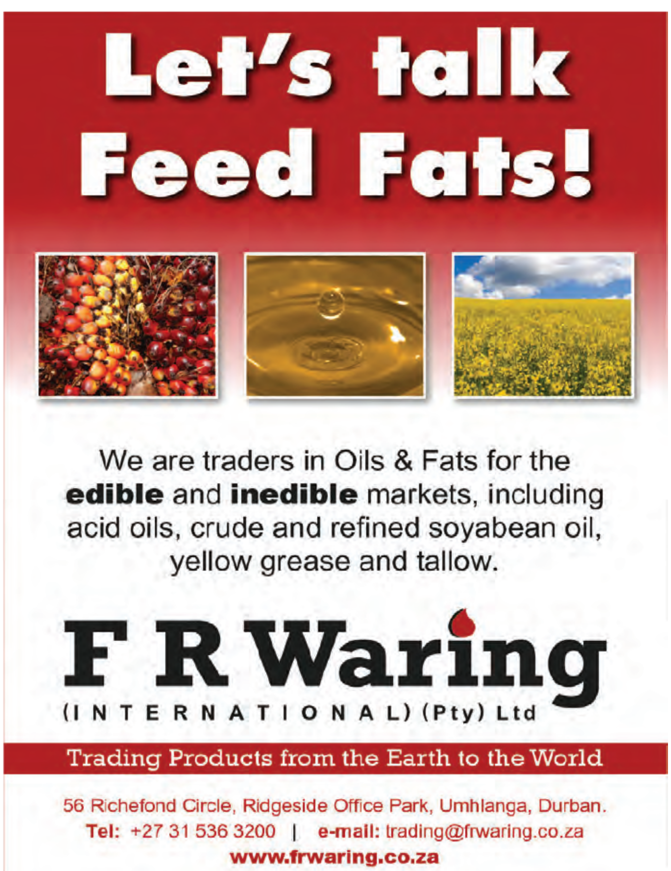
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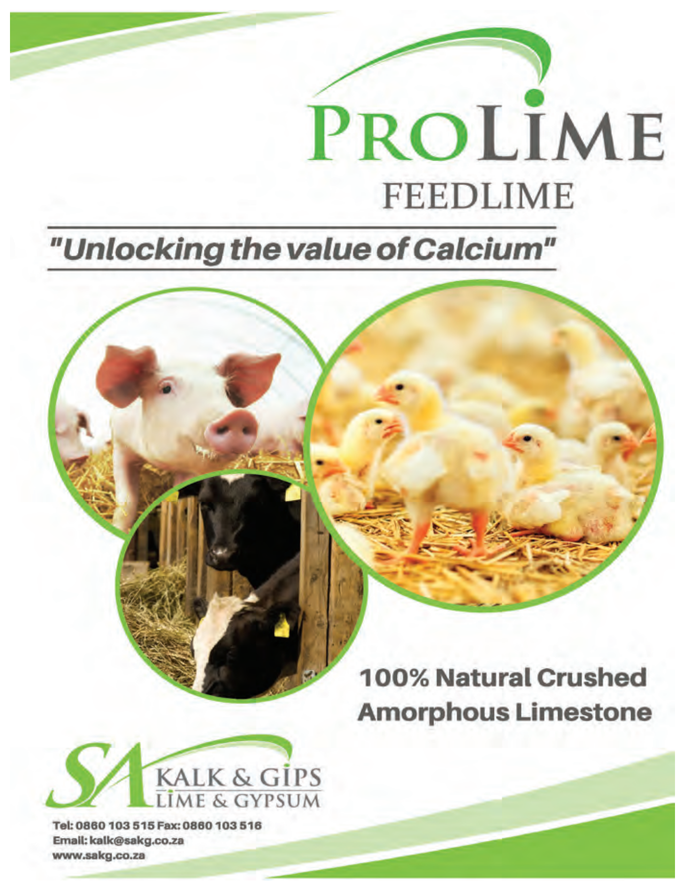
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Feeding postpartum cows successfully

By Samaneh Azarpajouh, All About Feed

Feeding management in high-producing cows is critical to adapt successfully to lactation, to maintain blood calcium and rumen health, and to sustain reproductive efficiency during the postpartum period. Proper diet formulation meets the nutrient requirements of cows, prevents over-consumption of energy, improves transition period outcome and increases fertility.

Impact of negative energy balance

Negative energy balance during early lactation occurs due to the lack of consuming sufficient energy-yielding nutrients from voluntary dry matter (DM) intake after calving to meet energy requirements for milk production. Negative energy balance detrimentally affects fertility and reproductive performance by influencing hypothalamic, pituitary and ovarian function, causing hindered resumption of ovarian cyclicity, affecting oocyte or corpus luteum quality, viability and function, and developing fatty liver.

Early ovulation after calving increases the fertility rate; however, negative energy balance decreases the pulse frequency of LH release, size and development rate of follicles, concentrations of oestrogen and progesterone, and the size of the corpus luteum.

In addition, blood-borne metabolites from responses to negative energy balance deleteriously affect oocytes and the corpus luteum. Furthermore, fatty liver and hepatic lipid infiltration, which are indirectly caused by extremely negative energy balance, reduce fertility and reproduction.

Adaption of cows

How does a cow adapt from gestation to lactation? Insulin and leptin have higher concentrations during the dry period than early lactation and promote energy storage in adipose tissue. Nutrient demands by the foetal calf and placenta peak three weeks before calving; however, DM intake decreases by 10 to 30% compared to the early dry period.

Ketosis: Low DM intake accompanied by stressors leads to negative energy balance before calving which worsens after calving. Around calving, the insulin concentration reduces, and growth hormone concentration increases to mobilise stored fat and support milk production. However, decreased DM intake and limited glucose supply due to negative energy balance, and the accumulation of liver fat, result in ketosis and fatty liver.

Mastitis: Loss of muscle mass starts before calving, and during the first week after calving muscle protein mobilisation is increased. During the transition period, immune system function is reduced which, in turn, leads to a high incidence of environmental mastitis and metritis and an increased rate of retained placenta.

Milk fever: Lactation requires a tremendous amount of calcium and causes low blood calcium at calving, milk fever and displaced abomasum by reducing smooth muscle function of the digestive tract. In addition, low blood calcium compromises immune cell function.

Feeding strategies

DM intake plays an important role in the depth and duration of negative energy balance. Therefore, feeding strategies for fresh cows should focus on promoting appetite and DM intake after calving. Proper diet formulation is based on considering the nutrient requirement of most cows and providing a balanced diet.

To promote good appetites and DM intake after calving, diminish environmental stressors, increase cow comfort, prevent excessive body condition score (BCS), inhibit over-consumption of energy relative to requirements during the dry period, provide sufficient fibre, prevent excessive starch intake after calving, and increase the dietary cation-anion difference in the fresh cow diet.

Proper dietary formulation during the dry period or close-up period will maintain or enable rumen adaptation to higher grain diets after calving and improve early lactation productivity. Cows need to be fed a low-energy far-off diet for five weeks followed by a higher-energy diet for the last three weeks before parturition for sufficient ruminal adaptation.

Subacute ruminal acidosis

This is a concern in fresh cows and is caused by a sudden increase in dietary energy density. Subacute ruminal acidosis decreases DM intake and nutrient digestibility. Furthermore, an adequate physical form of the diet, starch content and fermentability are essential to stimulate ruminal activity and chewing behaviour.

Diets for fresh cows

A moderate starch content (approximately 23 to 25% of DM) with moderate fermentability, along with adequate effective forage fibre is appropriate for fresh cows. Feeding a glucogenic diet until cows resume ovarian cyclicity, followed by a higher fat diet during the breeding period, improves reproductive success. Supplementing polyunsaturated fatty acids such as calcium salts of safflower oil and calcium salts of fish oil improves uterine health, and increases pregnancy rates and milk production.

Conclusion

Application of targeted feeding strategies to improve DM intake in fresh cows decreases health problems, minimises negative energy balance and improves fertility. However, little is known about the diet formulation for the immediate postpartum period; thus, further research is required to optimise transition success and subsequent reproduction.

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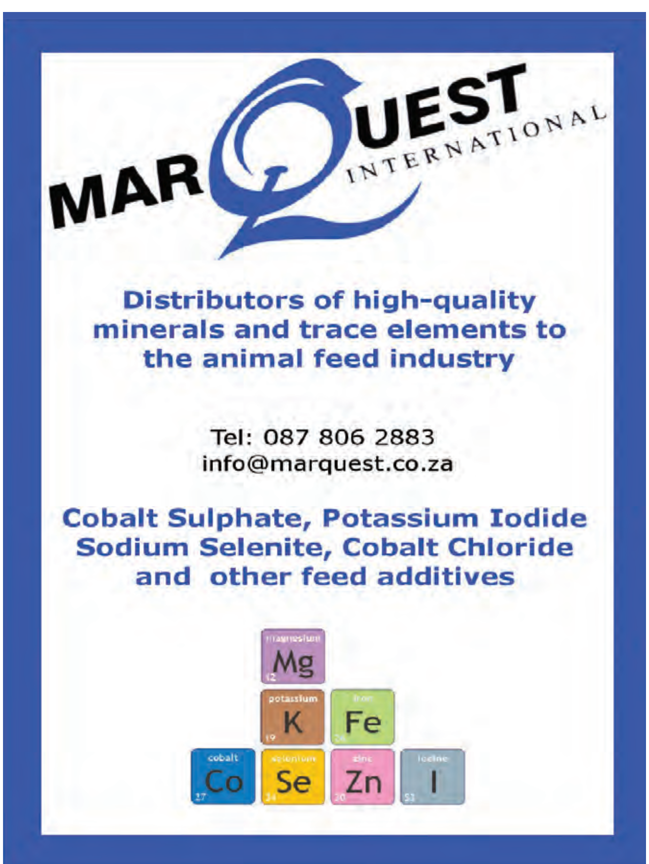
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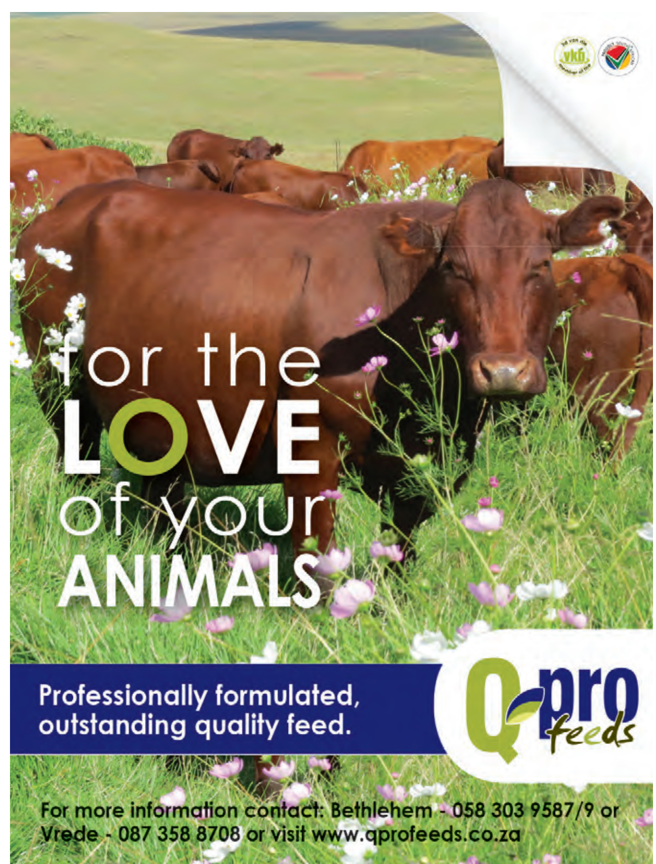
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The role of feed-grade amino acids in the bioeconomy: Contribution from production activities and use in animal feed

By Viktoriya Sturm, Martin Banse and Petra Salamon

A sustainable and circular bioeconomy is an option for a better future (Sturm and Banse, 2021; Chavarria *et al*., 2020; Fritsche *et al*., 2020). Policymakers in the European Union (EU) recognise this and confirm the importance of the contribution a sustainable and circular bioeconomy can make to achieving the sustainable development goals (SDGs) as well as the Paris Agreement by the EU's 2018 Bioeconomy Strategy update.

With the European Green Deal, important steps of integration of bioeconomy in other EU policies related to biodiversity, circularity, climate change, food systems, forest protection and restoration, and renewable energy are underway.

For the assessment of the status and the development of the bioeconomy, a monitoring system is needed. One of the EU's 2018 Bioeconomy Strategy update actions foresees the development of an EU-wide, international coherent monitoring system to track economic, social and environmental progress towards a circular and sustainable bioeconomy.

The European Commission (EC) Joint Research Centre (JRC) is leading this action, in collaboration with several Commission Services and stakeholders. The approach for the EU bioeconomy monitoring framework was developed (Kilsedar *et al*., 2021; Robert *et al*., 2020) and the first release of the EU Bioeconomy Monitoring System 1 was launched in November 2020; further improvements should follow.

At the same time, some bioeconomy monitoring activities are already taking place at the level of EU member states and there are even initiatives to set up a national bioeconomy monitoring system (Lier *et al*., 2018).

Thus, in 2016, the Federal Government of Germany initiated the development of a comprehensive bioeconomy monitoring system. Research projects based on this initiative resulted in a conceptual proposal for setting up a monitoring system in Germany and generated the first results (Banse *et al*., 2021; Iost *et al*., 2020; Bringezu *et al*., 2020). Furthermore, ongoing bioeconomy research projects result in additional proposals and recommendations concerning establishing a bioeconomy monitoring system (Kardung *et al*., 2021).

Despite some differences in the proposed approaches for setting up a monitoring system and indicators used to track the developments in the bioeconomy, one is common for all of them – the need for a sound database.

To test and validate proposed methodologies (Piotrowski *et al*., 2019) case studies were conducted. One of the conducted case studies focusses on the dynamics on the markets for feed-grade amino acids (Sturm *et al*., 2021). Amino acids contribute to a bioeconomy through associated producing activities as well as through their use in different applications.

Relevant producing activities are related to the production of bio-based amino acids mainly through the fermentation process, although other approaches to produce amino acids from bio-based feedstocks are also gaining attention (Deng *et al*., 2018; Tian *et al*., 2021; Song *et al*., 2020; Hirasawa and Shimizu, 2016).

Contribution to the GDP, value-added or employment associated with these activities could be directly assigned to the bioeconomy. Besides direct contribution to the bioeconomy through production activities, amino acids also contribute significantly to the bioeconomy through their use.

The areas of application of free amino acids include the use in animal feed, food and dietary supplements, pharmaceuticals, cosmetics and as precursors for bio-based plastics and other chemicals. However, the use in animal feed is currently the most important application category and we focus on this by looking into feed-grade amino acids.

Table 1: Order of limiting amino acids. (Source: Toride, 2004)

	First	Second	Third
Growing pigs	Lys	Thr	Trp
Broilers	Met	Lys	Thr

Introduction of amino acids

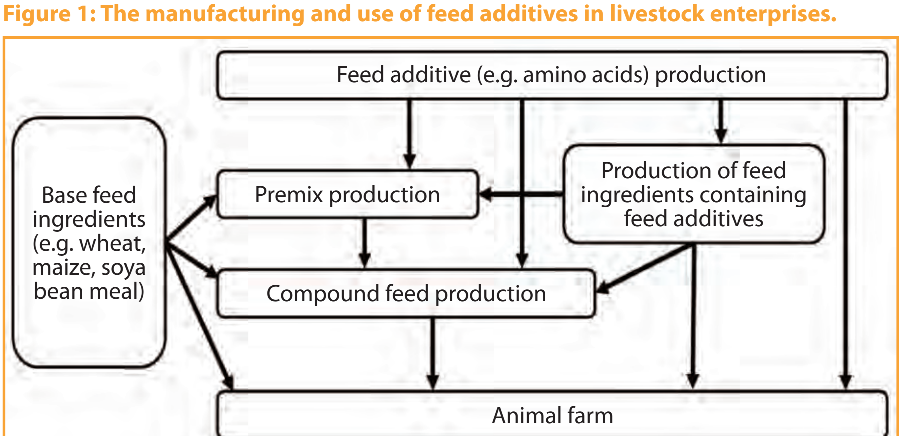
The industrial application of amino acids for feed has an almost 60-year history (Toride, 2004). First, in the late 1950s and 1960s, methionine (Met), produced by chemical synthesis, began to be used in poultry feed. Production of lysine (Lys) by a fermentation process was started in the 1960s. In the late 1980s, threonine (Thr) and tryptophan (Trp) also produced by the fermentation process were introduced.

The order of the introduction of amino acids in animal feed reflects the typical discrepancy between the requirements for amino acids by species and their actual presence in feed. The first amino acid, the absence of which interrupts protein synthesis of the other amino acids, is called the 'first limiting' amino acid. *Table 1* shows, based on Toride (2004), the orders of limiting amino acids in pig and broiler feeds, composed of maize/wheat and soya bean meals.

With feed formulation becoming increasingly advanced, further amino acids such as valine (Val), isoleucine (Ile), leucine (Leu), arginine (Arg), histidine (His) and cystine (Cys) are considered to be used in feed. Besides the introduction of new free amino acids in feed for monogastric animals (poultry and swine), the introduction of amino acids in feed for ruminants (in particular dairy cows) takes place. Coated Met and Lys, which can escape microbial degradation in the rumen, are already available on the market (Sturm *et al*., 2021).

Feed additives in general and amino acids specifically are usually not used on farms as such, and the feed additive value chain is composed of multiple actors, as described based on FAO (FAO, 2019) in *Figure 1*. It can be implied that the use of amino acids for animal feed could be covered by producers of compound feed, as the vast majority of amino acids are supplied to livestock producers through this channel.

Figure 1: The manufacturing and use of feed additives in livestock enterprises.



Assessment tools

Generally, LCA analysis is an appropriate tool to assess the contribution of amino acids to the bioeconomy through their use in animal feed. The establishment of an appropriate database and methodologies, however, is very demanding and quite challenging.

The Global Feed LCA Institute (GFLI) is developing an LCA database and tool, which aims together with the underlying UN FAO LEAP-based methodology to be the reference for assessing and benchmarking feed industry impacts and improvement in LCA calculations. The GFLI database consists of the LCA of raw materials from various regions in the world. Currently, this LCA database does not incorporate free amino acids used in animal feed, but it might be the case in the future.

Some firms, i.e. producers of feed additives such as Evonik and BASF, have already developed dedicated tools that make use of LCA and should enable the assessment of the environmental impact of both feed and the final animal protein product (Sturm *et al*., 2021). These tools are also supposed to be able to assess what effects feed additives such as amino acids introduced into animal feed have.

Effect of amino acids

Positive effects from the use of free amino acids in feed come from both bio-based and fossil-based free amino acids. The main contribution comes from the use of the first limiting amino acid. Therefore, in the case of pigs, the main effect comes from bio-based free amino acids (mainly Lys), while in the case of poultry, the main contribution is associated with the use of fossil-based free amino acids (Met).

At a certain point, when the use of a second and subsequent limiting amino acid is required, the additional positive effect is attributed to the use of two or more free amino acids. The effect from further use of an additional unit of the first limiting amino acid decreases in favour of the second and subsequent limiting amino acids.

Thus, due to the cross-cutting effects, the cumulative impact is not equivalent to the sum of effects from the use of each free amino acid. This makes the analysis even more challenging, without even taking into consideration that positive effects could also be a result of interaction from the use of free amino acids with other feed components, for example other feed additives.

The assessment of the impact of the use of free amino acids in the feed will hopefully be more profound in the future due to the expected progress in LCAs of feed components, as well as due to the development of dedicated tools. The amount of literature showing the positive environmental effects from the use of amino acids in animal feed are growing (Selle *et al*., 2020; Kebreab *et al*., 2016; Reckmann *et al*., 2016).

However, it could hardly be expected that the quantification of the absolute impact from the use of free amino acids in the feed will be possible. Most of the studies conduct comparative life cycle assessments and analyse the performance of different diets, without or with a certain amount of free amino acids, and cannot be used to generalise which environmental effects the total amount of amino acids used in feed has in the EU.

Results

Contribution to the bioeconomy through the use in animal feed

Farmers in the EU use feed-grade amino acids mainly by using compound/mineral feed. Therefore, the producer's demand for compound/mineral feed for feed-grade amino acids could be seen as a good proxy for the total demand of feed-grade amino acids.

Table 2: Occurrence of amino acids in animal feed (pigs and poultry) in the EU.

Species	Common practice	Partly used	Selective assignment
Poultry			
Laying hens	Met, Lys	Thr, Val, Ile	
Broilers	Met, Lys, Thr	Val	Ile, Arg, Leu, Cys, His
Turkey	Lys, Thr, Met		
Pigs			
Fattening pigs	Lys, Thr	Val, Trp	
Piglets	Lys, Thr, Met, Val, Trp		Ile, Leu, Cys, His
Sows	Lys, Thr	Met, Trp	Arg

Met = Methionine; Lys = Lysine; Thr = Threonine; Trp = Tryptophan; Val = Valine; Ile = Isoleucine; Arg = Arginine; Leu = Leucin; Cys = Cystine; His = Histidine.

The EU producers of compound feed use the free amino acids directly or in the form of 'premixtures' (mixtures of feed additives or mixtures of one or more feed additives with feed materials or water used as carriers). The premixes used in the formulation of feed in the EU are mainly produced in the EU; hardly any import of premixes from non-EU countries takes place. However, EU producers of premixtures export their products to other EU countries as well as to non-EU countries.

The use of amino acids in animal feed is driven by the level of feed efficiency that should be achieved and the quantity of animals. In the EU, conventional livestock farming shows a high level of feed efficiency, which goes along with a relatively intensive use of amino acids in feed. The demand for individual amino acids depends on the number of individual species as a requirement for supplemented amino acids in feed, and varies depending on the species and age of animals.

Table 2 shows the current occurrence of amino acids in animal feed. The differentiation is made with regard to the species, their age and husbandry objectives as well as the frequency of use. According to *Table 2*, the use of Met, Lys, Thr, Trp and Val in feed for poultry and pigs is already a 'common practice' or at least 'partly used'. But as the formulation of feed is getting more and more advanced, the use of further amino acids such as Ile, Leu and even Arg, Cys and His is taking off.

Currently, the use of amino acids in feed for calves, dairy cows or fattening bulls plays only a minor role in the EU. The use of coated Lys and Met in feed for dairy cows could be the next important future application area of amino acids in animal feed in the EU. Free amino acids, in particular the special formulation of Met, are also used in feed for fish and aquacultures. However, in the EU the importance of this application is quite low.

Besides the level of feed efficiency and the numbers of individual species, there are further important drivers for the use of free amino acids in animal feed. One of them is the expansion of organic livestock husbandry. The point is that free amino acids practically cannot be used in organic livestock farming. The Commission's Farm-to-Fork and the Biodiversity Strategy include the target of reaching 25% of agricultural land under organic farming by 2030. If this target will be applied to animal husbandry, and if the rules on the use of amino acids in organic livestock husbandry will be maintained, the use of (and demand for) feed grade amino acids in animal feed is expected to decline.

Another important driver on the market of feed-grade amino acids is the growing awareness of negative environmental effects of livestock farming, especially with regard to nitrogen emissions. As amino acids help to raise feed efficiency and to reduce N-leakage, the use of amino acids is supposed to increase if regulations to reduce N-leakage are implemented. The use of Life Cycle Assessments (LCAs) for amino acids as proposed by the FAO (FAO, 2019) could possibly reveal further positive effects of feed-grade amino acids. By taking such effects into account, policies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could further foster the use as well as the production of amino acids in the EU.

Discussion

Despite the fact that amino acids have been identified as potentially important bio-based products (ECOSYS, 2011; Lammens *et al*., 2017; Spekreijse *et al*., 2019), their contribution to the EU bioeconomy has not yet been quantified. Our analysis discloses the main hurdles in data collection as well as the methodological challenges associated with such assessments and provides a first attempt to close existing gaps.

There is an urgent need for detailed production data to assess the contribution of amino acids to the bioeconomy. 'Feed-grade amino acids' form a product or application category which does not have a counterpart in official statistical classification. The first step required to conduct an assessment is to identify relevant products and to find out under which activities their production is reported in the official statistics. In our case, we considered all 20 proteinogenic amino acids, and identified under which codes they are reported in official statistics.

Our analysis reveals that 20 amino acids are assigned to ten codes in trade statistics which are linked to nine codes in production statistics. Lys is the only amino acid used in feed that has its own dedicated code in the trade as well as in production statistics. The production and even trade of other amino acids are reported under a number of different codes, each covering a range of products. This fact makes the use of official statistics for other amino acids practically impossible.

But even data for Lys, which has its dedicated code, reveal significant gaps because of the confidentiality of data, and only a rough estimate for production in the entire EU is available. Therefore, the official statistics only provide parts of a database necessary for a comprehensive analysis. The main problem is not only a lack of information specifically on bio-based production, but general data gaps on amino acids caused by a lack of dedicated codes for amino acids and confidentiality of data.

Our finding can be generalised as follows: Data collection and monitoring of the contribution of a specific product group to the bioeconomy is a very challenging and demanding task, especially if no clear link (mapping) to the classification within production and trade statistics is established.

Amino acid production

We conducted a literature review and interviews to collect further information. Our results reveal that the production of feed-grade amino acids in the EU is dominated by three companies. Met produced from fossil-based feedstock is the most important amino acid produced in the EU. Some other bio-based feed-grade amino acids produced through fermentation (Lys, Trp, Ile, Leu and Arg) are produced in the EU by a single producer and their production volumes are quite low.

The production of bio-based amino acids generates about €50 million value-added and provides jobs for about 400 employees. The bio-based share in the total production of feed-grade amino acids in the EU is about 15% and low compared with a share of 70% at the global level. The main reason is the low level or absence of production of Lys and Thr in the EU, because of its disadvantages as a production location due to higher production costs as well as higher environmental standards.

As a consequence, the contribution from the production of bio-based feed-grade amino acids to the bioeconomy in the EU is quite low and is not expected to increase if current operating conditions remain unchanged.

Contribution of amino acids

The assessment of the contribution of feed-grade amino acids to the bioeconomy through their use in animal feed is even more challenging, as in addition to the data gaps identified previously (results in problems by estimation of use quantities), methodological challenges exist.

Generally, this contribution arises in two ways. First, amino acids help to rise feed efficiency and, thereby, decrease the total demand for feed (and negative effects associated with the production of feed), as well as to reduce nutrient leakages (and associated emissions). Second, free amino acids may contribute to reducing the protein content in the feed. A reduced crude protein (CP) diet is seen as one of the options to reduce the demand for soya bean (meals) in the EU. That would not only decrease the dependency of the EU on imported soya bean (meals), but would also help to reduce the overall negative impact coming from feed production, especially associated with land use change and deforestation.

We argue that an LCA analysis could be an appropriate tool to assess the contribution of amino acids to the bioeconomy through their use in animal feed. Currently, the GFLI partners are developing an LCA database and tool, which aims to serve as a reference for assessing and benchmarking feed industry impact and improvement in LCA calculations for feed. As soon as amino acids are included in the database, a more profound assessment will be possible.

In order to get an impression of the contribution of free amino acids resulting from their use in animal feed, we calculate their theoretical already existing positive impact on land use change. This rough theoretical calculation shows that the use of amino acids in feed already helps to avoid the use of millions of hectares of arable land for the production of animal feed.

If these various positive environmental effects stemming from the use of amino acids in animal feed will be recognised in the future, their use in animal feed would increase not only because of the positive effect on costs of animal husbandry.

However, besides this positive driver for the use of amino acids in feed, there might be negative drivers such as a decline in European livestock farming driven by a decreasing demand for animal products or an expansion of organic agricultural production in the EU, which does not tolerate the use of free amino acids in feed.

The contribution of bio-based amino acids to a more sustainable EU bioeconomy is much more significant if not only the contribution through the related production activities, but also the contribution through their use in animal feed is considered.

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ENQUIRIES
072 905 5111
028 050 1385
admin@expo.org.za
Nooitgedacht Road,
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Mycotoxin biomonitoring: Are we there yet?

By Caitlyn de Vos and Marthie Nickols, Vitam International

In the field of mycotoxin surveillance, there are several ways to determine risk. In-feed analysis is the gold standard; however, it is limited by the non-homogenous distribution of mycotoxins in feed. Additionally, by the time mycotoxicosis is suspected on-farm, the causative feed ingredient is often no longer available for analysis.

As technology continues to evolve, we are discovering alternative analytical methods to detect mycotoxins. One of these is the application of biomarkers that assess mycotoxin exposure directly in the animal by analysing blood or other body fluids (e.g., bile, urine, faeces) and tissues.

Mycotoxin biomarkers can be classified into two categories: mechanism-based and exposure-based. Mechanism-based biomarkers refer to a biological response caused by the mycotoxins, such as alterations in protein, enzyme or gene expression levels. Exposure-based biomarkers describe the measurement of the parent mycotoxin and/or its metabolites in biological matrices. Unfortunately, there are still many factors limiting the use of mycotoxin biomarkers on-farm.

Limitations of biomarker testing

Sampling time

Mycotoxins differ in both physical and chemical properties, leading to considerable differences in their kinetic profiles (i.e., the extent to which the toxins are absorbed, metabolised and eliminated from the body). It is further complicated by time after a meal and animal species. In short, a given sampling time point might be ideal to detect one mycotoxin biomarker in blood but not others, while a specific biomarker can show different characteristics across different animal species, meaning that one biomarker might not be suitable for all.

The right biomarker and matrix

Species-dependent and toxin-dependent differences in mycotoxin metabolism complicate which biomarkers to screen for in which biological matrix. Poorly absorbed mycotoxins are hard to detect, whereas others might be metabolised to higher concentrations than the parent mycotoxin. Unfortunately, the kinetics of many mycotoxins are not yet fully understood in livestock species, impeding the selection of suitable biomarkers for metabolites and emerging mycotoxins, which can be detected with in-feed sampling.

Influencing factors

Even for major mycotoxins of agricultural importance, such as deoxynivalenol, factors influencing the levels of biomarkers are poorly explored and understood. Several factors, including sex, age, production stage, nutritional status, co-exposure to multiple mycotoxins or other feed contaminants, and disease, can influence biomarker levels. Individual animals can show marked variations in biomarker levels when exposed to the same feed and sampled at the same time point. This complicates risk assessment and prevents the comparison of biomarker results among groups, production cycles or farms.

Lack of reference values

At present, compared to in-feed mycotoxin analysis, there is a lack of established biomarker reference values or proficiency tests to evaluate mycotoxin biomarker methods. These reference values are crucial for the correct interpretation of biomarker results and to determine the health risk posed to the animals. In general, research on the accuracy or relevance of blood mycotoxin testing is limited.

The analytical method

Biological matrixes such as blood can be difficult to handle and can interfere with analysis. This is further complicated by the extremely low concentrations (parts per trillion) at which biomarkers occur. The chosen analytical method and the first-hand experience of the laboratory are critical for reliable biomarker analysis. In comparison, the analysis of feedstuffs for mycotoxins is a well-established and reliable method that can detect at least 50 mycotoxins.

In conclusion

Analytical methods for the assessment of mycotoxin biomarkers in the blood are becoming more time- and cost-effective. However, several limitations still hinder the practicality and usefulness of this type of testing on-farm. Advancements in research and the establishment of interpretation thresholds will hopefully allow for the reliable application of mycotoxin biomarkers at farm level in the future. Until then, in-feed mycotoxin analysis will remain the most practical, reliable and accurate method for assessing and managing mycotoxin risk on-farm.

For enquiries, send an email to caitlynv@vitam.co.za or marthien@vitam.co.za.

AFMA INTERVARSITY WRITER'S CUP 2023: WINNER ROUND 3 / OWN RESEARCH

The effect of ionophore use and essential oil compounds in calf diets on animal growth and the prevalence of antibiotic resistant *Escherichia coli*

By M Gouws and L Steyn

The need for alternative growth promoting and disease preventing feed additives to ionophores in calf starter feed, has become an area of interest in many European Union (EU) countries since the ban of the subtherapeutic use of antimicrobial drugs.

The World Health Organisation considers antimicrobial resistance as one of the three largest threats to human health. Increased legislation on antibiotic use in the livestock sectors of many countries and concern of a rise in antibiotic resistant pathogens urged the investigation into alternatives to ionophores such as essential oil compounds.

The aim of the study was to determine if either monensin or a blend of essential oil compounds could yield similar health and growth benefits while influencing the prevalence of antibiotic resistant *Escherichia coli*.

Background

Ionophores are antimicrobial compounds used in calf nutrition to control coccidiosis and aid in growth and health of the calf (Baldwin *et al*., 2003; Tahmasbi *et al*., 2014). Early nutrition and management practices are crucial for growth, health and productivity of both replacement heifers and surplus veal calves (Tahmasbi *et al*., 2014), and monensin has been readily acknowledged for its anticoccidial and growth promoting effects (Edrington *et al*., 2003; Van Baale *et al*., 2004).

Due to the increased concerns over antimicrobial resistant (AMR) pathogens, the use of ionophores in animal nutrition has received scrutiny and led to the ban of all growth promoting antimicrobial drugs in the European Union in 2006 (Sánchez *et al*., 2021). Furthermore, European pharmaceutical companies are phasing out growth promoting antimicrobial drugs, which sparked conversation among researchers to investigate alternative non-antibiotic feed additives (National Department of Health, 2021).

Essential oils (EOs) are complex phenolic and volatile compounds produced by herbal plants as secondary metabolites (Spisni *et al*., 2020). These EO compounds have been studied for their anticoccidial and antibacterial properties. Carvacrol, capsaicin, cinnamaldehyde and thymol

have shown promising growth-modulating action in lambs, poultry and piglets (Castillo *et al*., 2012; Spisni *et al*., 2020).

Furthermore, EO compounds have often been overlooked in calf nutrition and as a result limited research is available on the practical application of an EO compound blend that, when added to the diet, could successfully be compared to ionophores to aid in health and growth performance of pre-weaned calves (Salazar *et al*., 2019).

Calves that received a garlic or oregano EO supplement were observed to have decreased morbidity and increased milk intake (Ghosh *et al*., 2010; Tapki *et al*., 2020; Chen *et al*., 2021). Calves that received a thymol and eugenol EO showed increased weight gain and nutrient absorption in the gastrointestinal tract (GIT) (Kazemi-Bonchenari *et al*., 2018; Kekana *et al*., 2020).

Antibiotic resistance can be defined as the ability of a bacteria to withstand an antibiotic agent designed to kill or inhibit the growth of the bacterium by targeting specific processes that are essential for its survival (Simjee, 2019). Livestock farming practices are often seen as reservoirs for antibiotic resistant (ABR) pathogenic bacteria that can impact both human and animal health.

High levels of therapeutic or subtherapeutic antibiotic use is a common practice in dairy operations and exposure to the antibiotics have been shown to alter the microbiota in the GIT of neonatal calves (Pardon *et al*., 2012; Manishimwe *et al*., 2017). Furthermore, calf rearing systems have been identified as one of the sectors with the highest abundance of ABR bacteria (Amin and Seifert, 2021).

Although ionophores are not considered as medically important antimicrobial drugs, animal-only drugs can lead to the resistance of pathogenic bacteria to medically important antibiotics through shared resistance mechanisms, or resistant gene transfer (Alarcon and Omenaca, 2004; Wong, 2019).

Materials and methods

Treatments and animal management

The trial was conducted on two farms in the Western Cape of South Africa. Jersey bull calves (n = 24; Farm A) and Ayrshire heifer calves (n = 39; Farm B) were randomly allocated to one of three treatment groups: (CON) no added elements to either the liquid or solid diet; (EOC) added garlic extract (0,6g/day per calf) to the liquid diet and an added blend of cinnamaldehyde, carvacrol and capsaicin (150mg/kg dry matter; DM) to the starter feed diet; and (MON) no added elements to the liquid diet, and added monensin (30mg/kg DM) in the starter feed diet.

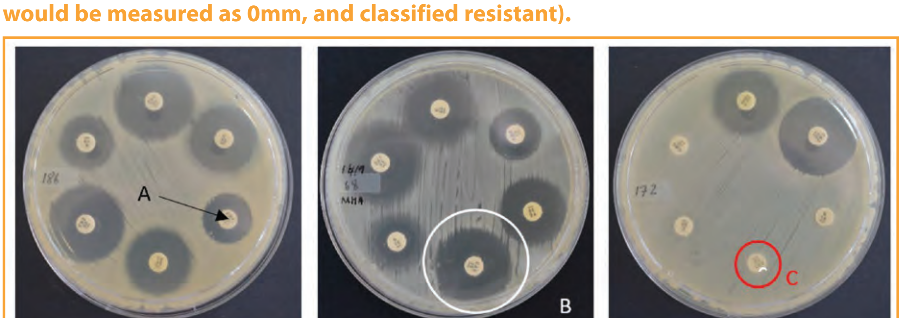
All calves received veterinary treatment if necessary, and all antibiotic administration and supportive therapy (electrolytes, anti-inflammatory medication, etc.) as well as disease status were fully recorded throughout the study. Intake and faecal scores were recorded daily, whereas bodyweight of the individual calves was taken weekly, and rectal swabs were taken on day 1, 30 and 56 of the trial.

Furthermore, faecal samples of individual calves were obtained daily between 20 to 40 days of age to determine the prevalence of *Eimeria* spp. oocysts between the treatment groups by using a modified McMaster technique (LNE10-300; Zajac *et al*., 2010). Furthermore, eighteen Jersey bull calves were slaughtered at 60 days of age to determine rumen wall thickness, papillae density, rumen papillae length (PL) and rumen papillae width (PW).

Faecal swabs were taken for the isolation of *E. coli* whereafter a Kirby-Bauer disc diffusion method was used to determine antibiotic resistant isolates (Humphries *et al*., 2018; Van den Honert *et al*., 2021). Isolates were tested for resistance to sulfamethoxazole-trimethoprim, chloramphenicol, ampicillin, tetracycline, ceftazidime and streptomycin.

The zone diameters around each antibiotic (*Figure 1*) were measured to determine susceptibility of the isolate to the respective antibiotic based on the Clinical and Laboratory Standards Institute (2022) zone diameter interpretive standards.

Figure 1: Mueller-Hinton agar plates with antibiotic discs (A) after a 24 hour incubation period; B and C display the zones around the antibiotic discs, the diameter of the zone would be measured to determine resistance (i.e., zone C would be measured as 0mm, and classified resistant).



Discussion

Animal growth, health and rumen development

The addition of a pungent garlic extract in the milk or MR did not affect the milk intake of the calves. Similar starter feed intake, average daily gain, and feed conversion ratio between the three diets were observed for both the Jersey bull calves and the Ayrshire heifer calves.

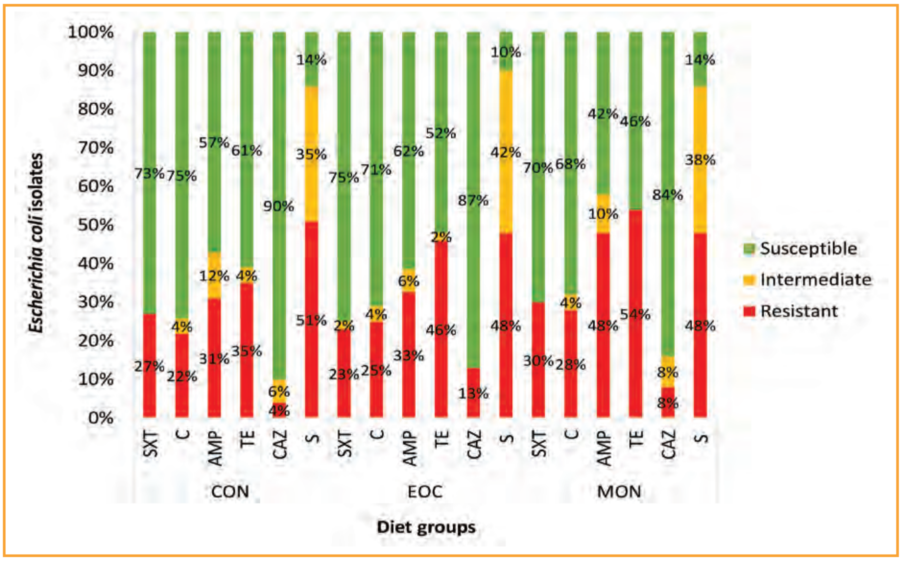
Eimeria spp. oocysts were present in 66,7% of Jersey bull calves and all the oocyst counts were within normal ranges, 16 to 1447 oocysts per gram of faeces (OPG; Makau *et al*., 2017; Lopez-Osorio *et al*., 2020). The addition of either an EO compound blend or monensin to the diet of the bull calves had no significant effect on the number of oocysts present ($P = 0{,}214$).

Rumen papillae density, PL and PW were less than indicated in literature for calves aged 60 days (Ragionieri *et al*., 2016). It can be theorised that high frequency of disease may have had an effect on overall nutrient absorption and therefore subsequent growth and rumen development (Baldwin *et al*., 2003). Furthermore, the diet had no effect on rumen papillae density, PL or PW.

***Antibiotic resistant* E. coli**

E. coli is abundant in soil, water and the GIT of all livestock (Amin and Seifert, 2021; Formenti *et al*., 2021; Martínez-Vázquez *et al*., 2021) and can transfer antibiotic resistance elements (genes) to other gram-negative bacteria (Alarcon and Omenaca, 2004). It was therefore regarded

Figure 2: Representation of the Kirby-Bauer disc diffusion susceptibility test results of all isolates from South African dairy calves (Jersey and Ayrshire) fed one of three diets.



as a representative indicator of antibiotic resistance of gram-negative bacteria in this study (Gregova and Kmet, 2020).

There was no diet influence on the prevalence of *E. coli* in the faecal matter of the calves ($P = 0{,}09$). Furthermore, 32% of the isolates were resistant to at least one or more of the antibiotics tested in the study, 10% indicated intermediate resistance, and 58% were susceptible to the antibiotics tested (sulfamethoxazole-trimethoprim, chloramphenicol, ampicillin, tetracycline, ceftazidime and streptomycin).

Figure 2 provides a complete summary of the antibiotic resistance profiles across all the isolates that were tested against antibiotics commonly used in the livestock sector. Isolates from the CON diet group displayed the most resistance to streptomycin (51%) followed by tetracycline (35%) and ampicillin (31%). The EOC diet resulted in a similar resistance pattern, but a slightly increased resistance to tetracycline (54%) and ampicillin (48%) were observed from the MON diet group.

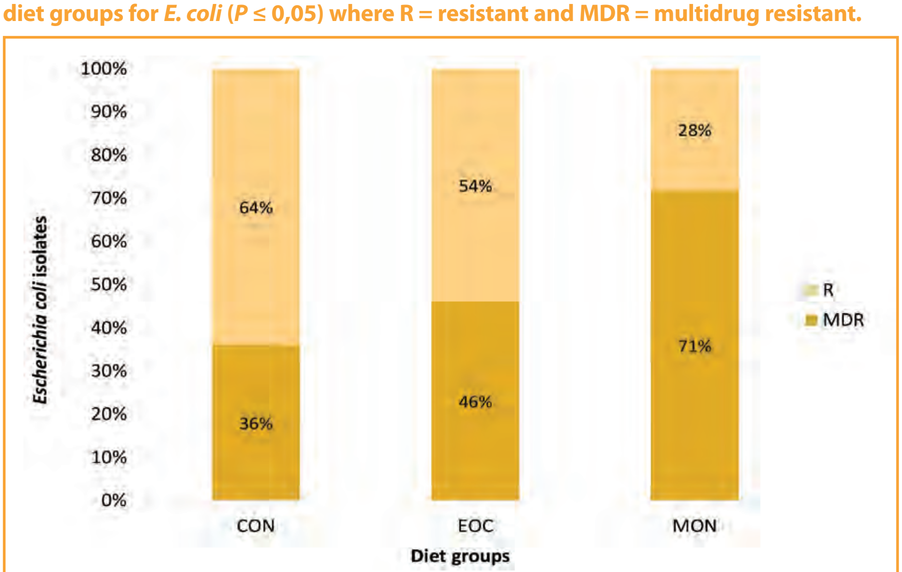
Multidrug resistant E. coli

Antibiotic resistance classification considers the level of resistance of ABR isolates, where multidrug resistant (MDR) isolates are resistant to three or more different classes of antibiotics. To further understand the impact the diets may have on antibiotic resistance, only the resistant *E. coli* isolates were further observed for multidrug resistance.

An increase in MDR *E. coli* ($P = 0{,}02$) was observed for the MON diet compared to the EOC and CON diets (*Figure 3*). These findings suggest that even though no increase in the prevalence of general ABR *E. coli* were found, isolates from the MON fed group did indicate resistance to more classes of antibiotics than the EOC and CON diet groups.

This contradicts previous research with the theory that subtherapeutic use of antibiotics have no effect on antibiotic resistance (Thames *et al*., 2012), but rather supports the current hypothesis that ionophores included in calf starter diets result in increased antibiotic resistance of *E. coli*.

Figure 3: Averaged antibiotic resistance classification of the dairy calves across three diet groups for *E. coli* ($P \leq 0{,}05$) where R = resistant and MDR = multidrug resistant.



Conclusion

Overall growth and health benefits associated with either a diet containing monensin or a blend of capsaicin, carvacrol, cinnamaldehyde and garlic extract were similar. No benefits were associated with either diet as no decrease in *Eimeria* spp. oocysts, and no increased rumen development could be linked to any of the diets. However, increased multidrug resistant *E. coli* was associated with a diet that contains monensin, which further confirms the theory that ionophores do contribute to the increased burden of antibiotic resistance at farm level.

Michelle Gouws formulated and executed this article as part of her MScAgric research thesis. Dr L Steyn was responsible for the supervision and development of the research. Gouws graduated with her MScAgric Animal Sciences degree in March 2023, and is currently pursuing a PhD in Agriculture at Stellenbosch University.

References available on request.
For more information, send an email to Michelle Gouws at 19903812@sun.ac.za.