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Animal Feed Manufacturers Association

Quarterly magazine of the Animal Feed Manufacturers Association

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High stakes in the animal feed industry

By Anina Hunter, chairperson, AFMA

Animal feed is one of the most critical components of successful animal production, yet it is also one of the most challenging areas to manage. There are many risks involved when providing feed to animals, ranging from nutritional imbalances to environmental contamination of feed and feed utilisation due to the animal's health status. These challenges have a direct impact on production efficiency, livestock health and welfare, and can affect the profitability of the animal's performance.

Despite these challenges, the South African animal feed industry is well equipped with world-class knowledge and facilities to overcome obstacles and provide the correct balance and feed nutrition to livestock.

The biggest challenges the industry is facing are critical infrastructure limitations, high commodity prices and disposable income available in the market. These challenges limit industries and their ability to pass on the additional costs to consumers, while ensuring affordable food and food security.

Anina Hunter.

Failing to supply the basics

One of the hottest topics in South African news of late is the 'energy crisis' debacle. This does not only impact electricity – it also affects water supply to producers and halts operations if not available. Producers cannot successfully operate during periods without load shedding if they experience water restrictions. This results in further downtime, adding to producers' costs.

These pressures are further exacerbated by an aging water supply system – waterpipes, -lines and other infrastructure have not been maintained over the years, with water losses of up to 60% in some cases.

Skyrocketing commodity prices

The rising prices of animal feed commodities is another challenge that adds to the animal feed industry's list of woes. There has been a steady increase in the prices of these commodities, driven by the increasing demand for meat, dairy and eggs. Higher prices directly affect the cost of animal production that, up to now, could not be passed on to the consumer in full.

As the prices of animal feed commodities rise, production costs follow suit. This increase in costs can lead to decreased profitability and increased strain on livestock producers.

South African companies have been relatively successful in buffering some of the impact of high commodity prices through least-cost formulations and lower density diets while maintaining animal production on the farm. The challenge is to develop feeding strategies that are tailored to the individual needs of animals, ensuring that their nutrient requirements are met, and that companies remain profitable.

This can only be achieved if the best quality feed is supplied to producers – this is where producers and manufacturers of raw materials play a pivotal role.

Feed safety first

Producers and manufacturers of raw materials must focus on their product quality to ensure proper management and elimination of environmental feed contamination. Contaminants such as mycotoxins, heavy metals and chemicals can enter animal feed through feed ingredients, processing and storage. These contaminants can have a negative impact on animal health, performance and production efficiency. To mitigate the risks associated with environmental contamination, producers must ensure that their farming and manufacturing practices and storage facilities are of the highest standard.

The availability of electricity, water and raw materials are crucial in the feed-to-food value chain, and any restrictions can lead to animal welfare problems, ultimately leading to food security challenges and malnutrition in the South African population. (It is a fact that the average South African child does not receive adequate protein to ensure normal brain development.)

Some relief in the works

The budget speech addressed some relieve strategies government has put in place to lighten the burden. Among these are the Eskom debt relief to ensure capital is utilised for generator maintenance, diesel refunds, tax relief for the installation of renewable energy, focus on water infrastructure and road maintenance.

Government plays a critical role in ensuring food security and for this reason the challenges must be overcome as soon as possible. I hope this is not too little too late, but rest assured that South Africans are resilient and will find ways to be a beacon in the darkness.

For more information, contact the author at email Anina.Hunter@rclfoods.com.




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NEWS & views

AFMA FORUM
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SAVE THE DATE: AFMA Forum 2023
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 mark the date in your diary to be part of this highlight on the annual agricultural calendar. Watch the press for details.

Slowing broiler growth with nutritional interventions

According to the National Chicken Council, the United States poultry industry processed over 9,22 billion broilers in 2020. To accomplish this, the poultry industry relies on a vertically integrated production system to ensure the seamless flow of poultry products from the breeder farm, through the hatchery, to the broiler farm and on to the processing plant.

Problems arise when disruptions occur in the supply chain. Modern commercial broilers are fast growing, averaging 60g per day over the course of the production period with maximum growth occurring three weeks post-placement.

Some role-players have, however, received requests for nutritional and feeding interventions to slow broiler growth. The degree or magnitude of broiler growth slowdown will be driven by the severity of the disruption keeping the birds from moving off the farm to the processing plant. The steps needed to slow broiler growth by one or two varies greatly compared to those needing to slow growth by as much as six or even ten days.

The primary goal when applying a growth intervention strategy is to slow growth and maintain carcass quality without adversely affecting bird health and elevating mortality. However, these interventions may have an impact on carcass and breast meat yield. – *Poultry World*

Fish welfare legislation urgently needed

Fish welfare legislation is urgently needed to protect farmed fish from cruelty, says Compassion in World Farming. A new scientific study published recently shows that the number of farmed fish slaughtered globally has risen dramatically over the last 12 years – from 61 billion fish in 2007 to 124 billion in 2019.

The total number of fish farmed is likely to be much higher because many die during rearing. The study highlights that most farmed fish (70 to 72%) have no legal protection at all and less than 1% have any species-specific legal protection at slaughter. – *Press release, Compassion in World Farming*

Bobby Lawrence.

Kemin welcomes two new faces

Dr Daniël van der Merwe (Pr.Sci.Nat. 122652) was recently appointed technical service manager for ruminants at Kemin Industries. He holds a PhD in animal science from Stellenbosch University where his research is focussed on modelling the production characteristics of feedlot lambs.

Bobby Lawrence has been promoted to national sales manager, ruminant essentialities. He is known for his hands-on approach to the ruminant industry and extensive on-farm experience, which includes working alongside producers and nutritionists alike. – *Kemin press release*

Dr Daniël van der Merwe.

Novus acquires Agrivida

Global animal health and nutrition company, Novus International Inc, recently announced its acquisition of biotech company Agrivida Inc. Through the purchase, Novus takes ownership of the proprietary INTERIUS™ technology Agrivida developed to embed feed additives inside grain. This move by Novus comes two years after it began a commercial partnership with Agrivida to support the sale of the start-up's flagship product, GRAINZYME®.

Says Novus president and CEO, Dan Meagher: "With this technology we believe we can revolutionise the feed additive industry through the expression of high-value, functional proteins inside grain, providing new products that are sustainable, both environmentally and operationally."

The acquisition includes Agrivida and its operations in Massachusetts and Nebraska. The Agrivida team, including founders R Michael Raab and Jeremy Schley-Johnson, will join Novus, bringing with them their innovative approach to developing functional proteins. – *Press release, Novus*

Probiotics in feed market set to expand

The global probiotics in animal feed market are likely to benefit from the increasing awareness regarding animal health and welfare. The market was valued at US$3,56 billion in 2018. It is predicted that the market will reach US$6,24 billion by 2026, thereby exhibiting a CAGR of 7,30%.
The benefits of probiotics in animal nutrition have created a high demand for these products across the world. The growing emphasis on animal health and welfare and the increasing trend of commercial livestock farming have led to the availability of new products in the marketplace. The growing focus on research and development in probiotics to substitute antibiotic growth promoter feed additives has led to the increasing popularity of probiotics in animal feed.

The use of probiotics in poultry farming and breeding is expected to enhance the productivity and performance of feed products. The report states that the rising uptake of probiotics over various domains will aid the growth of global probiotics in the animal feed market in the coming years. – *Fortune Business Insights*

BASF and Cargill to expand partnership

BASF and Cargill recently announced their expanded co-operation, adding the United States to their existing feed enzymes development and distribution agreement. Together, the two companies are committed to bringing innovative enzyme-based solutions to the market, generating distinctive value for animal feed customers. By combining the enzyme research and development strengths of BASF with Cargill's knowhow in application and broad market reach, the partners will form a joint innovation pipeline for animal protein producers.

With the expanded geographical reach, BASF and Cargill aim to bring the voice and commercial insights of US protein producers to craft the next generation of enzymes jointly.

In 2021, BASF and Cargill moved the relationship beyond pure distribution agreements into the joint development of new enzyme technologies and applications. This extended partnership builds upon the successful go-to-market collaboration between the companies across Argentina, Brazil, Mexico, Portugal, Spain, the Middle East and Africa. As part of the partnership, BASF and Cargill will co-develop, produce, market and sell customer-centric enzyme products and solutions. – *BASF*

Growth in South African poultry slaughter capacity

Despite major global and local challenges, the South African poultry industry has been able to improve its slaughtering capacity by 15% from 19,5 million broilers per month to 22,5 million broilers per month over the past three years. Over the same period, the industry was able to grow from R56 billion per annum to R59 billion per annum.

"This is fantastic, because the sector's growth remained stagnant for a decade before the signing of the Poultry Sector Masterplan in 2019," Izaak Breitenbach, general manager of broiler division of the South African Poultry Association (SAPA), told journalists during a virtual meeting. The significant growth rate was mainly due to investments into the industry.

"The master plan had called for an investment of R1,5 billion to be concluded by 2022. However, South Africa's poultry industry has already invested R1,8 billion, with an additional R600 million earmarked to be invested by the end of 2024."

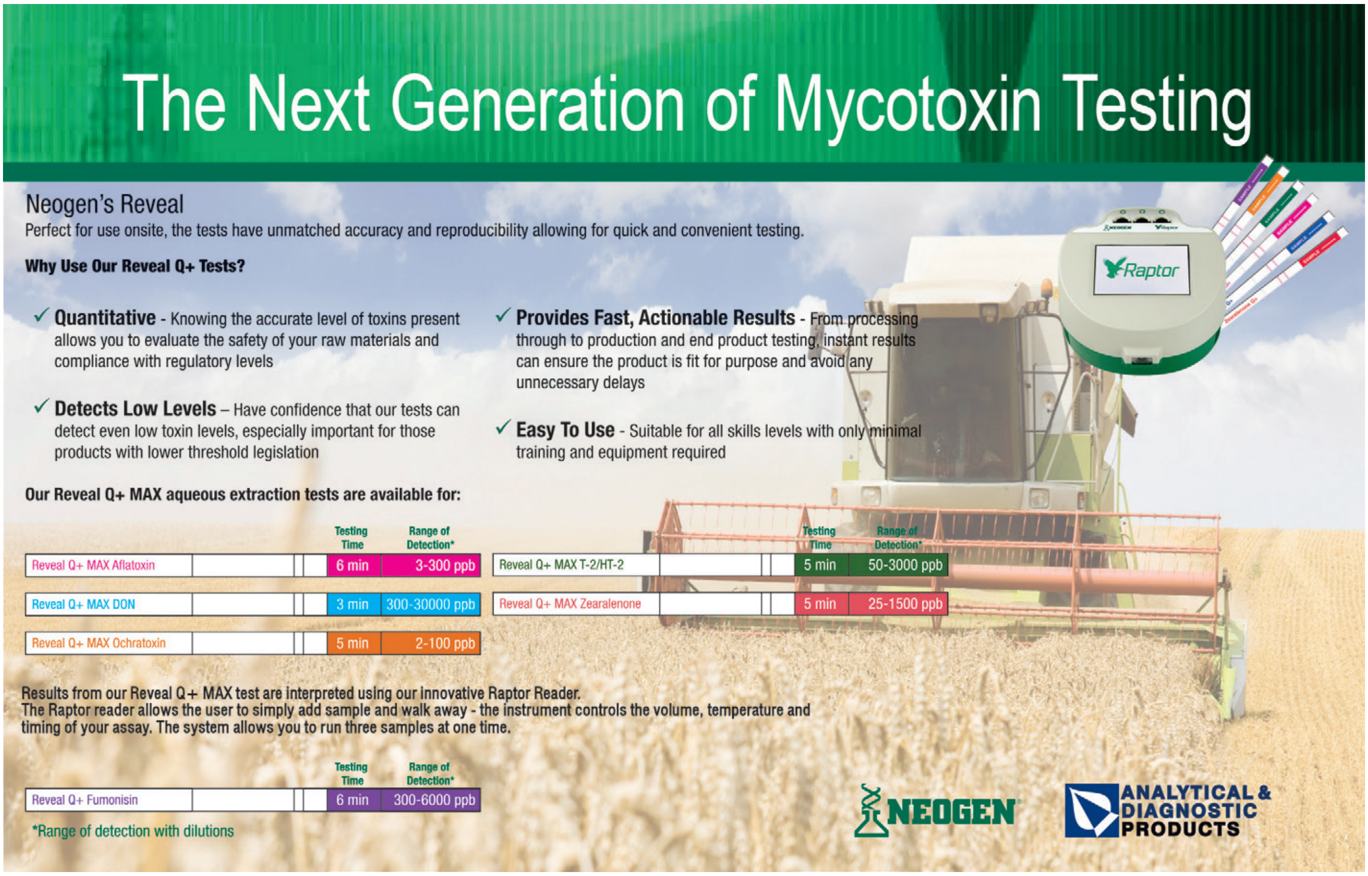
While SAPA was confident that the long-term outlook for the poultry sector was very good, the short-to-medium-term forecast was rather bleak, Breitenbach added. – *Susan Marais, Plaas Media*

Evonik and Dr Eckel to co-operate

Evonik and Dr Eckel Animal Nutrition have entered into a partnership effective 1 January 2023. This agreement will enable Evonik's animal nutrition business line to expand its product portfolio in the gut health solutions area to include the phytogenics product class. Evonik plans to launch a first product from the partnership with Dr Eckel in Europe in the first quarter of 2023.

Phytogenics are plant-based feed ingredients which play an important role in alternative solutions to antibiotic use in livestock farming. The use of antibiotic growth promoters in animal feed has been banned in the European Union since 2006.

"It often needs complex solutions to maintain health and productivity in the barn without antibiotics," says Gaetano Blanda, head of the Animal Nutrition business line at Evonik. "In combination with our existing gut health solutions, phytogenics will help farmers achieve this goal." – *Press release, Evonik* ❖



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Proposed regulation amendments regarding maize intended for sale in South Africa

By Lucius Phaleng, trade advisor, AFMA

The grading of maize products is regulated by the *Agricultural Product Standards Act, 1990 (Act 119 of 1990)* which provides for the standardisation of quality norms. These norms include quality attributes, packaging, marking, and labelling.

Grading standards are intended to ensure fair business practices and a competitive marketing environment for grains and oilseeds. Fair and competitive trade practices are fostered by the right application of grading standards, to the overall benefit of customers and agricultural business.

The purpose of the said regulations is to, inter alia, set out norms and standards according to which compositional properties, quality standards (classification) and requirements relating to packaging, grading, and labelling of maize intended for sale are to be inspected by the designated assignee. The grading regulations ensure reliable and accepted descriptions of crop quality and value and promote economic health and prosperity in agriculture.

Notice for public comments

In October 2022, the South African government invited public comments on the proposed regulations and amendments relating to the *Agricultural Product Standards Act: Regulations: Grading, packing, and marking of maize intended for sale in South Africa.*

The public notice came after the maize forum submitted the proposed revised regulations on behalf of the grain industry. All interested parties were invited to submit written comments and representations concerning the proposed revised regulations.

Consultation process

After publication of these proposed regulatory amendments, AFMA engaged in extensive member consultations and strongly maintains its duly communicated stance forwarded to the Department of Agriculture, Land Reform and Rural Development (DALRRD), stating that it does not support proceeding with the amendments.

It was made clear that the current regulations should remain in place until the maize industry has collectively and objectively identified the changes that are best for food safety and quality throughout the entire value chain.

The production of mycotoxins in water-damaged maize kernels was the main concern in this regard. Few studies exist on co-occurring water damage, discoloured kernels, and fungi resulting in multi-mycotoxins contamination (Giorni *et al*, 2019). Therefore, it is critical to investigate the effect of water-damaged and discoloured kernels on mycotoxin contamination as it might pose health risks to maize consumers and lead to economic losses.

The study conducted by the Southern African Grain Laboratory NPC (SAGL) was limited as it is silent on how the materials were sourced from undisclosed areas and no mycotoxins test was conducted on either water-damaged or discoloured kernels. In addition, yellow maize was excluded from the investigation because it could not be sourced.

The way forward

Considering the reasons advanced by all stakeholders in favour of and against proposed amendments, AFMA believes that cognisance should be taken of the potential threat of mycotoxins as a result of the proposed changes. Also, further research must be conducted considering, inter alia, the representativeness of samples and cooking quality of maize products derived from water-damaged and discoloured kernels.

Given the aforesaid, it was decided to completely withdraw the proposed amendment until such time as the industry has collectively engaged and consensually agreed on the new proposed amendments to the grading regulations. Furthermore, no dispensation will be entertained flowing from the discussions.

For references and more information, email the author at trade@afma.co.za.

Sustainable livestock production: Challenges and impact on the feed sector

By Tony McDougal, All About Feed

Feeding livestock in harmony with circularity supports the environment and climate protection, according to a leading academic.

Prof Wilhelm Windisch, past chair of animal nutrition at the Technical University of Munich School of Life Sciences, challenged the myth that livestock was considered by many to 'pollute the environment' and 'compete with humans for food'.

Prof Windisch told the *Shothorst Feed Research* webinar that he didn't want to fight against these narratives, but to look at the myth of animal feed being unsustainable.

The world's agricultural area is shrinking rapidly – down from 3 800m^2 in 1970 to a predicted 1 500m^2 metres by 2050. This was prompting some to call for a halt on livestock production, which Windisch described as an interesting question, as he highlighted that agriculture produces biomass that is non-edible for the most part – grassland, crop rotation (clover) and co-products (straw).

Large swathes of grassland cannot be converted to arable land producing vegan food because:

- The climate is too humid or dry and/or there is a short vegetation period.
- The topography is not conducive to livestock (i.e. it is too steep or floods easily).
- Conversion would release dramatic amounts of CO_2.

Non-edible biomass

Absolute grassland covers major proportions of the total agricultural areas including 40 to 70% of Alpine regions and around 30% within Central Europe. So, in future it will be important to make the best use of non-edible biomass.

One kilogram of vegan food generates at least 3 to 5kg of non-edible biomass, containing a large amount of plant nutrients (nitrogen and phosphorus). Among the strategies to recycle, plant nutrients were put directly back into the soil, but this was inefficient and produced high emissions; fermentation to biogas with residues kept as storable fertilisers for precision farming or feeding to livestock.

Manure is a storable fertiliser and is much more efficient, he said, and represented a win-win situation as it also produces food. "We can produce food without competition to humans."

High-quality food protein

"Livestock delivers high-quality food protein and kilocalories from a given agricultural area equivalent to 50 to 100% of primary vegan food without food competition, solely from circulation of inevitably occurring, non-edible biomass, simultaneously delivering fertilisers and promoting vegan production."

Carbon footprint of bread

Windisch touched on the trade-off between emissions, efficiency and food competition, pointing out that the carbon footprint of protein from bread was higher than for chicken and eggs and on a par with pork when looking at per gram of protein.

Feed from non-edible grassland

He said it was important to look at what kind of land was used. Poultry CO_2 equivalent was derived from feed from arable land, but were also the strongest food competitors. Beef, which has the highest carbon footprint due to the high quantity of methane produced, had feed from non-edible grassland.

But while "feeding ruminants just seems to be inefficient and dirty, at absence of food competition, however, it is the most efficient way to make use of non-edible grassland."

Methane and CO_2 emissions

One issue that causes problems is methane production, although there are signs (Kuhla *et al.,* 2022) that current ruminant head counts and their methane emissions have already fallen below the pre-industrial level in Germany.

While it is a strong greenhouse gas, it is quickly degraded with a half live within eight years. "At constant head counts, ruminants do not increase atmospheric CH_4 concentration and hence do not heat up the atmosphere. Carbon dioxide is extremely stable and accumulates in the atmosphere. Once emitted from fossil fuel energy sources, it does not stop heating up the atmosphere."

He suggested that agriculture should:

- Look to stop using fossil energy use on farm and switch to ruminants.
- Build up carbon sinks such as faster grassland, clover/alfalfa and agro-forestry.
- Maintain ruminant production at minimised methane burdens.

Rise in emissions

Abstinence from livestock feeding, he argued, does not relieve the environment or climate; it destroys food delivered from livestock for free and forces the doubling of 'vegan' harvests on limited arable land, which in turn would prompt a severe rise in emissions per unit of nutrients.

Concluding he said, the impact of livestock feeding on the environment and climate had two steps:

- **Feeding within circularity:** Fully sustainable, but limited and low-input production capacity.
- **Feeding through food competition:** A burden to the environment and climate but highly productive.

Article courtesy of *All About Feed*. Visit *www.allaboutfeed.com* for more information.

Safe use of food by-products and biowaste in the feed production chain

By LWD van Raamsdonk, N Meijer, EWJ Gerrits and MJ Appel

Reuse or recycling are some of the essential retention options recognised in circular economy approaches. An attempt to embed the linear feed and food production chain in the general concepts of sustainability and circular economy revealed common elements as well as principally different issues between feed/food and non-food strategies.

Regrading of former food products as feed ingredients is an important, if not essential, development in reducing large volumes of biowaste, and to achieve a footprint as small as possible for animal husbandry. An analysis of legal requirements provides opportunities as well as legal restrictions for reusing former food as feed.

The main focus is on European Union (EU) legislation, since this system is among the strictest legal frameworks globally. The specific issues include feed and food safety, possible adverse effects of strict loop closing, the frame of the biological background and legal requirements. Dedicated concepts are developed for reaching solutions.

Feed and food safety covers four domains including biology (e.g. prions, viruses), chemical compounds (e.g. pesticides, antibiotics, heavy metals, dioxins), microbiology (pathogenic bacteria and viruses, zoonoses), and physical objects (e.g. microparticles, packaging material). Physical hazards should receive extensive attention for the frequent presence of packaging material in former food products.

Legislation should allow and encourage innovations and technologies for regrading of by-products of the feed and food production chain. The WISE principle (witful, indicative, societal supportive, enforceable) for legal developments should be used for optimising the relationship between the legal framework, assurance of feed safety, and support of necessary innovations. Biological principles will add considerably to the concept of circular bioeconomy.

Technological innovations for upgrading biowaste and former food products will result in suitable feed ingredients. The evolutionary distance between animals and their biological needs should be part of the design of strategies and of the legislative process.

The requirement of circularity for production and usage loops should be applied diversely. The approach of food webs as found in nature should be explored for feed and food production. Genetic distance among species in loops or webs can be used as guidance for route diversification.

Footprint and food security

Agriculture in the current era is challenged by two major incentives: minimisation of the ecological footprint and maximisation of the food security for the global human population. In both cases, a broad interpretation should be applied for these issues. Food production includes the feed production chain, and it should be subjected to safety for a large diversity of chemical and physical compounds, hygiene requirements and to sufficient nutrition.

The ecological footprint differs widely among the different types, procedures and geographic regions of food production. Livestock husbandry ranges among the most disputed production chains of human food because of the large ecological footprint, and for the production of waste, including greenhouse gasses, manure and slaughter by-products (Van Kernebeek *et al.*, 2016; Springmann *et al.*, 2018). At the same time, cattle husbandry is for a range of cases the most obvious way of land use, most notably for marginal regions which are predominantly suitable for vegetable biomass production by grasses (Squires *et al.*, 2018).

An option is to connect both issues, by converting the by-products of food production with a major share in the ecological footprint to valuable feed products (Waarts *et al.*, 2011). Waste reduction is one of the measures used to improve the sustainability of food production (Springmann *et al.*, 2018). This option is a basic principle for the initiatives to achieve a circular bioeconomy as far as food production is at stake (European Commission, 2008).

Legal framework

At EU level, production and use of animal feed are regulated in *European Commission, 2002 (General Food Law)*. Article 3, paragraph 4 defines animal feed as "all substances and products, including additives, processed, partially processed or unprocessed, which are to be used for oral feeding to animals".

Further provisions are laid down in *European Commission, 2009*. Annex III of this Regulation provides a list of materials that are prohibited as animal feed (ingredient), including manure, construction and urban waste, glass, household waste, treated seeds, skins (leather) and wood, and packaging material. Products or by-products that are suitable for, and in practice used as ingredients in animal feed, are listed in the *EC Animal Feed Ingredient Catalogue (European Commission, 2013)*.

Ingredients of compound feed are in a diverse range of cases actual residual materials with a long history of application, predominantly originating from the food production chain: oil production (expellers, pulp), flour and starch (chaff, bran, groats, hulls, middlings), legumes (hulls, flakes), forage (straw, silage), beer or more general ethanol production (distiller's grains, solubles), bakery products (dough, over-date products), residual streams containing animal by-products or consisting entirely of it (slaughter by-products, food products which partly consist of animal products), and a number of smaller streams.

The intention of the *Feed Catalogue* is not to authorise the use of new ingredients or to prohibit the ones that are not included, but to facilitate that a listed ingredient complies with the definition as included in the catalogue and should be labelled appropriately. Waste is defined as materials for which the producer has the "intention to discard or is required to discard" (*European Commission, 2008*, Article 3, point 1).

Concepts of circular economy

Circular economy (CE) and its forerunners are concepts with a long history and a diverse interpretation (Cooper, 2011;

Table 1: Sports of the ladder of Moerman for valorisation of former food products, compared to three other classifications: retention options for non-food residual materials (Reike *et al.,* 2018), materials for insect rearing as mentioned by European Food Safety Authority, 2015, and categories of animal by-products in *Regulation (EU) 1069/2009*.

Action or purpose (ladder of Moerman)	Retention options (Reike et al., 2018)	Materials mentioned by EFSA (2015)	Regulation (EU) 1069/2009
Prevention	R0: Refuse R1: Reduce		
Alternative use for human food	R2: Resell, Re-Use		
Conversion to human food			
Use in animal feed	"Regrade"	Unused food products not containing meat/fish (B)	Vegetal
		Organic waste from e.g. gardening, wood (part of F)	
		Intended by-products (A)	Cat 3, subjected to specific legislation
		Unused food products containing meat/fish (B)	
Industrial use (biobased economy)	"R8: Recover (components)"	By-products from slaughterhouse (C)	
		Food waste from human consumption (D)	Cat 2
Processing for fermentation	R6: Repurpose	Animal manure, intestinal content (E)	Cat 2
		Organic waste from e.g. gardening, wood (part of F)	
		Human manure, sewage sludge (G)	
Composting	R7: Recycle		Cat 2
Sustainable energy	R8: Recover (energy)		Cat 1
Burning (incineration)			
Land fill	R9: Remine		

Olive shading: actions as directed to human food; pink shading: destinations of animal by-products within the scope of Directive (2008)/98/EU article 2 part 2b (general waste directive).

Whitney, 2015; Reike *et al.*, 2018). The report of the Club of Rome (Meadows *et al.*, 1972) could be considered a turning point in the discussion of the role of mankind in using, depleting and spilling of natural resources. Reike *et al.* (2018) recognised three phases in the evolution of CE concepts.

Up to 1990 the paradigm was to deal with waste in terms of reducing, reusing and recycling (CE 1.0). The emphasis was on the output side by principles such as 'polluter pays'. During the next evolutionary phase in the following two decades input and output of production processes were linked (CE 2.0). The aim was to achieve a win-win situation for both economy and environment (escaping the resource trap; see also Geissdoerfer *et al.*, 2017).

In this second phase, circularity was increasingly part of the strategies. Finally, the insight was developed that resource depletion should not limit economic growth. Therefore, during the years after approximately 2010, both new and revitalised older concepts are applied in the third phase of CE in order to achieve a decoupling of growth and environmental load (UNEP, 2011).

The production chain of feed and food can only be compared to any other (industrial) production process to a certain extent. Retention options in CE for optimising production chains and closing loops are frequently presented as cascades or ladders (Reike *et al.*, 2018). These options, up to ten in modern representations (*Table 1*), can be organised in three groups: client/user choices, product upgrade and downcycling.

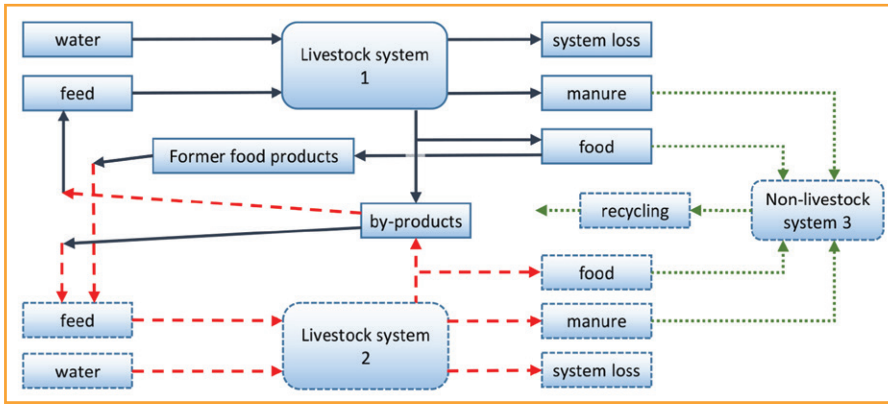
The client/user choices (refuse, reduce and resell) are comparable to the first three options in the ladder of Moerman (*Table 1*). At the lower end of the options, downcycling includes re-mine (landfill), recovery of energy, re-cycle and re-purpose ('re-wording' from Reike *et al.*, 2018). In contrast, the middle segment of the retention options, product upgrade, shows large conceptual differences between biological and non-biological types of products.

Relationship between species

In the view of the biological dimension of feed and food production, genetic distances between species, either consumers, producers or producer-consumer relationships, are being used in several ways. An example is the establishment of close relatives to human beings (e.g. 96% congruence between human and chimpanzee genomes) and the resulting choice of a representative model system for testing human susceptibility for diseases.

For example, macaque apes were used as animal model for testing the infectivity of chronic wasting disorder prions for their close relationship with humans (Race *et al.*, 2018).

Figure 1: Scheme of a livestock production system (1) with routes for recycling of food loss and animal by-products (blue straight arrows). Another livestock production system (2) can be used for creating a food web and preventing excessive accumulation of hazardous compounds (red dashed arrows). Other non-livestock systems (3), such as fermentation, can be connected as well (green pointed arrows). System loss is pointing to gasses and liquids which are leaked to the environment or collected for other, e.g. industrial, purposes.



Food webs, closed and local loops

The major aim of transferring a linear economy to a circular economy is to make closed loops for minimising the ecologic footprint, and assuring economic profit. Elements recovered from old batteries can be used for the manufacturing of new batteries. Automotive parts or materials can be used for the production of new automobiles. Metal alloys or glass can be melted to produce new metal or glass products.

In these and comparable situations recycling, recovering and reutilisation do not principally pose additional risks compared to the use of virgin materials. However, installing this type of closed short loops in the feed and food production chains could result in additional risks because of the possible short genetic distance between producing and consuming animals, or even lack of any distance. The use of ruminant slaughter by-products in the feeding of ruminants in the United Kingdom resulted in an epidemic of mad cow disease (Prince *et al.*, 2003).

A short loop will result in re-entering food waste in the same production system. In a general sense, such short loops or reuse of resources will have the potential hazard of accumulation. It is therefore recommended to apply principles of food webs and 'route diversification' of materials in the production of feed and food (*Figure 1*).

Local use of by-products can be an instrument for circular agriculture. After a period of economic growth and the correlated need to maintain or enhance profits by achieving international trade, a movement into the opposite direction occurred in the last decades. In the view of a decrease in urban farming in Europe and North America, and an increase in other regions of the world, a proposal for exchange of urban farming technology was published in 1980 (Smit, 1980). Since then, small-scale farming or urban farming is a developing part of the entire sector of agricultural production.

Technical solutions for conversion

The current EU legislation has basically two sets of options for processing animal by-products when reusing as a feed ingredient is prohibited. These are fermentation or composting by organisms with a position at the lower end of the biological classification (bacteria, moulds), or non-biological chemical processing.

In general terms of retention options, these can be indicated as repurposing or recycling (*Table 1*).

Insects are only distantly related to the classic vertebrate farmed animals (ruminants, pigs, poultry). Nevertheless, the restrictions in feeding of insects are currently fixed according to the legal principles applying to these conventional livestock animals. It is, however, part of the nature of specific insects to extract nutrients and energy from sources which are currently prohibited or strongly restricted for conventional livestock animals as a feed material.

Heat sterilisation

Processing of animal material is a general way to eliminate or minimise food and feed safety issues. The severity and extent of the processing procedures can vary, depending on the material, intention of the treatment and on the animal species (-group) intended for consumption. Sterilisation can aid to the safety of former food products (FFPs).

European Commission, 2011, Annex IV provides a range of seven described procedures for sterilisation intended to deactivate prions. The most frequently applied procedure, which is obligatory for ruminant material include heating at 133°C during 20 minutes (Annex IV, method 1).

Hydrolysation, fermentation

Processes of hydrolysation of proteins are mentioned in legislation in two different ways. These procedures include alkaline or heat treatment (*European Commission, 2011, Annex IV*), and treatment with acid or enzymes (*European Commission, 2013*). In contrast to acid, alkaline or enzyme processes, the sole treatment with heat, as applied to feather meal, is primarily meant to cut the S-bonds in the tertiary structure of a protein in order to enhance digestibility, without shortening the chain length.

A specific type of biological conversion is fermentation. A range of fermented products exist of e.g., rice, wheat, grains or cereals in general, soya, sugar beet, potato, milk and the micro-organisms itself. Fermentation is not mentioned in the animal by-product regulations for the production and use of feed materials, as a range of other processing methods. Fermentation is mentioned as part of the process of biogas production (*European Commission, 2011, Annex IV, Chapter IV, Section 2 [C], part 2 [e]*). An example of fermentation is the enhancement of the digestibility of straw, a by-product of the production of cereals.

The use of catering waste, kitchen waste and household waste is prohibited in the feed production chain as mentioned in several *EU Regulations*. Most notably the risks on transfer of food-and-mouth disease (FMD) and African swine fever (ASF) were associated with the feeding of catering waste ('swill') among other sources (Knight-Jones and Rushton, 2013; Halasa *et al.*, 2016). An overview of microbial health hazards of swill as a feed-stock is provided by Dame-Korevaar *et al.* (2021).

Future directions

Fruit and vegetable materials are primarily used for fermentation (Van Raamsdonk *et al.*, 2011). One of the opportunities of urban farming is to use food-grade products such as bakery, vegetables and fruits from local shops for livestock feeding in urban farms, under the assumption that these products will be discarded exclusively for economic reasons and avoiding safety issues.

Another development with possible future consequences is the increasing availability of novel foods (in the context of *European Commission, 2015*), which might result in new types of by-products. However, from a biological perspective, these novel foods will be based on known nutritional needs, which means that they will consist of proteins, fatty acids, carbohydrates, vitamins, minerals and other micronutrients, and recognisable units based on mixtures of these components such as viruses.

As stated, safety is a cornerstone for the production of feed and food, reflected in a range of legal measures. If by-products, complying to feed standards and with sufficient microbiological conditions do not contain unauthorised animal proteins, use as feed ingredient is legalised.

Concluding remarks

The strategies outlined in this article would allow certain materials to be shifted towards higher levelled sports at the ladder of Moerman – after proper conversion (*Table 1*). The WISE principle is intended as framework for the development of legislation by addressing several requirements (Van Raamsdonk *et al.*, 2017; Van Raamsdonk *et al.*, 2019): witful (reasonable legal principles), indicative (clear limits between prohibition and authorisation), societal supportive (public health, environment, economy), and enforceable (presence of suited monitoring methods).

Comparable principles were discussed by Robert Maxwell, as part of the risk evaluation of bovine spongiform encephalopathy (BSE) in the UK (see Randall, 2009). The issue of how to manage and improve the relationship between policy, politics and science was specifically named (see Randall, 2009: p 78). The existence of a legal framework meeting the societal demands, or to be more precise, which facilitates new technological opportunities, with continuing assurance of feed and food safety, needs to be optimised (Thieme and Makkar, 2017).

If safety assessments of new opportunities do not result in approval of a higher valued use, new assessments of the regraded products should be planned in the future, e.g. within five years, in order to keep pace with the rapidly evolving technological innovations. A 'safe-by-design' approach, in which awareness of food and feed safety is implemented throughout the development of new products, should be employed to curtail monitoring costs to check for compliance.

The balance between 'environmental, economic and safety' aspects should be included in such assessments (Barros *et al.*, 2020; Focker *et al.*, 2022). In general, information extracted from the biological fundament of our feed and food production chain, such as the position of animals and feed materials in biological classification, and information from food webs for designing loops for recycling, is vital for a sustainable feed and food production.

Smart processing systems, targeted at either achieving sufficient safety of by-products or extracting and optimising specific parts (fatty acids, proteins), are necessary for a circular agriculture.

This article has been shortened for publication in *AFMA Matrix*. For enquiries or a list of references, email nathan.meijer@wur.nl.

Let nature bring coccidiosis to its knees: Natural coccidiosis management in poultry

By Marthie Nickols, poultry development manager, Vitam International

Protozoal infections such as coccidiosis are widespread in livestock production. They cause huge economic impairment across the poultry industry, amounting to more than $3 billion annually.

A single-celled parasite, *Eimeria*, causes coccidiosis in chickens. There are nine species in total, five of which are economically most important: *E. acervulina, E. maxima, E. tenella, E. necatrix* and *E. brunetti*. The main route of infection occurs through the ingestion of sporulated oocysts, most commonly via faecal-contaminated feed. The single-cell protozoan parasites may cause damage to the bird's intestine, impairing nutrient absorption and productivity.

The severity of the disease varies considerably and factors influencing it include pathogen characteristics, the number of ingested oocysts, host susceptibility, age, and immunity. Housing conditions are also of importance, e.g. higher stocking densities can increase the number of oocysts shed and the rate of transmission.

Infection and disease onset

The period from ingestion to shedding of new oocysts is four to seven days. Clinical signs are seen from four days post-ingestion. Coccidial oocysts are exceptionally environmentally stable and can remain infectious for over a year, making the pathogen additionally problematic.

Diagnostics depend on the species of *Eimeria* involved. Post-mortem examination can be used to identify clinical symptoms such as gut lesions. Secondary infections such as necrotic enteritis usually cause mortalities. Sub-clinially infected birds would be listless and have morbidity but no other clinical signs. It is the sub-clinical infection that causes major losses.

Chemical coccidiostats, ionophores, and vaccines are current prophylactics for coccidiosis, but they can come with major drawbacks: high costs and the emergence of pathogen resistance (even in a single broiler cycle). Inefficient on-farm management with traditional prophylactics and accidental coccidiostat changes by operations that change their feed company, increase the risk of resistance. Long-term solutions are needed.

Natural alternatives on the rise

Current coccidiosis prophylactic resistance necessitates new alternatives such as phytogenic feed additives. Saponins are diverse and occur in plants as secondary metabolites. They possess a wide spectrum of pharmacological effects including haemolytic, anti-inflammatory, anti-protozoal, antifungal, anti-bacterial, and anti-viral activities. However, not all saponins have the same biological activity, so choose wisely.

Saponins are usually found as mixtures from plant extracts or powders. Few extracts of saponin-containing plants are on the European Union Register of Feed Additives. Good quality products with consistent performance are important when considering phytogenic alternatives.

Recent studies and data have shown that saponin-rich plant extracts may be potent candidates to prevent *Eimeria* infestations. Saponins destabilise the protozoa's membrane and lead to cellular lysis. Resistance to saponin-rich products is low if the products contain the correct combination of various saponins.

Conclusion

The use of selected saponins represents an appropriate strategy for coccidiosis management and meeting the challenges of modern poultry production. Besides reducing the use of ionophore and synthetic coccidiostats and thus reducing the risk of emergence of drug resistance, supplementation of saponin-containing plant extracts shows beneficial effects on performance parameters in poultry.

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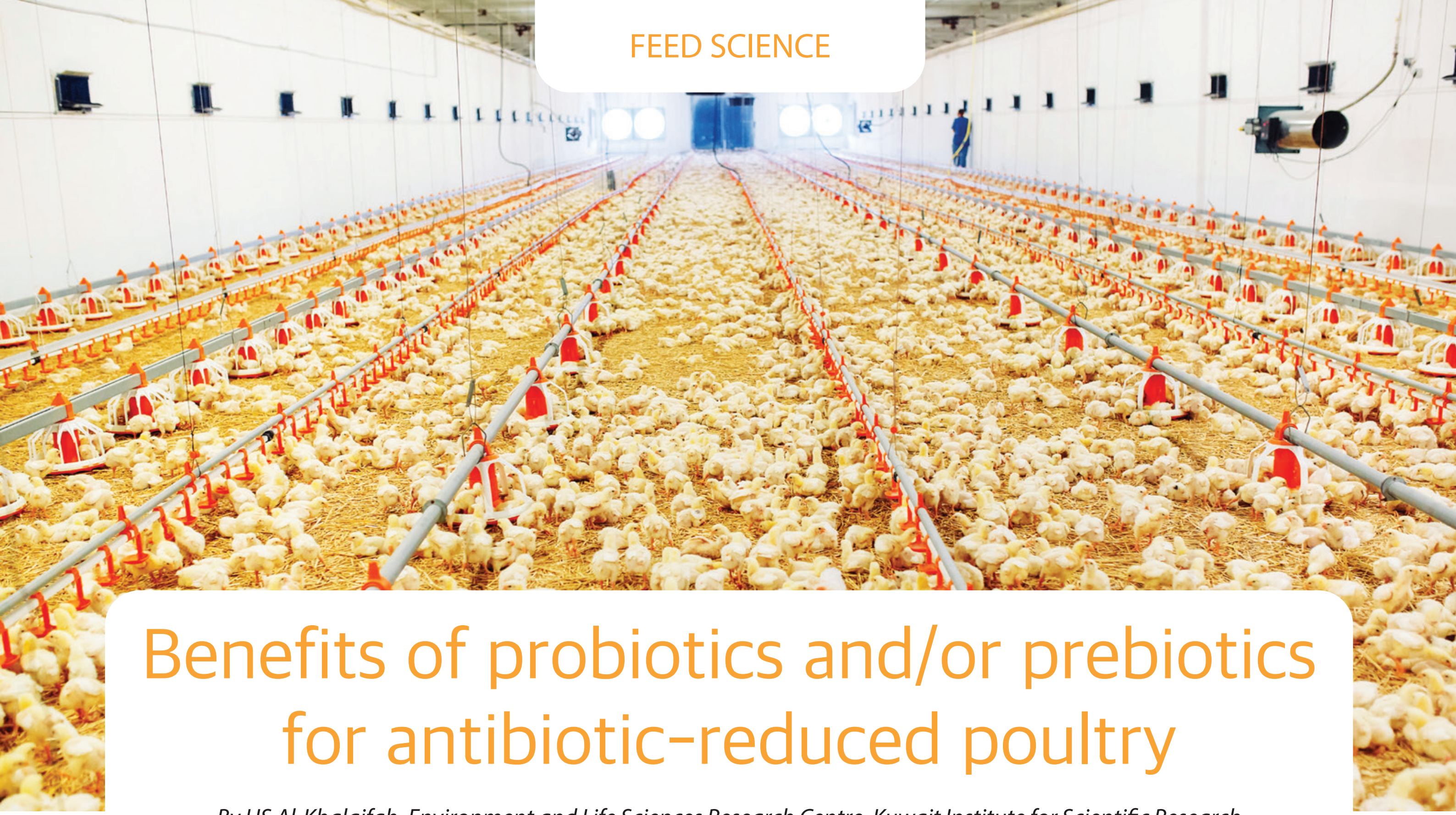

Benefits of probiotics and/or prebiotics for antibiotic-reduced poultry

By HS Al-Khalaifah, Environment and Life Sciences Research Centre, Kuwait Institute for Scientific Research

Over more than six decades dietary antibiotics have been used not only as a means to control infectious diseases, but also to improve growth performance and feed efficiency (Gadde *et al.*, 2018). The wider use of antibiotics as feed additives in the long run can contribute to the development of bacteria resistant to drugs used to treat infections which are of a potential risk if they are transferred to humans.

For this reason, the World Health Organization (WHO, 1997) and the Economic and Social Committee of the European Union (1998) concluded that the use of antimicrobials in food animals is a public health issue. The European Union banned the use of all in-feed antibiotics as growth promoters in 2006 (Castanon, 2007). In 2009, government agencies in the US such as the Food and Drug Administration (FDA) testified that the use of antibiotics for growth promotion should be eliminated (FDA, 2009).

However, probiotics have not been approved by FDA yet. Although safe and effective, this treatment cannot become the standard of care, nor can it be implemented in hospital formularies (Janvier *et al.*, 2013). Accordingly, the use of antibiotics has been minimised and replaced by effective dietary supplements such as probiotics and/or prebiotics that are claimed to enhance growth and positively modulate the immune response.

Benefit of probiotics and prebiotics

The inclusion of antibiotics as growth promoters was effectively banned in 2006, throughout the European Union, because some microbes developed resistance to these antibiotics (Europe Union Commision, 2005), and dietary probiotics and/or prebiotics have been used as immunomodulators and alternatives to antibiotics.

Probiotics and prebiotics may be classified as functional food; that which affects bodily functions in a positive manner so as to improve health, or if its effect extends to the physiological or psychological levels going beyond the traditional nutritional effect. Probiotics are live micro-organisms which, when administered in adequate amounts, confer a beneficial health effect on the host.

Lactic acid bacteria (LAB), *Bacillus* and *Bifidobacteria*, are the most common types of probiotics (Parvez *et al.*, 2006; Roberfroid, 2000). Prebiotics are non-digestible food ingredients that stimulate the growth and/or activity of beneficial microflora in the digestive system. Typically, prebiotics are carbohydrates (such as oligosaccharides), but they may also be non-carbohydrates.

Prebiotics are selectively fermented in the colon by beneficial bacteria such as *Bifidobacterium* and *Lactobacillus*. The probiotic bacteria exert competitive effect in the gut and produce bacteriocins, which have an antimicrobial effect on other bacteria (Patterson and Burkholder, 2003).

Effect on production parameters

There are many reports concerning the effect of using probiotics including *Lactobacillus*, *Bifidobacterium*, *Bacillus*, *Streptococcus*, *Pediococcus*, *Enterococcus*, and yeast such as *Saccharomyces cerevisiae* on the different performance parameters in chickens, including immune status and feed efficiency in broilers.

Bodyweight gain and increased feed intake as a result of dietary probiotics supplementation were reported. For example, Mountzouris *et al.* (2007), supplemented broiler chickens with a probiotics mixture at 1g/kg of feed from 1 to 42 days of age. The probiotic mixture included microbes that were isolated from the gut of healthy chickens. These included

Lactobacillus reuteri, Enterococcus faecium, Bifidobacterium animalis, Pediococcus acidilactici and *Lactobacillus salivarius*. The probiotic product had a total bacterial count, expressed as colony-forming units of 2×10^{12} CFU/kg of product.

It was shown that the dietary probiotics in the feed of broiler chickens significantly promoted growth performance. Similarly, supplementing broiler chickens with a probiotic mixture of *Bacillus licheniformis* and *Bacillus subtilis* spores at 0,05% of feed significantly improved the feed conversion ratio, compared to the unsupplemented control group, the supplemented probiotic contained $2{,}3 \times 10^8$ CFU/g *Bacillus licheniformis* and $2{,}3 \times 10^8$ CFU/g *Bacillus subtilis* spores in equal rates, at a level of 0,5g/kg (Midilli *et al.*, 2008).

In addition, *Bacillus coagulans*-supplemented broiler feed significantly improved final and daily weight gain, feed conversion ratio and survival rate, when compared to the unsupplemented control group, and is recommended to replace growth promoters in broiler production (Awad *et al.*, 2009; Cavazzoni *et al.*, 1998; Francesca *et al.*, 2010; Hume, 2011; Huyghebaert *et al.*, 2011; Kral *et al.*, 2012; Sohail *et al.*, 2011; Taheri *et al.*, 2010; Zhou *et al.*, 2010).

The beneficial effect of using prebiotics is well-documented in the literature. For example, Ammerman *et al*. (1989) investigated the effect of supplementing broiler chickens with 0,375% oligofructose on bodyweight gain and percentage carcass and breast weight. The authors reported that the supplemented diet produced heavier birds at 47 days of age, improved percentage carcass and breast weights, and reduced percentage fat pad, compared to the control group.

Immune response in poultry

Chickens reared under harsh environmental conditions such as high temperatures are subjected to immunological stress. Dietary probiotics and prebiotics have been used to modulate the immune response in poultry. Their effect may include immuno-stimulation, anti-inflammatory reactions, exclusion and killing of pathogens in the intestinal tract, and reduction of bacterial contamination on processed broiler carcasses. The balanced interaction between the intestinal microbiota, epithelium, and immune system provides resistance to enteric pathogens (Patterson and Burkholder, 2003).

Scientists from Nottingham Trent University fed 240 broiler chickens with different doses (200 and 800g/t) of a yeast-based feed supplement and monitored them every day for 42 days. Their findings suggest that the feed supplement containing a carbohydrate fraction antigen found in yeast resulted in the birds having greater natural defense to harmful bacteria entering their gut, and this effect could be age-dependent with younger birds having increased secretion capacity of mucin, a substance secreted by the gut lining, which can help defend against infectious agents (Lea *et al.*, 2012).

Probiotics were administered *in ovo* in broiler chicks with a mixed *Eimeria* infection, to study its effect on hatchability, performance, immune organ weights and lesion scores. They proceeded to inject 210 eggs with probiotic bacteria. Post-hatch, on the third day, half of the chicks were challenged with a mixed inoculum of *Eimeria*. Tissue samples and measurements were taken on days 3, 9 and 15. No significance was observed for hatchability, bodyweight, bodyweight gain or immune organ weights prior to *Eimeria* challenge.

However, the non-challenged birds, administered with the probiotics, showed significantly higher bodyweight, bodyweight gain or immune organ weights, whereas no differences were observed in the challenged groups. Also, birds receiving probiotics had lower mortality, with reduction in lesions. Overall, this is an indication that supplementation of probiotics in ovo may improve performance and offer immunity against infection (Pender *et al*. 2016).

Not always positive

The use of probiotics and prebiotics may not always provide a positive response in poultry production. In contrast to the afore-stated, laying hens and broilers infected with *Salmonella enterica serovar Enteritidis* (SE) were divided into groups fed control, probiotics, prebiotics and synbiotics. The results showed that laying hens and broilers fed probiotics and synbiotics did not influence SE infection (Murate *et al.*, 2015).

In a study on the digestive potency of the pancreas, cockerels of different ages were injected *in ovo* with a combination of probiotics and synbiotics. The results showed that the probiotics and synbiotics increased bodyweight but did not change the feed conversion ratio (Pruszynska Oszmalek *et al.*, 2015).

However, there are some studies in the literature showing no benefits of using probiotics and prebiotics in poultry production. For example, a total of 108 day-old commercial broilers chicks were divided into six groups: probiotic/prebiotic non-challenged, probiotic/prebiotic challenged, non-treated challenged and non-treated non-challenged. The study was conducted to test the protective properties of commercial probiotic (Lacto G) and prebiotic (Immunolin) on performance, lesion scores and immunological parameters in *Eimeria tenella* infected broiler chickens.

The results obtained showed that probiotic/prebiotic supplementation did not improve bodyweight or feed conversion ratio, lesion scores were significantly reduced, and to some extent the negative effects of coccidiosis was reduced (Abu-Akkada and Awad, 2015).

Proposed mechanisms by which the indigenous intestinal bacteria inhibit pathogens include competition for colonisation sites on the intestinal epithelium, competition for nutrients, and production of toxic compounds such as volatile fatty acids and bacteriocins or modulation of the immune system. The inhibition process may comprise one, several, or all of these mechanisms in a balanced way (Rolfe, 1991).

Carcass and meat quality

Studies on the modulatory effect of dietary supplementation with probiotics on the lipid profile in broilers are limited. Salma *et al.* (2007) reported that the dietary supplementation of bacteria (*Rhodobacter capsulatus*) could improve fatty acid profile in broilers. Yang *et al.* (2010) found that dietary *Clostridium butyricum* at $1{,}6 \times 10^{10}$cfu/g moderately decreased the ratio of n-6:n-3 fatty acids in breast muscles and increased eicosapentaenoic acid (EPA) and total n-3 fatty acids. In the same study, supplemented *C. butyricum* in the diet significantly reduced the shear force of broiler meat.

The supplementation of probiotics (*Bacillus, Lactobacillus, Streptococcus,*


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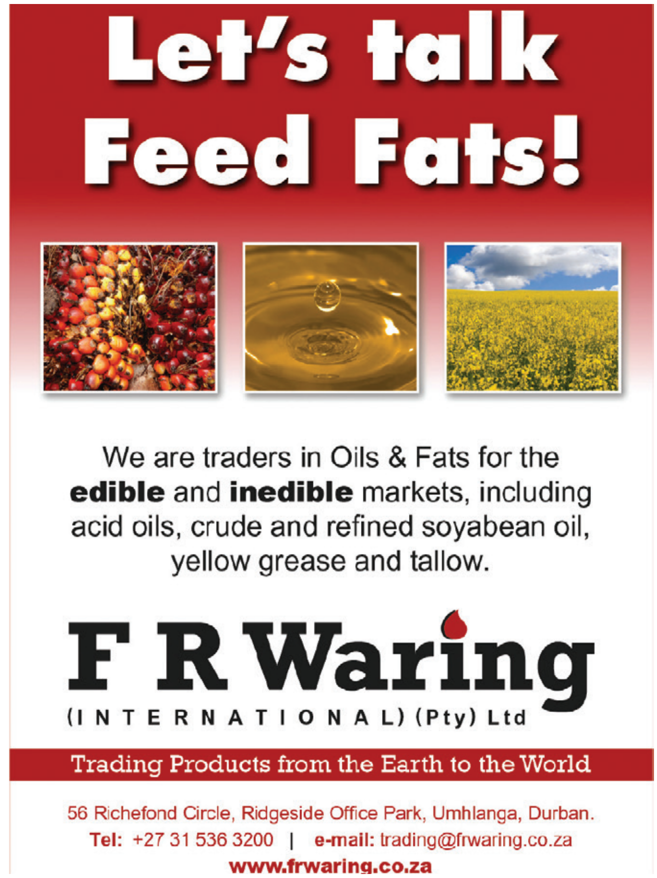
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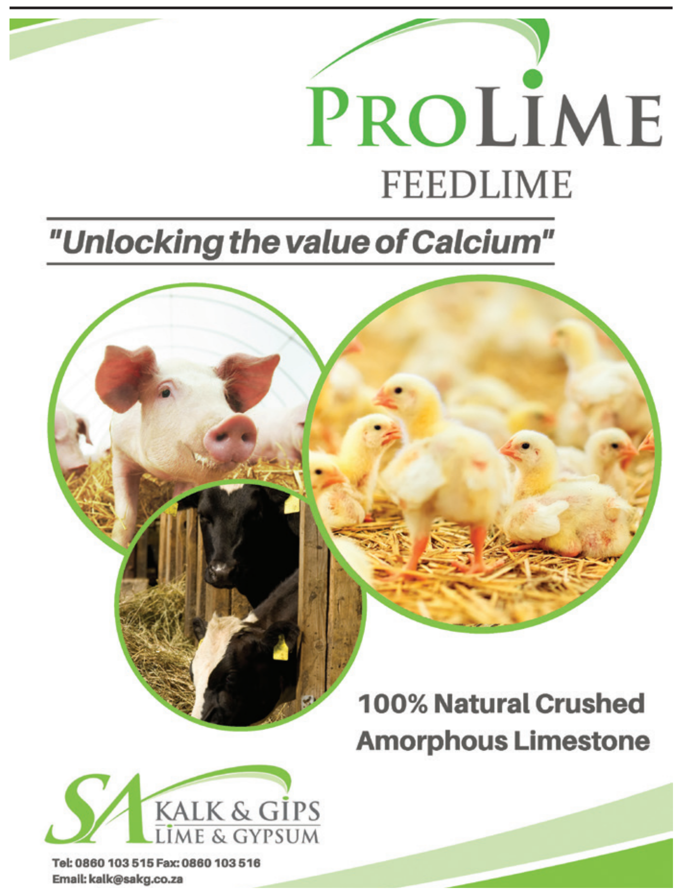
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Clostridium, Saccharomyces and *Candida* spp.) to broiler diets improved the characteristics of carcass and meat quality in male broilers (Endo and Nakano, 1999). Also, Mahajan *et al.* (2000) reported that the use of probiotics (*Lactobacillus acidophilus* and *Streptococcus faecium*) increased moisture, protein, ash, water holding capacity, emulsion capacity and stability in broiler meats. In general, probiotic supplementation improves meat quality of the produced broilers.

Oxidative status and heat stress

Sohail *et al.* (2011) studied the oxidative status of broilers under cyclic heat stress (HS) as modulated by supplementation of mannanoligosaccharides (MOS) and a probiotic mixture at 6×10^7 CFU per gram of product. The probiotic mixture contained *Lactobacillus plantarum, Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus rhamnosus, Bifidobacterium bifidum, Streptococcus thermophilus, Enterococcus faecium, Aspergillus oryzae* and *Candida pintolopesii.*

The author showed that heat stress increased total oxidants and total antioxidants and decreased paraoxonase and arylesterase, with no change in ceruloplasmin, aspartate aminotransferase, and alanine aminotransferase activities. Dietary supplementation decreased total oxidants and total antioxidants, with no effect on the activities of other enzymes. Heat stress did not influence serum copper, zinc and manganese concentrations of birds when compared with those in the thermoneutral group. However, MOS increased concentrations of all the trace minerals.

It was concluded that MOS or PM supplementation, alone or combined, may reduce some of the detrimental effects of heat stress. Also, the prebiotic mannanoligosacharide, with a mixture of probiotics, was found to enhance bodyweight, reduce feed consumption and improve the immunity of the gut in broiler chickens which were subjected to chronic heat stress (Sohail *et al.*, 2012).

Economic analysis of probiotic use

The decision to use specific types of probiotics and/or prebiotics is governed by the cost of these products and ease of their applications against their potential benefits to improve production performance and to increase overall profits. Currently, there is a wide spectrum of commercial probiotics and prebiotics for poultry available in the market. These additives are commonly used at small dosages.

In general, the cost of probiotics or prebiotics ranges from US$1 to 20/kg, depending on the commercial company and the active ingredients in the product (Young, 2008). If growth performance and feed efficiency are increased in commercial farming, then the costs of production are likely to be reduced. Also, if the chicken flock is able to resist disease and survive until they are of marketable size, the subsequent cost of medication and overall production costs would be reduced drastically.

For example, Torres-Rodriguez *et al.* (2007) reported that the economic analysis of using probiotics dietary supplement in turkeys indicated a lower cost per kilogram of live turkeys after including their probiotic cost, 59,90 and 58,37 cents/kg of live turkeys for the control and probiotic treatment respectively.

In the same study, the combination of a higher daily weight gain and a small reduction in the feed conversion ratio associated with the addition of the probiotic may have contributed to the lower cost of production, even after considering the costs for the addition of the probiotic, with an estimated additional income per turkey hen of about US 10 cents, representing an economic alternative to improve poultry production.

In Anjum *et al.* (2005) studied the economic efficiency of using protexin probiotic in broiler. The authors fed diets containing protexin at 100 and 110g/t in starter and 50 and 55g/t in finisher diets, respectively. The results of the study suggested that protexin supplementation is beneficial for better weight gains, feed efficiency and economic efficiency in broiler chicks.

Increase in live weight

The results of this study revealed that per-bird total return average on sale was US$1,59 at total average expenditure of US$0,982. The net per-bird income was US$0,611 on average. This indicated that supplementation of broiler starter and finisher diets with protexin at 100g/t in starter and 50g/t in finisher diets were economically beneficial and encouraging where treated groups generated more profit than the control group (Anjum *et al.*, 2005).

According to Rosen (1995), supplementing broiler diet with bacitracin probiotic increased in live weight from 1,5 to 2,6kg over weeks 5 to 8 of the growth period, this elevated the annual net profit per 1 000 birds by £60. In addition, Gutierrez-Fuentes *et al.* (2013) evaluated the effect of a commercial lactic acid bacteria-based probiotic (FloraMax-B11) on growth performance, bone qualities and morphometric analysis of broiler chickens.

The authors also estimated the cost benefit of using this probiotic in their study. The results showed an increase in bodyweight and improvement in feed conversion upon using the probiotic. The cost-benefit analysis showed that the increase in bodyweight of 100g, when converted to a cost benefit ratio, suggested that for every US$1 spent on this probiotic there was a cost benefit of 1:22,57.

However, there are studies in the literature showing no benefit of using probiotics and prebiotics in poultry feed. For example, a total of 200 day-old commercial broiler chicks were experimentally divided into five dietary treatments: control (T1), probiotic in the feed (100g/ton) (T2), prebiotic in the feed (500g/ton) (T3), probiotic + prebiotic (100g/ton+500g/ton) (T4), and probiotic + prebiotic (50g/ton+250g/ton) (T5). The economics of this usage was calculated in terms of return over feed cost (ROFC) and European efficiency index (EPEI). It was seen that a diet supplemented with symbiotic (100% level) proved to be more efficient in terms of EPEI and ROFC (50% level) than prebiotics or probiotic alone (Saiyed *et al.*, 2015).

In conclusion, the economic analysis data obtained from probiotic studies in broilers indicated that probiotic supplementation may not always be more feasible and economical to obtain maximum profitability from broiler production and hence further research in the field is currently ongoing.

This article has been condensed for publication in *AFMA Matrix*. For enquiries or references, email the author athkhalifa@kisr.edu.kw.

Sparing energy to reduce feed costs with supplements

By Lukas Bauer, Manager Technical Consultancy, Evonik

Feed has always been the biggest production cost faced by poultry producers and recent rises in global prices, combined with supply volatility, mean that producers have to continue looking for ways to maintain their margins.

The situation is particularly acute because the current global shortage of grain and seed-oil stocks has not been matched by a proportional rise in meat prices. The situation does not look likely to be reversed soon. The challenge facing nutritionists and poultry producers is how to maintain animal performance, meat quality and revenues by keeping feed costs down.

Non-starch polysaccharide-degrading enzymes

One obvious solution is to get more energy out of the same amount of feed. Non-starch polysaccharide-degrading enzymes or NSPases is one approach that has been shown to have the potential to do just that. These enzymes, derived from bacteria and fungi, can be added to feed to help break down soluble NSPs and release energy that would otherwise not be available to the bird.

Unfortunately, NSPases have several inherent drawbacks. Although they are present in most plant material used in animal feeds, the amount varies considerably between cereal sources and from harvest to harvest. So, deciding how much NSPase to add to each feed batch to give the best return on investment is not always straightforward.

NSPs consist of pentose and hexose sugars combined in different ways to produce a family of complex polymers such as xylans and glucans. The action of NSPases is very specific, so an enzyme that breaks down xylans will not degrade glucans. So again, to be optimally effective, you need to know the exact types and amounts of NSP in any given feed source, so the optimal types and amounts of NSPase can be added.

Finally, the NSP content of maize is relatively low, so the potential for increased energy yield from feeds based on these raw materials is relatively limited.

Guanidinoacetic acid

Over the past decade commercial trials have shown that guanidinoacetic acid or GAA supplementation to the feed increases the energy efficiency of broilers. The results have shown that bird performance can be maintained even when the energy value of the diet is reduced by up to 100kcal AME_n (N-corrected apparent metabolisable energy)/kg below breeder recommendations when supplemented with 0,06% GAA. This is equivalent to between 83 000 and 166 000kcal AME_n/kg GAA.

Unlike NSPase, which is active in the gut where it facilitates the digestion of soluble NSPs, GAA acts at a more fundamental cellular level, and therefore its energy sparing effect is not influenced in the same way by the feed composition. GAA is a precursor of creatine which is an energy carrier and energy buffer, and therefore an essential part of many biochemical reactions in many different tissues, especially in muscle. Creatine is essential for healthy muscle function and growth, especially in birds that are growing quickly.

Modern vegetable-based broiler diets contain very little creatine, and so birds must rely mostly on creatine that is synthesised by the body from the precursor GAA. Poultry can synthesise some of this GAA themselves from the amino acids glycine and arginine, but not enough to meet all their needs; the rest has to be obtained from the diet. Supplementing the diet with GAA can fill the gap and increases creatine concentration which improves the efficiency of energy usage and spares glycine and arginine in the diet for other bodily functions.

So why not cut out the middleman and just add creatine to the diet? This approach has been tried but is unfortunately not very practical because the thermal stability of creatine is not as good as GAA and, crucially, it is more expensive.

Energy sparing

A recent trial, published in *British Poultry Science* in 2022, confirmed that 600g/t GAA added to a wheat/barley/soya bean meal-based diet can offset the loss of performance induced by lowering the energy value of broiler feed (Pirgozliev *et al.*, 2022).

The study followed 1 280 one-day-old Ross 308 broilers to day 42 on commercial AME_n levels of 12,55, 12,97 and 13,18MJ/kg in their starter, grower and finisher diets respectively. Birds were divided into four groups:

- Positive controls received the standard diets (PC).
- Group two received the PC diet but with 0,21MJ/kg less energy content (NC1).
- Group three received the NC1 diet plus 0,06% GAA (NC1 + GAA).
- The final group received the PC diet with 0,42MJ/kg less energy plus 0,06% GAA (NC2 + GAA).

The energy content was reduced by decreasing the amount of soya bean oil and wheat while increasing barley.

At day 42, birds fed the energy-reduced, non-GAA diet had a lower weight gain compared to all other diets and a significantly lower final bodyweight compared to birds that received GAA supplementation. GAA fed birds also had a significantly higher poultry efficiency factor than those that received a calorie-reduced diet but no GAA (EPEF is calculated by the mean grams gained per day, multiplied by the percentage survival rate, and divided by the FCR x 10).

The authors concluded their results implying that "lower performance induced by a reduction of dietary AME_n in the range of 0,21 to 0,42MJ/kg was more than compensated by supplementing 600g/t GAA to the feed".

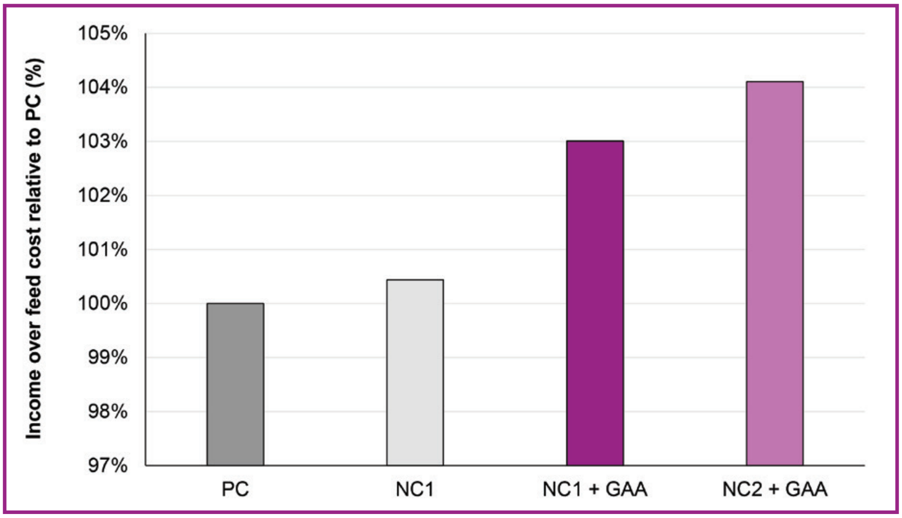
Based on the results of the Pirgozliev study, with the energy sparing of GAA, it would be possible to save between €2 and €7 per ton of feed, i.e., about 1 to 2,4% of feed cost. As might be expected, simply reducing the energy content of feed like in the NC1 treatment, reduced the feed costs by more than adding the GAA supplement like in NC1 + GAA.

However, when feed costs and animal performance were both included in the cost calculations, income over feed cost improved by 3% in the NC1 + GAA group compared to the PC and by 2,6% compared to the NC1 diet. Feed costs of the NC2 + GAA diets were the lowest compared to all other diets in the trial even though GAA was supplemented.

The further reduction of 50kcal AME_n/kg compared to NC1 was reducing the diet cost more than the GAA supplementation of 0,06%. Additionally, the income over feed cost in the NC2+ GAA groups was the highest as the performance of the birds fed the NC2+ GAA diets was not significantly different compared to the PC and the NC1 + GAA treatments and significantly improved compared to the NC1 treatment (*Figure 1*).

The afore-mentioned results confirm those of a similar trial of 11 400 Ross 308 birds in commercial conditions which found that GAA supplementation (0,06%) significantly improved final bodyweight and FCR by 1,77 and 1,66% respectively in fast-growing modern broilers (Ceylan *et al*., 2021).

Figure 1: Income over feed cost (IOFC) calculation.



Based on the diets and performance data from Pirgozliev et al. (2012) (wheat and soya bean meal based) with long-term average feed prices. IOFC is expressed as a percentage to the IOFC of the PC. Positive control (PC; recommended ME levels of 3 000, 3 100 and 3 150 kcal/kg feed in starter, grower, finisher diets), negative control 1 (NC1 = PC -50 kcal/kg), NC1 + GAA (NC1 + 0,06% GAA), NC2 + GAA (NC2 = PC -100 kcal/kg + 0,06% GAA).

Return on investment

Simply reducing the energy content in poultry feeds may not be a viable option for cost-saving in feed formulation. Assuming that energy is the most limiting factor in the feed, poultry will eat to meet their energy needs and lowering energy will result in increased feed conversion.

Previous studies have suggested that the increase in feed conversion is the response of the broilers adjusting their feed intake and energy expenditure to maintain energy balance and bodyweight. Therefore, reducing dietary energy without negatively affecting performance and still generating an economic return requires an additional strategy.

An economical solution that enables a reduction in the energy value of the diet while maintaining bird performance is the dietary supplementation of GAA. In this context, a recent study with GuanAMINO® supplementation showed an energy sparing of 57, 79 and 91kcal/kg in starter, grower and finisher diets respectively.

This result confirms what we know about the energy sparing potential of GAA which can be from 50kcal up to 100kcal/kg feed. Considering the energy matrix of GuanAMINO® in the feed formulation can contribute to significantly decrease the inclusion of oil, which can be quite expensive in feeds.

At a time when the cost of feed is high, the returns for any supplement that enables a reduction in the energy content of the diet while maintaining bird performance is likely to be worth evaluating. For instance, a saving of just 2c/kg live weight for a 25 000-bird house with a 34-day growing period and 2,25kg weight gain is equivalent to an increased profit of around US$1 125 per cycle. GAA could provide some of the savings that producers are looking for as feed prices remain high.

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Examining feed preference of different pellet formulations for application to automated milking systems

By AL Carroll, KK Buse, JD Stypinski, CJR Jenkins and PJ Kononoff

There are approximately 50 000 automated milking systems (AMS) worldwide, and AMS is a sector within dairy production that continues to grow in popularity (Maculan and Lopes, 2016). Motivating animals to enter the milking unit by offering palatable, and usually pelleted, mixes of ingredients meets the need of animals to consume nutrients and increases the number of visits to the robot, milking frequency, and production (Migliorati *et al.*, 2009).

Although reduced visits to the robot could be a function of pellet preference and pellet quantity, experiments have suggested that the quantity of pellets does not affect the number of visits to the robot or required fetching (Halachmi *et al.*, 2005; Bach *et al.*, 2007). This could be because animals are more motivated by feed preference alone and not the amount of pellets offered. Therefore, inclusion of preferred ingredients may have a greater impact. For example, increasing the inclusion of molasses or other highly palatable ingredients has been linked to increases in the number of voluntary visits and decreases in fetching (Rodenburg and Wheeler, 2002).

To differentiate the effects of pellet preference and quantity, an experiment by Migliorati *et al.* (2005) tested a fenugreek (*Trigonella foenum-graecum*) flavouring and pellet allowance. Results of this experiment indicated that the amount of pellets within the AMS had no effect on visits per day; however, fenugreek did increase the number of visits and decreased the interval between visits while numerically increasing the milk yield.

In general, palatability is believed to be a factor that influences visits with milking to the AMS (Madsen *et al.*, 2010) and could have practical implications on feed cost related to pellet formulation strategies, but data are lacking in comparing differing pellet formulation strategies on feed preference. The objective of this experiment was to examine the preference of four pelleting formulation strategies. We hypothesised that the most preferred feed would be the pellet designed with feedstuffs indicated to be palatable in dairy cattle.

Study design

Eight multiparous lactating Jersey cattle (289 ± 25,3 DIM, 26,0 ± 2,45kg of milk yield, 19,36 ± 1,29kg of DMI) were utilised for the taste preference experiment in a manner described by Erickson *et al.* (2004).

This study was not conducted in an AMS; rather, cows were housed in tie-stalls with continuous access to water and fed 85% of the previous week's average intake once daily at 10:00. After milking at 18:00 and returning from the exercise pen at 19:30, feed was removed from the individual feed bunks and animals were offered 0,50kg of each pellet treatment in a randomised arrangement and separated within 30,48cm × 40,64cm plastic tubs. These were offered for 60 minutes or until a single treatment was fully consumed.

Feeds were offered for nine days total as described, with all four pellets


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allocated from day one to four. After the initial feeding period, the most preferred feed was removed, and three feeds were offered for three days. Then, the process was repeated for two days. Treatments were ranked sequentially with one being most preferred and four being least. This is the same way in which they were removed during the experiment.

The four pellets examined included a single pellet of dry corn gluten feed (CGF); a pellet including feedstuffs considered to be highly palatable (53,2% wheat middling, 15,7% dried distillers grains, 15,2% cane molasses, and 1,81% oregano [FLVR]); a high-energy pellet (ENG) consisting of 61% corn grain and 26,2% wheat middlings; and a pellet containing feeds commonly included in the concentrate mixture of a TMR including 43,1% corn grain, 26,3% dried distillers grains, 3,18% soya bean meal, and 5,6% vitamin and mineral premix (CMIX).

The CMIX was included as a treatment because we observed that a similar formulation and offering was provided on a commercial AMS dairy in Nebraska.

Pellet composition

As expected, the pellet treatments differed in chemical composition, but the objective of the current experiment was not to test the impacts of chemical composition or nutrient concentration on preference. The authors also recognise that in AMS, nutrition pellets are generally formulated to balance the nutritional needs of the animal with the partial total mixed ration, but in the current experiment, the partial total mixed ration was the same for animals consuming all treatments.

Although nutrient provision and incentive to enter the AMS systems are goals of practical AMS diet formulation, we recognise that since a small quantity was offered relative to on-farm AMS systems and TMR access was limited to 85% of the previous week's intake, we believe we did not affect nutrient intake to an extent that physiological factors would affect preference behaviour.

As expected, CGF contained more CP (21,0%) than CMIX, FLVR, and ENG (18,6, 17,7, and 14,2%, respectively). Amylase-treated NDF and aNDFom were similar for CGF and FLVR (31,7 and 31,2 versus 31,5 and 31,1), likely as a function of the approximate 38,7% and content of wheat middlings in the FLVR treatment (NASEM, 2021). These concentrations were increased relative to the CMIX pellet at 24,1% and the ENG at 19,8%, and were likely due to the dilution of fibre-containing components with mineral inclusion in the CMIX, as well as increased corn grain content in both CMIX and ENG.

Increasing the corn grain content within CMIX and ENG had a subsequent impact on the starch content, increasing the concentrations to 49,3 and 31,5% starch relative to those of CGF and FLVR at 15,6 and 21,3%. Also, ash content was increased with the CGF pellet and the CMIX pellet at 7,84 and 5,38%. These values fall in line with the NASEM (2021) ash values for corn gluten feed, and we formulated the CMIX pellet to contain vitamins and minerals.

Pellet preference

Feed preference was ranked, with **1** being most preferred and **4** being the least, and then analysed for mean and standard deviation. The resulting preference ranking for CGF, FLVR, CMIX, and ENG was 1,5 ± 0,463, 2,50 ± 0,926, 2,88 ± 0,835, and 3,13 ± 0,991, respectively (*Table 1*). Based on the preference ranking, a pellet containing a single feed ingredient being observed to be most palatable in the present study was surprising.

Dry corn gluten feed contains a portion of steep water, distillers solubles, and corn bran, all which undergo a heating process during drying as well as steam extrusion during pelleting. During heating, Maillard reactions occur where available carbohydrate complexes react with AA, creating aromatic compounds, such as furosine, which may contribute to the sweet sensory perception (Jo *et al.*, 2018). While the sweet aroma may have been enticing to cows, the sweet taste was likely not the primary driver; the FLVR contained molasses, and as a result, more sugar (NRC, 2001).

Similar to sweet taste preference in dairy cattle, umami tastes are highly preferred, as displayed by preference for monosodium glutamate (Nombekela *et al.*, 1994; Roura and Navarro, 2018). Glutamate is a primary, taste-active umami flavour in food additives, such as monosodium glutamate, formed through fermentation of starch sources similar to dry and wet milling practices, which produce corn gluten feed (Nombekela *et al.*, 1994). Therefore, we speculate that the umami flavour is likely present in CGF due to the addition and condensation of distillers solubles from starch fermentation and may have been preferable to cows.

Table 1: Preference scores of four different pelleting strategies in lactating Jersey cattle.

	Treatment[1]			
Item	**CGF**	**FLVR**	**ENG**	**CMIX**
Cow ID				
1	1	2	4	3
2	2	3	1	3
3	1	3	3	2
4	1	2	3	4
5	1	3	4	2
6	2	1	3	4
7	1	4	3	2
8	1	2	4	3
Sum	10	20	25	23
Mean	1,25	2,5	3,13	2,8
SD	0,463	0,926	0,991	0,835

[1]Rank of pellet formulations fed at 0,5kg per treatment with 1 as most preferred and 4 as least preferred. CGF = corn gluten feed (pellets); FLVR = high palatability oregano; ENG = energy balance containing 20% of the energy required for an animal milking 32,7kg/d and consuming 24,2kg/d DMI; CMIX = typical pellet containing vitamins and minerals.

It should be noted that it is possible that a factor affecting preference of CGF was pellet size (Beauchemin, 1991). In the case of the current study, pellet size was different between treatments. Specifically, the CGF was manufactured and pelleted at the wet corn milling plant, resulting in a pellet width of 9,53mm, whereas the pellet width of remaining treatments was 6,35mm.

The CGF treatment was designed to be a simple, low-cost alternative to the remaining treatments that were strategically formulated. It is possible that pellet length played a role because it has been observed to be associated with feed intake (Beauchemin, 1991), and a linear increase in eating rate with increasing pellet length has also been observed (Spörndly and Åsberg, 2006).

When interpreting data related to feed preference, one animal factor to consider

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is previous exposure to feeds. All animals part of this experiment originated from a commercial farm and had previously been fed both wet and dry corn gluten feed within their lifetime. Although animals had not been exposed to oregano leaf, they had been previously fed DDGS, corn, and molasses, which made up 45,1% of the pellet.

Increasing inclusion of corn gluten feed has been observed to increase DMI in lactating cattle when replacing other concentrates including corn grain, soya bean meal, and soya bean hulls (Mullins *et al.*, 2010). This supports the notion that although previous exposure could influence preference, other factors likely contribute to the drive to consume the CGF pellet.

Probability of pellet choice

Table 2 displays the probability an animal will choose a pellet based on the observations made during the preference test and if that probability differs from a mean value of no choice at 25% (Erickson *et al.*, 2004). The probability of first choice for the CGF pellet was observed to be 78,6 ± 0,601%, which was different from the mean value of no choice at 25%.

The second and third choices averaged 9,38 ± 0,438% FLVR and 7,11 ± 0,439% CMIX and did not differ (P > 0,19) from the mean values of 25%. The FLVR pellet was likely preferred over the CMIX as the addition of oregano to feed has been previously indicated to improve preference of hydrolysed feather meal; feather meal has historically been considered an unpalatable feed ingredient (Buse *et al.*, 2021).

Another contributing factor in the observed preference ranking is that animals prefer pellets over feeds in the form of a meal (Krogstad *et al.*, 2021). Therefore, the increased fines caused by vitamin and mineral inclusion in the CMIX pellet containing 5,38% ash decreased the pellet hardness. This is further supported by the observation that pellet hardness decreased approximately 62% from 20,8 ± 7,05kg in the CGF to 7,9 ± 2,94kg in the CMIX and it is possible this may have negatively affected feed preference.

Based on the taste preference experiment, we conclude that animals would choose the ENG pellet 4,94 ± 0,453% of the time and this proportion was significantly (P = 0,04) lower than the mean value of no choice at 25%. The ENG pellet within the current experiment contained similar base ingredients to that of the CMIX and FLVR, but an increased proportion of corn grain containing starch.

Unlike humans, cattle contain no salivary amylase, which converts dietary starch into sugar (McDougall, 1948). As such, the starch contained within the pellet would not be enzymatically degraded to sugars, which then could be perceived by type II sensory cells on the tongue (Lee and Owyang, 2017). Also, animals consumed 85% of the previous week's intake; therefore, the increased energy content of the ENG pellet was not a driver in feed preference in the current experiment. Thus, we speculate that CMIX contained a less desirable flavour relative to all other treatments.

Conclusions

The aim of this experiment was to examine four different pelleting strategies for preference in lactating Jersey cattle. Results suggest that animals exhibit a high degree of preference for CGF pellets. Alternatively, cows appeared to exhibit lowest preference for a pellet containing predominantly corn and wheat middlings.

Overall, little work has been done aimed at describing feed preference in dairy cattle, although it has direct implications within AMS systems, as previously described. Further exploration on this topic could expand our ability to utilise target chemical compounds within palatable ingredients to improve intake of nutritious yet more unpalatable feeds (Buse *et al.*, 2021).

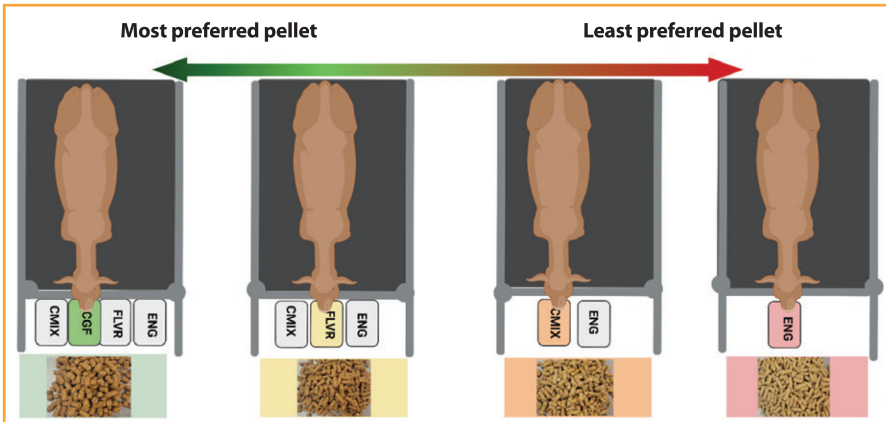
Also, considering feed preference in pellet formulation strategies may aid producers in designing pellets that consider both cost and feed preference. Therefore, further research is needed in preference to define compounds found in feeds as well as describe their influence on preference in cows.

Table 2: Probability animals will choose a given pelleting strategy first, based upon the preference of lactating Jersey cattle.

Treatment[1]	μ[2]	SE[3]	Z[4]	P[5]
CGF	78,6	0,601	3,04	<0,01
FLVR	9,38	0,438	-0,69	0,49
ENG	4,94	0,453	-2,08	0,04
CMIX	7,11	0,439	-1,32	0,19

[1]Pellet formulations: CGF = corn gluten feed (pellets); FLVR = high-palatability oregano; ENG = energy balance; CMIX = typical pellet containing vitamins and minerals.
[2]μ = estimated percentage chance a diet will be chosen when all treatments are presented.
[3]SE = standard error for the percentage chance a treatment will be chosen first.
[4]Z = percentage chance that a treatment being chosen first is different from the percentage of no choice at 25%.
[5]P-value: indicates that the preference value differs from the percentage chance an animal would choose one of the four feeds at random (25%).

Figure 1: Lactating Jersey cattle's preference for the four different pelleting strategies.



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Lifetime performance improves when feeding rumen-protected methionine

By Drs Danielle Sherlock, ruminant R&I science manager, and Daniel Luchini, head of ruminant R&I, Adisseo

Extensive research over the last three decades shows that feeding supplemental, encapsulated methionine increases the lifetime performance of dairy cows with the benefits first seen in milk, milk protein and milk fat production. By precisely balancing amino acid levels in the ration, nutritionists can economise the total protein level fed.

Methionine is an essential amino acid and nutrient and typically is the first limiting amino acid in today's dairy rations. Dairy cows cannot synthesise methionine in the quantity required for milk production, health and reproduction, and feedstuffs cannot totally fill the nutritional requirement even when protein is overfed. The unique biochemical role of methionine has led to it being dubbed the enabler of all protein synthesis. It is heavily involved in a multitude of key metabolic and immune pathways.

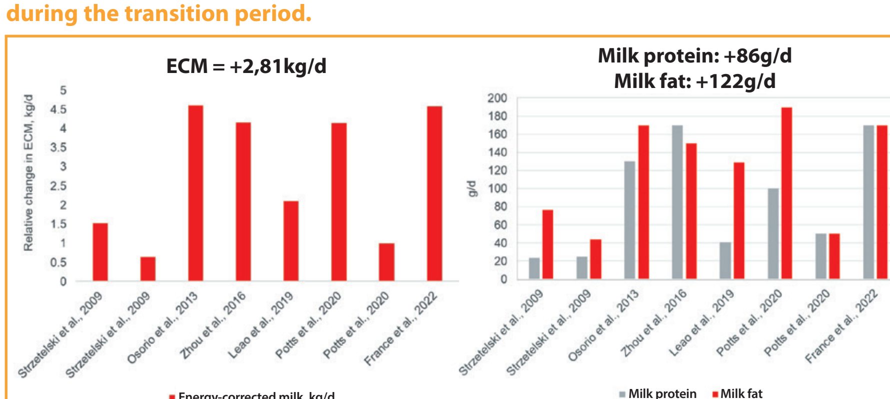
Longer-term health benefits

Rumen-protected methionine was introduced during the 1990s to support increases in production. In 2001, the National Research Council (NRC) in its publication *Nutrient Requirements of Dairy Cattle* concluded that methionine was one of the most limiting amino acids in dairy diets. More recently, the National Academies of Sciences, Engineering and Medicine (NASEM) released its *2021 Nutrient Requirements of Dairy Cattle*.

In between these two, multiple research groups recognised methionine's longer-term health and reproduction benefits, and advancements in ration formulation software enabled better optimisation and balancing of amino acids. Today's software delivers least-cost, non-linear solutions. Nutritionists can cost-effectively focus on providing individual amino acids to meet requirements rather than overfeeding protein.

Different universities have researched the functional role of methionine

Figure 1: Effect on milk performance when supplementary RP-Met are provided during the transition period.



beyond protein synthesis over the last two decades. Their research has spurred trials to investigate methionine supplementation from the transition period through the entire lactation period. These trials help define the extent to which amino acid balancing improves lifetime performance.

Advantages of methionine

During the transition period, dairy cows experience the most health issues. Early trials at the University of New Hampshire suggested that methionine supplementation during the transition period improved milk production, sparking interest in understanding more about the role of methionine at transition.

Trials followed at the University of Illinois, Cornell University and University of Wisconsin. Across these studies, increases in dry matter intake, milk yield, milk fat and protein have been reported with methionine supplementation during the transition period.

Such improvements in production were linked to the effects of methionine on metabolism and immune responses. Specifically, methionine derivatives have antioxidant properties. Cows fed the rumen-protected methionine source have higher antioxidant status (glutathione) and better immune (oxidative burst) and liver function, which prepares them to withstand the transition period and results in higher dry matter intake for healthier cows with increased milk production and composition.

By setting the cow up right in the transition period, enriching diets with methionine bears dividends throughout lactation. Gains in energy-corrected milk generated prior to peak lactation will hold through the rest of lactation. As milk yields decline in later lactation, the gain in component yield will be seen as a further increase in milk component percentage.

Continuing into lactation, amino acid balancing maintains elevated levels of energy-corrected milk and minimises embryonic deaths. Ration protein levels can also be reduced strategically to avoid overfeeding of protein and excess nitrogen excretion. During the last third of lactation, amino acid balancing allows the feeding of very low protein rations to continue maximising nitrogen efficiency and maintaining milk performance.

Balancing the essential amino acid levels in dairy rations now has been well-proven as an effective nutritional tool at all stages of lactation.

Since the beginning of amino acid research, many trials conducted under controlled conditions have expanded on the role of methionine as an essential amino acid and functional nutrient with benefits throughout the entire lactation of dairy cows, underscoring the positive benefits of amino acid balancing on lifetime performance.

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Association of distance to swine concentrated animal feeding operations with immune-mediated diseases: An exploratory gene-environment study

By Montserrat Ayala-Ramirez, Nathaniel MacNell, Lucy E McNamee, John A McGrath, Farida S Akhtari, Matthew D Curry, Askia K Dunnon, Michael B Fessler, Stavros Garantziotis, Christine G Parks, David C Fargo, Charles P Schmitt, Alison A Motsinger-Reif, Janet E Hall, Frederick W Miller and Shepherd H Schurman

Concentrated animal feeding operations (CAFOs) are a source of environmental pollution and have been associated with a variety of health outcomes. Immune-mediated diseases (IMD) are characterised by dysregulation of the normal immune response and, while they may be affected by gene and environmental factors, their association with living in proximity to a CAFO is unknown.

The objectives of the study were to explore gene, environment, and gene-environment (GxE) relationships between IMD, CAFOs, and single nucleotide polymorphisms (SNPs) of prototypical xenobiotic response genes AHR, ARNT, and AHRR and prototypical immune response gene PTPN22.

The exposure analysis cohort consisted of 6 464 participants who completed the *Personalised Environment and Genes Study Health and Exposure Survey* and a subset of 1 541 participants who were genotyped. The association between participants' residential proximity to a CAFO in gene, environment, and GxE models was explored. Individual associations in a transethnic model using METAL meta-analysis were recombined.

In white participants, ARNT SNP rs11204735 was associated with autoimmune diseases and rheumatoid arthritis (RA), and ARNT SNP rs1889740 was associated with RA. In a transethnic genetic analysis, ARNT SNPs rs11204735 and rs1889740 and PTPN22 SNP rs2476601 were associated with autoimmune diseases and RA.

In participants living closer than 1,6km to a CAFO, the log-distance to a CAFO was associated with autoimmune diseases and RA. In a GxE interaction model, white participants with ARNT SNPs rs11204735 and rs1889740 living closer than 13km to a CAFO had increased odds of RA and autoimmune diseases, respectively. The transethnic model revealed similar GxE interactions.

The results suggest increased risk of autoimmune diseases and RA in those living in proximity to a CAFO and a potential role of the AHR-ARNT pathway in conferring risk. We also report the first association of ARNT SNPs rs11204735 and rs1889740 with RA. The findings, if confirmed, could allow for novel genetically targeted or other preventive approaches for certain IMD.

Environmental factors

Immune-mediated diseases (IMD) are heterogeneous disorders characterised by dysregulation of the normal immune response and inflammatory pathways that can lead to tissue damage, increased disability, and mortality (El-Gabalawy *et al.*, 2010). IMD include autoimmune diseases such as hypothyroidism, hyperthyroidism, rheumatoid arthritis (RA), and other diseases that include allergies and asthma.

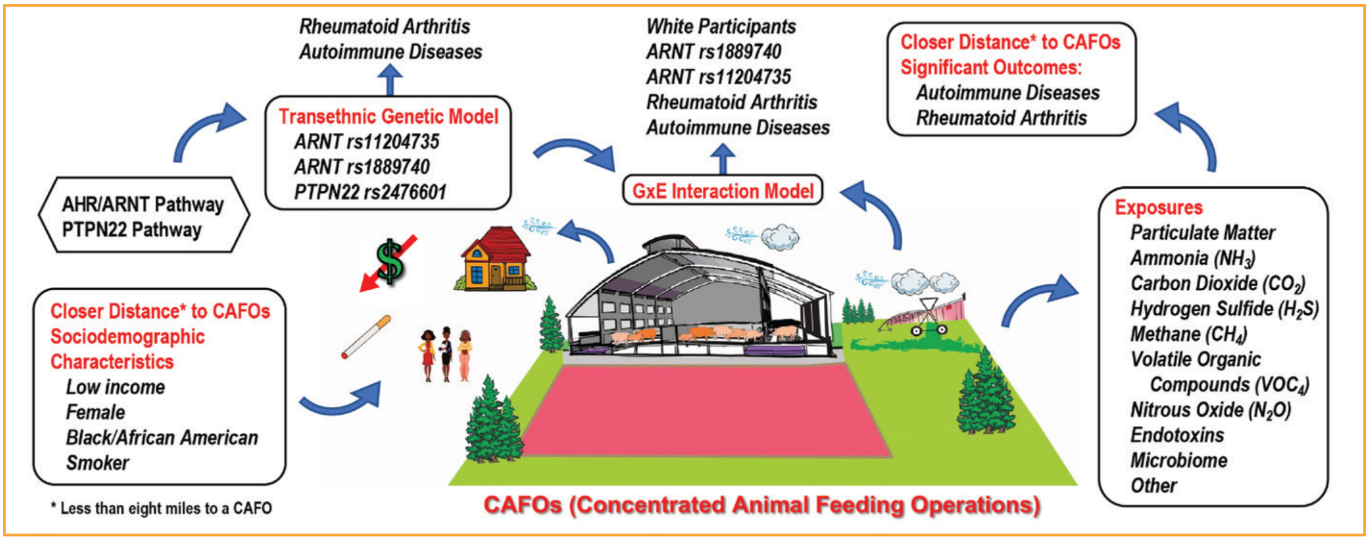
The association of IMD with susceptibility loci (Kumar *et al.*, 2014; Parkes *et al.*, 2013) and environmental exposures (Kreitinger *et al.*, 2016; Vojdani *et al.*, 2014) common across diseases suggests shared pathogeneses. Recent studies have reported that particulate matter present in air pollution may contribute to the development and exacerbation of autoimmune diseases such as multiple sclerosis, systemic lupus erythematosus (SLE), and RA (Alsaber *et al.*, 2020; Cakmak *et al.*, 2021; Roux *et al.*, 2017).

Further, environmental exposure to ammonia has been associated with decreased lung function in children with asthma (Loftus *et al.*, 2015); hydrogen sulphide has been suggested as a pro-inflammatory mediator in RA (Muniraj *et al.*, 2017); and endocrine disruptors have been associated with SLE flares and increased prevalence of autoimmune diseases (Ahmed et al., 1999; Yurino *et al.*, 2004).

An animal feeding operation (AFO) is an agricultural facility where animals are confined and fed for at least 45 days each year and where no crop is sustained within the facility (US Environmental Protection Agency, 2022). A CAFO is an AFO with >1 000 animal units enclosed for at least 45 days a year although facilities with between 300 and 1 000 animal units can also be considered a CAFO if they meet specific criteria (US Environmental Protection Agency, 2001).

North Carolina is home to over 2 500 permitted AFOs (Quality, 2019), including 2 276 swine CAFOs in 2019, with most located in the eastern part of the state in low income and minority communities in which high mortality rates have been

Figure 1: Summary of findings. Gene-environment interactions in participants living less than eight miles from a concentrated animal feeding operation: exposures and outcomes.



reported (Kravchenko *et al.*, 2018; Nicole, 2013; Son *et al.*, 2021; Wing *et al.*, 2000).

Introduction

CAFOs are well-known sources of water and air pollution (Domingo *et al.*, 2021) and are associated with several environmental and population-level health risks (Blunden and Aneja, 2008; Brown *et al.*, 2020; Burkholder *et al.*, 2007; Glibert, 2020; Kravchenko *et al.*, 2018; Nicole, 2013; US Environmental Protection Agency, 2005). While the specific relationships between IMD, exposure to a CAFO, and gene-environment (GxE) interactions (Heederik *et al.*, 2007) are unclear, exploring potential associations may enable the identification of groups at risk of IMD and, in turn, support continued development of preventive measures.

In this study, relationships between IMD and living less than 13km from a CAFO were explored in the National Institute of Environmental Health Sciences (NIEHS) Personalised Environment and Genes Study (PEGS) cohort, which was previously known as the Environmental Polymorphisms Registry (EPR) (National Institute of Environmental Health Sciences, 2022a).

Proximity to a CAFO can approximate potential exposures related to CAFOs such as various types of particulate matter (PM), volatile organic compounds (VOCs), aerosols, and gases. GxE interactions in participants living within 13km of a CAFO were investigated, specifically associations for single nucleotide polymorphisms (SNPs) of protein tyrosine phosphatase nonreceptor type 22 (PTPN22), a prototypical immune response gene, and aryl hydrocarbon receptor (AHR) and AHR nuclear translocator (ARNT), which are prototypical xenobiotic response genes.

Discussion

The findings expand the current understanding of the environmental and public health impacts of CAFOs on nearby communities. To the authors' knowledge, associations between living less than 13km from a CAFO and autoimmune diseases such as RA, as well as associations with SNPs such as ARNT SNP rs11204735 and ARNT SNP rs18897740, have not been previously reported (*Figure 1*).

We investigated the association between proximity to a CAFO and IMD phenotypes and assessed evidence of GxE interactions between exposure to a CAFO and IMD-associated SNPs. This expands the current understanding of the impacts of CAFOs because most research to date on livestock and swine CAFOs has focused on associations with asthma and allergies (Loftus *et al.*, 2020; Mirabelli *et al.*, 2006; Radon *et al.*, 2007; Rasmussen *et al.*, 2017; Schulze *et al.*, 2011), soft tissue infections, antibiotic resistance, and infectious outbreaks (Beaudoin *et al.*, 2012; Beresin *et al.*, 2017; Pedati *et al.*, 2019).

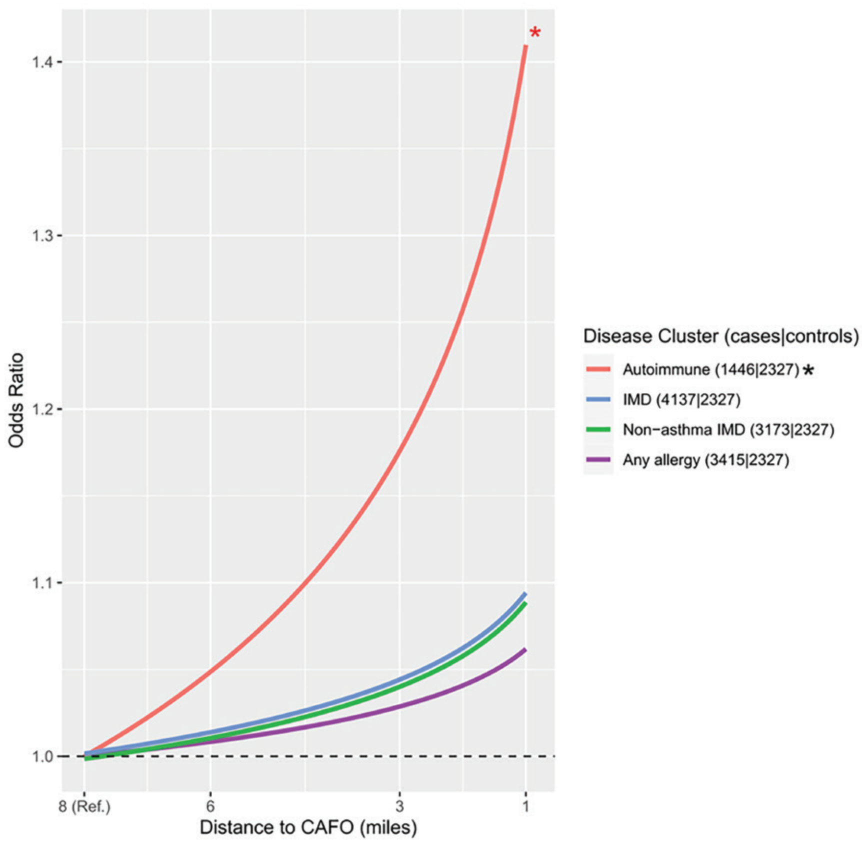
Genetic associations have been described for IMD and autoimmune diseases, with multiple genetic risk loci identified, including some minor mutations and hundreds of SNPs that overlap across diseases (Cotsapas *et al.*, 2011; Richard-Miceli and Criswell, 2012). However, the association of environmental exposures with IMD and autoimmune diseases is less well understood (Javierre *et al.*, 2011; Pollard *et al.*, 2018; Selmi *et al.*, 2012; Wahren-Herlenius and Dorner, 2013). Confirming these exposures as risk factors and discovering the mechanisms for GxE interactions may enable the development of preventive and therapeutic approaches (Hunter, 2005).

In our genetic and transethnic genetic models, intronic ARNT SNP rs11204735 was associated with autoimmune diseases and RA, and intronic ARNT SNP rs1889740 was associated with RA in both models and autoimmune diseases only in the transethnic model. The association of ARNT SNP 11204735 with autoimmune diseases in the transethnic genetic model is consistent with our prior investigation of IMD associated SNP loci in the PEGS cohort, considering differences in the analyses in terms of the cohort, assessment of environmental associations, and model adjustments made to control for confounding bias (Schurman *et al.*, 2020).

Adaptive cellular responses

The ARNT gene encodes a protein that forms a complex with AHR and participates in xenobiotic metabolism (Larigot *et al.*, 2018). ARNT is also involved in the hypoxia inducible factor (HIF)-alpha signalling pathway, which senses and enacts adaptive cellular responses to hypoxia. Phenotypic

Figure 2: Continuous model for associations between proximity to a concentrated animal feeding operations and immune-mediated disease phenotype by disease cluster (*p < 0,05).



associations with intronic variants have been described for autoimmune diseases (Kochi *et al.*, 2018; Saevarsdottir *et al.*, 2020) and RA (Pakzad *et al.*, 2021).

While intronic variants do not encode for proteins, they have important cellular functions that include gene expression regulation, transcription initiation, and chromatin assembly (Jo and Choi, 2015).

Additionally, PTPN22 rs2476601 was associated with autoimmune diseases and RA in the transethnic genetic model. PTPN22 rs2476601 was associated with RA in prior work by our group using an allelic model and by others using dominant, recessive, and allelic models (Abbasifard *et al.*, 2020; Begovich *et al.*, 2004; Schurman *et al.*, 2020). PTPN22 encodes a protein involved in CTLA4 signalling and immune system pathways associated with various autoimmune diseases (Abbasifard *et al.*, 2020; Criswell *et al.*, 2005; Jiang *et al.*, 2012; Ramu *et al.*, 2019; Schurman *et al.*, 2020).

In our prior work, we found a linkage disequilibrium, which is the non-random association between alleles at different loci, in the Black/African American, Hispanic, and white groups for ARNT rs11204735 and ARNT rs1889740, between 0,50 and 0,84. Accordingly, we assume these associations are not likely entirely independent (Schurman *et al.*, 2020; Slatkin, 2008). In the continuous model, there was a higher frequency of autoimmune diseases and RA in participants who lived less than 13km from a CAFO.

IMD result from complex interactions between the environment and the host's non-genetic and genetic factors, and there is growing evidence of shared components among conditions included under the IMD umbrella (Acosta-Herrera *et al.*, 2019; Ter Horst *et al.*, 2016). In the context of swine CAFOs, associated adverse health effects may be related to airborne exposures and water contamination from lagoons and sprayfield systems used for waste management (Mallin and McIver, 2018).

Exposure to pollutants

The most common air pollutants associated with CAFOs are ammonia (NH_3), hydrogen sulphide (H_2S), carbon dioxide (CO_2), VOCs, methane (CH_4), and particles from micro-organisms (Glibert, 2020; Heederik *et al.*, 2007; Ni *et al.*, 2012). These pollutants are components of airborne PM and may affect the host's immune response via the respiratory tract (Miyata, 2011; Tripathy *et al.*, 2021). Exposure to PM related to traffic, wildfires, and biomass burning as well as biological PM from livestock has been associated with increased inflammatory and oxidative stress responses via various pathways, including AHR (Bekki *et al.*, 2016; Castaneda *et al.*, 2018; Liu *et al.*, 2020; Wu *et al.*, 2018).

In addition, urban PM has been associated with RA (Park *et al.*, 2021), with a proposed role of IL6 and COXII through mitogen activated protein kinase (MAPK) signalling activation (Tsai *et al.*, 2020). Furthermore, ammonia produced from the metabolism of urea from urine is known to activate inflammation pathways (Janus kinase (JAK)- signal transducer and activator of transcription (STAT) and MAPK) and increase inflammation markers (Wang *et al.*, 2020).

High ammonia emissions are present in swine hoop houses and the lagoons near the swine houses used in manure management systems (Liu *et al.*, 2014). Aerosolisation depends on the temperature of the manure and the air, wind velocity, and humidity, and these factors contribute to variations in reported data on ammonia emissions.

In a study in Eastern North Carolina, the populations with the greatest exposure to NH_3 lived within 1 to 5km from one or more swine CAFOs (Wilson, 2007). Ammonia is also a common component of chemical fertilisers, the use of which has been associated with RA in agricultural settings (Parks *et al.*, 2019).

In a study of large dairy farms with adjacent waste irrigated fields in Washington State's Yakima Valley, there were higher concentrations of pollutants associated with animal waste products, such as cow allergens and endotoxins, in the indoor- and outdoor-settled dust of proximal homes (≤0,4km) compared to that of distal homes (>4,8km) (Williams *et al.*, 2011).

Although water-based exposures from CAFOs are not well understood, inorganic and organic contaminants, coliphages, and hepatitis E virus have been reported

in surface water, groundwater, and water close to a CAFO (Brown *et al.*, 2020; Burkholder *et al.*, 2007; Casanova *et al.*, 2020; Gentry-Shields *et al.*, 2015).

Because the density of nearby CAFOs, the number of swine at each CAFO, and the number of lagoons at a CAFO may influence exposures to pollutants, we performed an additional analysis using a density function based on swine lagoon density (which we found to be proportional to the number of swine). The results from this sensitivity analysis not only confirmed the findings from our main analysis but also revealed two additional gene associations in white participants between ARNT SNPs rs1889740 and autoimmune diseases and ARNT SNPs rs11204735 and asthma.

Because the density of nearby CAFOs, the number of swine at each CAFO, and the number of lagoons at a CAFO may influence exposures to pollutants, we performed an additional analysis using a density function based on swine lagoon density.

Additionally, we observed similar environmental associations as those in the main analysis, with additional significant associations between CAFO lagoon density and IMD and asthma. These novel associations provide evidence that lagoon density should be considered when estimating the effects of CAFO pollutants on IMD and asthma (e.g., antigen release). These novel associations provide evidence that lagoon density should be considered when estimating the effects of CAFO pollutants on IMD and asthma (e.g., antigen release).

Potential limitations

This study has potential limitations. First, both the exposures and clinical outcomes were assessed at a single point in time when participants completed a self-reported survey and thus detailed data on the timing of exposures relative to the development of IMD are not available. Second, although the probability of misclassifying proximity to a CAFO is low as we excluded all incomplete addresses, we used residential proximity to the nearest CAFO as a proxy for exposure to a complex mixture of exposure components that include PM, VOCs, aerosols, and gases.

However, this metric does not capture any increase in exposures due to living in proximity to multiple CAFOs. Mixtures differ across individual CAFOs as they are affected by operation-specific and seasonal factors, including the number and life stage of swine kept at the facility, facility and waste system design, and local microclimate.

Additionally, due to the difficulty of estimating the operational dates of individual CAFOs and the multiple residential addresses provided by many participants, we did not model the length of time participants were exposed to a CAFO (i.e., the number of years each participant lived at their current address in conjunction with the number of years each CAFO was operational), which could influence cumulative exposures.

Further, proximity metrics do not capture the range of other agricultural exposures that may be common in areas hosting CAFOs, including chemical compounds and biological agents used as pesticides and fungicides, and their effects on the environment and personal microbiome of nearby residents. Third, self-reporting bias resulting from using data from questions about diagnoses by doctors or other health professionals may have affected our findings despite the measures we took to decrease the likelihood of misclassification.

We excluded approximately 15% of eligible participants due to missing data, most commonly because of missing data on IMD. Selection bias may have occurred due to missing data, and also because less healthy participants may have been more motivated to complete the Health and Exposure Survey than healthy participants. However, because the Health and Exposure Survey is administered confidentially and the study outcomes are chronic diseases and not isolated illnesses, it is unlikely that respondents inaccurately recalled past events.

Fourth, our cohort may not be representative of the overall population of North Carolina, potentially limiting the generalisability of the findings. Fifth, because we defined IMD to include relatively common conditions such as allergies, the majority of the study cohort was classified as cases (for an approximate 2:1 case-control ratio). This may have resulted in lower precision (greater standard error) and greater bias than would have occurred with more controls (Sturmer and Brenner, 2001).

Compared to the exposure cohort, the genotyped cohort was more likely to be male, Black/African American, younger, overweight/obese, and lower income. Although we adjusted for these factors in the regression models, these differences could lead to different apparent associations in the two cohorts by influencing underlying disease risks.

Accordingly, we did not perform additional environment-only analyses on the smaller exposure analysis cohort. Due to the number of tests performed, we did not fit models assessing GxE interactions on an additive scale. Finally, the sample size for some phenotypes was small, limiting the accuracy of statistical estimates.

Although we adjusted for basic demographic factors (sex, race/ethnicity, income, and BMI) and rural/urban residence, we did not consider other determinants of personal exposures, including farm work or other agricultural exposures. Larger, prospective studies are needed to confirm our results and identify additional genetic and environmental risk factors and mechanisms for IMD.

Conclusion

Our results suggest interactions between living in proximity to a CAFO and IMD and the potential role of the AHR-ARNT pathway in conferring increased risk. We report the first association of intronic ARNT SNPs rs11204735 and rs1889740 with RA. Our findings contribute to the growing body of evidence indicating that it is essential to manage emissions from CAFOs and minimise their public health and environmental impacts.

This article has been shortened for publication in *AFMA Matrix*. For the full article, contact details and references, visit *www.sciencedirect.com/science/article/pii/S0160412022006146?via%3Dihub.*

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AFMA INTERVARSITY WRITER'S CUP 2023: WINNER ROUND 2 / LITERATURE REVIEW

Vitamin D-elight

By Gerhard Claassen, University of Pretoria, Chemuniqué (Pty) Ltd

Vitamin D nutrition does not demand the same amount of attention at the table of nutritionists as its more 'important' nutrient counterparts such as carbohydrates, amino acids, minerals, and enzymes. Vitamin D is often formulated into diets as a one-size-fits-all solution; however, there is much more to the story. Contrary to popular belief, vitamin D is not a single entity, but rather a description for a group of analogues that share a similar structure and function.

All vitamin D analogues perform the same functions in the body, although they differ physiologically in their metabolism and their homeostatic regulation. The purpose of this review is to highlight the importance of vitamin D in animal nutrition and to give new insight into vitamin D analogues, their metabolism, regulation, and their application in industry.

Background

Vitamin D is a series of fat-soluble steroid derivatives that have the physiological ability to perform hormonal functions when in their active state (Adams and Hewison, 2010; De Paula and Rosen, 2012). Vitamin D can be broadly grouped into ergocalciferol or vitamin D_2, which is the predominant form in plants, and cholecalciferol or vitamin D_3, which is the predominant form in animals.

Approximately 90% of vitamin D is present in the body as vitamin D_3 and only 10% as vitamin D_2 (Ibrahim *et al.*, 2022). Both vitamin D_2 and D_3 are biologically inactive prohormones and do not perform any physiological functions in the body. These molecules can only act upon receptors and initiate the cellular processes associated with the well-known functions of vitamin D once they have been metabolically altered in the kidney or liver (Del Valle *et al.*, 2011; Bilezikian *et al.*, 2021).

Vitamin D_2 and D_3 are only the precursor molecules for 1α,25-dihydroxyvitamin D_3, the active form of vitamin D in mammals – better known as calcitriol. Calcitriol exerts its effects by binding to the vitamin D receptor (VDR) of target genes that are located on numerous types of cells and tissues across the body (Pike *et al.*, 2017).

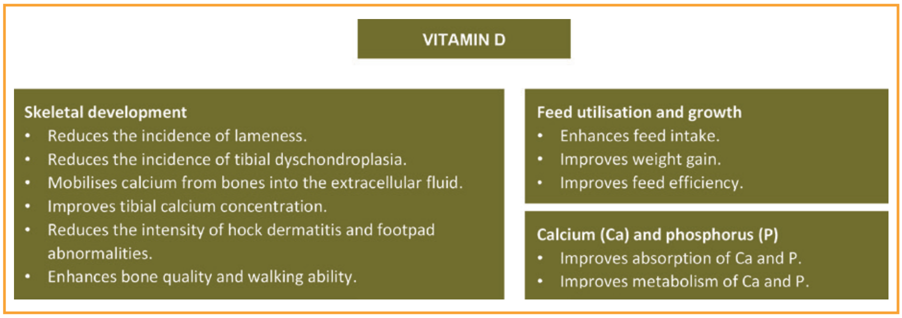
The complete scope of vitamin D target genes has not been defined in animals; however it has been shown that approximately 3% of the human genome is regulated by VDR (Caprio *et al.*, 2017), which includes numerous cells and tissues such as those of the bone (Goltzman, 2018), kidney (Yang *et al.*, 2018), intestine (Wang *et al.*, 2012), immune system (Colotta *et al.*, 2017; Koivisto *et al.*, 2020; Martens *et al.*, 2020), cardiovascular system (Wu-Wong *et al.*, 2006; Norman and Powell, 2014), and brain (Eyles *et al.*, 2014).

Although vitamin D is used by the body for a variety of purposes and in many tissues, its role in the absorption of calcium (Ca) and phosphorus (P) is one of its most vital functions and is of the greatest importance to the animal nutrition industry. Several analogues of vitamin D are commercially available, all of which differ in their efficacy and ability to regulate intestinal Ca and P absorption. The rest of this review will focus on the different analogues of vitamin D and how producers can benefit from vitamin D supplementation.

Vitamin D metabolism

Vitamin D_3 is non-essential and can be naturally synthesised in the skin from

Figure 1: The physiological functions of vitamin D (adapted from Khan *et al.*, 2021).

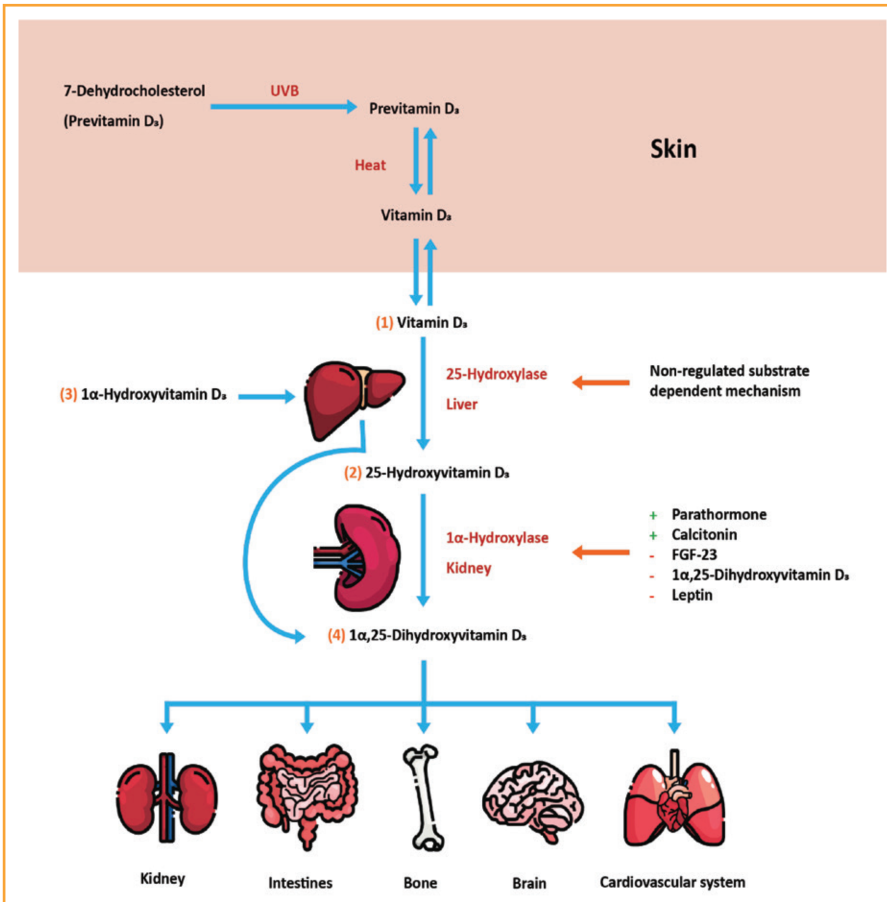


cholesterol upon radiation by ultraviolet (UV) light (Jäpelt and Jakobsen, 2013). In the skin, previtamin D_3 is formed from 7-dehydrocholesterol upon radiation from UVB light (Tian and Holick, 1995). This presents a problem in most modern poultry and pig production systems where animals are kept entirely indoors and only receive limited, if any, UV exposure, and thus diets must be supplemented with synthetic forms of vitamin D_3 to meet vitamin D requirements.

After the formation of previtamin D_3, the molecule undergoes thermal isomerisation to produce vitamin D_3 (Tian and Holick, 1995), which is biologically inactive. The hydroxylation of vitamin D_3 to 25-hydroxyvitamin D_3 is the first of two hydroxylation steps required to transform vitamin D_3 into 1α,25-dihydroxyvitamin D_3, the hormonally functional form of the molecule. This step can be mediated by several hydroxylase enzymes; however, the CYP2R1 enzyme has been identified as the main responsible enzyme and is located in the liver. This enzyme is not regulated, but rather is reliant on a substrate-dependent mechanism (Saponaro *et al*., 2020).

The second hydroxylation step involves the addition of a hydroxyl group in the 1α-position to the 25-hydroxyvitamin D_3 molecule to create 1α,25-dihydroxyvitamin D_3, the physiologically active and functional metabolite of vitamin D. This is done mainly in the kidney by the 1α-hydroxylase enzyme, CYP27B1 (Bikle *et al*., 2018). Only after the second hydroxylation step is complete the molecule is able to bind to vitamin D receptors and perform the distinct functions of vitamin D.

Figure 2: The metabolism, regulation, and target tissues of 1α,25-dihydroxyvitamin D_3 (adapted from Saponaro *et al*., 2020).



Vitamin D_3

The Vitamin D_3 requirements of poultry are not well defined and there are some discrepancies regarding the true vitamin D requirements of broilers. Aviagen recommends that Ross broilers are fed 5 000 IU of vitamin D_3 per kilogram feed in the starter phase, gradually decreasing to 4 000 IU/kg in the finisher phase (Aviagen, 2022), while the recommendation of Cobb is 5 000 IU vitamin D_3/kg throughout the rearing cycle (Cobb-Vantress, 2022).

The National Research Council (NRC) recommendation for vitamin D_3 in broiler feed is 200 IU/kg (NRC, 1994); however, this figure is likely outdated as the genetic makeup of broilers have changed significantly over the past 19 years. Whitehead *et al.* (2004) found that up to 10 000 IU vitamin D_3/kg is required to maximise bodyweight and tibia breaking strength on day 14; however, 5 000 IU vitaminD_3/kg was sufficient to significantly reduce tibial dyschondroplasia (TD) and maximise tibia ash.

Fritts and Waldroup (2003) found that 2 000 to 4 000 IU vitamin D_3/kg was adequate to maximise tibia ash and reduce the incidence and severity of TD. A similar conclusion was reached by Sun *et al.* (2013), who found that 2 000 to 4 000 IU vitamin D_3/kg improved bird's walking ability and tibia quality, and reduced footpad or hock dermatitis.

Bodyweight (BW) and feed conversion ratio (FCR) were improved when 2 000 IU vitamin D_3/kg were added to the diet of broilers between day one and day 21 (Gómez-Verduzco *et al*., 2013). Rao *et al.* (2006) showed that tibia ash, feed efficiency, and BW gain were improved to the level of the positive control when 2 400 IU vitamin D_3/kg was added to the diet of broilers between day two and day 42; however, bone mineralisation only improved to the level of the positive control at 3 600 IU vitamin D_3/kg.

Later, Rao *et al.* (2009) showed that 1 000 IU vitamin D_3/kg was adequate to maintain bone mineralisation, BW gain, feed intake, and leg abnormality scores of birds that were fed diets containing 5g calcium/kg and 2,5g non-phytate phosphorus/kg up to five weeks of age. Colet *et al.* (2015) concluded that there is no added benefit on performance, TD incidence, bone quality parameters, or carcass yield in broilers between day one and day 21 when increasing vitamin D supplementation above 3 500 IU/kg.

From these studies it can be concluded that the vitamin D requirement of broilers is in the range of 1 000 to 4 000 IU/kg and that the 200 IU/kg recommended by the NRC can be treated as the minimum vitamin D requirement to prevent premature mortality; however, most research on this topic was performed more than a decade ago, with none being conducted in a South African commercial setting. Therefore, new research is needed to establish the exact requirement of broilers.

25-Hydroxyvitamin D_3

An advantage of 25-hydroxyvitamin D_3 (25-D_3) is that it is a polar molecule, affording it a higher solubility in aqueous solution and allowing the molecule to be passively absorbed in the small intestine, which improves its bioavailability (Guo *et al.*, 2018; Vazquez *et al.*, 2018). This is in contrast with vitamin D_3, which requires micelle formation for absorption.

Furthermore, vitamin D-binding proteins – the carrier proteins responsible for vitamin D transportation in the blood – have the highest affinity for 25-D_3, meaning that it is more effectively transported through the blood than vitamin D_3 (Soares Jr *et al.*, 1995); 25-D_3 also has a longer serum half-life than calcitriol, and is commonly used to evaluate an animal's vitamin D status (Sezer and Behzat, 2021).

The biological activity of vitamin D_3 is dependent on its ability to raise the circulating level of 25-D_3. When fed at the same rates, 25-D_3 supplementation consistently results in significantly greater blood concentrations when compared to vitamin D_3 alone (Turner, 2013). Vitamin D supplied in the form of 25-D_3 is also not dependent on hydroxylation by 25-hydroxylase enzymes; however, 25-D_3 requires hydroxylation by 1α-hydroxylases in the kidney, which is considered the rate-limiting step in the synthesis of the biologically active metabolite, calcitriol (Portale and Miller, 2000).

This is a result of the kidney's 1α-hydroxylase enzymes, which are tightly controlled by several hormones and serve as the primary site of 1-hydroxylation. Renal 1-hydroxylase enzymes have been reported to be upregulated by parathormone and calcitonin, while the enzyme is inhibited by fibroblast growth factor-23, calcitriol, and leptin (Saponaro *et al.*, 2020). However, 1α-hydroxylase activity have been reported in tissues of the skin, parathyroid glands, the macrophages of the immune system, and in bone mesenchymal stem cells (Adams *et al.*, 1983; Bikle, 1994).

Most producers aim to benefit from the advantages of 25-D_3 without completely replacing existing vitamin D_3 in diets and, therefore, 25-D_3 is commonly added to broiler diets as a supplement in addition to vitamin D_3, although it rarely serves as a complete replacement for vitamin D_3. 25-D_3 can potentially improve performance and cellular immune response, reduce the incidence of TD, and improve bone mineralisation in broilers (Fritts and Waldroup, 2003; Koreleski and Swiatkiewicz, 2005; Michalczuk *et al.*, 2010; Gomez *et al.*, 2012; Vazquez *et al.*, 2018)

1α-Hydroxyvitamin D_3

1α-Hydroxycholecalciferol (1α-D_3) is a synthetic form of vitamin D_3 that differs from vitamin D_3 in that it has been hydroxylated in the 1α position. After ingestion, 1α-D_3 is rapidly hydroxylated by liver 25-hydroxylase enzymes to produce the biologically active vitamin D metabolite, 1α,25-dihydroxyvitamin D_3 (Bouillon and Reid, 2013).

A great benefit of 1α-D_3 is that the metabolite has equal efficacy as the active metabolite, but is less expensive to synthesise (Warren *et al.*, 2020). 1α-D_3 has been used clinically for decades in patients with chronic kidney disease and to treat bone abnormalities and mineral imbalances (Vervloet, 2014). Vitamin D that is supplied in the form of 1α-D_3 requires 25-hydroxylation by the 25-hydroxylase enzymes of the liver, and therefore bypasses 1α-hydroxylation by 1α-hydroxylase enzymes in the kidney, which is regarded as the rate-limiting step in the synthesis of 1α,25-dihydroxyvitamin D_3 (Portale and Miller, 2000; Ringe *et al.*, 2005).

This is a benefit as 1α-D_3 can rapidly increase calcium uptake into the blood. However, in doing so, the molecule bypasses a critical regulatory step in the metabolism of vitamin D, thereby increasing the probability of hypercalcemia and vitamin D toxicity (Warren *et al.*, 2020). Because of the possible undesirable effects of 1α-D_3, the typical dosage is between 2,5 µg/kg and 10 µg/kg, which is considerably lower than that of vitamin D_3 and 25- D_3.

1α-D_3 has been shown to improve blood-ionised calcium status during the starter phase, reduce the incidence of TD, improve bone mineralisation and performance, as well as upregulate genes responsible for calcium and phosphorus absorption (Ebrahimi *et al.*, 2016; Han *et al.*, 2016; Han *et al.*, 2017; Han *et al.*, 2018; Yang *et al.*, 2019; Landy *et al.*, 2020; Warren *et al.*, 2020).

1α,25-Dihydroxyvitamin D_3

1α,25-Dihydoxyvitamin D_3 (1α,25-D_3) is the physiologically active metabolite and is also commercially available. 1α,25-D_3 is unregulated and able to bind to VDR of several target genes across the body and immediately initiate the physiological effects of vitamin D. However, it is more costly to produce than the other analogues that offer similar benefits (Warren *et al.*, 2020).

The benefits of including 1α,25-D_3 include improved performance and bone mineralisation, reduced bone abnormalities, and mitigation of the effects of calcium and phosphorus restriction on bone development (Roberson and Edwards, 1996; Vieites *et al.*, 2018; Wu *et al.*, 2022).

Conclusion

Vitamin D is a series of fat-soluble steroid derivatives that perform hormonal functions when in their active state. Vitamin D_3 is the most common analogue of vitamin D that is supplied in broiler diets and requires two hydroxylation steps before becoming physiologically active. Several other analogues are commercially available, including 25-D_3, 1α- D_3 and 1α,25- D_3, which differ in their regulatory pathways and can therefore provide an additional benefit when supplemented into broiler diets.

Producers should consider both the benefits and drawbacks, the recommended supplementation levels, and the cost associated with each product before deciding which will benefit their production system the most.

For references and more information, email the author at gerhard@chemunique.co.za

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Effect of reduced concentrate supplementation rates on the production parameters of pasture-based lactating Jersey cows in winter

By J de Beer, L Steyn and JHC van Zyl, Faculty of AgriScience, Department of Animal Science, and T Kleynhans, Faculty of AgriScience, Department of Agricultural Economics, Stellenbosch University

Pasture-based dairy systems mostly rely on intake of pasture as the largest contributor to the diet. This is due to the fact that pasture normally has the lowest cost of all available feed resources (Bargo *et al.*, 2003; Abrahamse, 2009; Stojanovic, 2018).

Grazing dairy cows are often fed an additional concentrate supplement high in readily fermentable carbohydrates to increase pasture dry-matter intake (DMI) and milk yield. In the past, milk yield per cow and/or milk yield per hectare have been key indicators of the efficiency of pasture-based dairy systems (Bargo *et al.*, 2003).

The use and level of concentrate supplementation input has continued to increase over the last 20 years in an effort to maximise income through increased milk yield. Bargo *et al.* (2003) reasoned that although production cost is higher with higher inputs, the higher milk yield obtained as a result diluted the fixed input cost.

However, as Milkbench (2013) stated, an increase in concentrate supplementation to increase milk yield is only justified if both the milk price and the feed price are favourable. This concept has been challenged in countries

such as South Africa, where pasture-based dairy farms have struggled and are still struggling to remain profitable as purchased feed prices continue to increase.

Milk-to-feed ratios

Recently the Milk Producers' Organisation (MPO, 2021) reported dangerously low milk-to-feed price ratios of 1,04, 1,06 and 1,01 for November 2020, December 2020 and January 2021, respectively. The milk-to-feed price ratio steadily increased throughout 2021 to reach a maximum in June 2021 of 1,37. The ratio, however, decreased again to a lower level of 1,09 at the end of December 2021.

However, even with a more favourable milk-to-feed price ratio of 1,13, Meeske *et al.* (2006) found that the highest margin over feed cost (R583 cow/month) was obtained at a relatively low level of concentrate supplement feeding of 2,4kg cow/day. In comparison, the lowest margin over feed cost (R519 cow/month) was obtained on high levels of concentrate supplement feeding of 7,2kg cow/day (Meeske *et al.*, 2006).

Several studies reported that decreasing the amount of concentrate supplemented to lactating dairy cows improves the profit margin over feed cost when cows graze sufficient good quality pasture (Meeske *et al.*, 2006; Dillon, 2007; Hanrahan *et al.*, 2018).

The objective of this study was to determine the effect of reduced concentrate supplementation on the production parameters of pasture-based, lactating Jersey cows in winter conditions.

Table 1: Mean milk yield, milk composition and body parameters of cows receiving two different levels of concentrate supplement (n = 12).

Parameter*	Treatment[1]		SEM	P-value
	LC	HC		
Milk yield (ℓ cow/day)	19,98	20,38	1,13	0,60
ECM yield (kg cow/day)	22,24	22,91	1,19	0,70
FCM yield (kg cow/day)	20,37	20,41	1,22	0,98
Total solids (g/kg)	132,00	129,80	02,90	0,60
Milk protein content (g/kg)	37,80	38,10	0,90	0,82
Milk protein yield (kg cow/day)	0,73	0,77	0,03	0,33
Milk fat content (g/kg)	41,60	38,70	2,40	0,41
Milk fat yield (kg cow/day)	0,79	0,79	0,05	0,96
Milk lactose content (g/kg)	48,10	48,00	0,30	0,84
SCC (x 1 000 cells/mℓ)	313,04	168,92	101,34	0,33
MUN (mg N/dℓ)	9,11	6,56	0,29	<0,01
BW average (kg/cow)	391,31	399,08	9,29	0,56
BW change (kg/cow)	18,08	10,92	7,71	0,52
BCS average	2,43	2,27	0,06	0,07
BCS change	0,12	0,29	0,12	0,34

[1]LC = cows receiving 4kg concentrate supplement and 12kg DM pasture per day; HC = cows receiving 8kg concentrate supplement and 8kg DM pasture per day.
**ECM = energy corrected milk; FCM = 4% fat corrected milk; SCC = somatic cell count; MUN = milk urea nitrogen; BW = bodyweight; BCS = body condition score.*

Results and discussion

Fat and protein content

Milk yield, ECM yield and FCM yield remained unchanged regardless of supplement treatment (P > 0,05). Results indicate that yield can be maintained even at low supplementation levels. Similarly, Afzalzadeh *et al.* (2010) suggests that the optimal dietary non-fibrous carbohydrate (NFC) inclusion in dairy diets for maximum yield and digestibility is between 30 to 40% DM.

The total diet of cows in the LC treatment contained 665,62g NFC kg/DM (equivalent to 33,67% of total diet; *Table 1*). This suggests that the cows in the LC treatment did have enough NFC inclusion to sustain yield throughout the winter trial.

Treatment did not have an effect on either milk fat content (P = 0,41) or milk fat yield (P = 0,96). Meeske *et al.* (2006) and McEvoy *et al.* (2008) both reported no effect of concentrate supplementation rate on milk fat content of Jersey cows grazing high-quality pastures. Milk fat yields, however, increased linearly with increases in concentrate supplementation rate as a result of increased milk yields.

McEvoy *et al.* (2008) reasoned that the lower fibre content in high-quality pasture would result in low physically effective NDF, causing a decrease in milk fat content because of reduced rumination. Although herbage intake in the current trial was ± 2,97kg/day higher for cows in the LC treatment (*Table 2*), the possible lack of physically effective NDF resulted in an unchanged milk fat content.

Treatment did not influence either milk protein content (P = 0,82) or milk protein yield (P = 0,33). Milk protein content and total protein yield is mostly influenced by the amount of energy consumed, as well as the density and source of energy in the diet (Bargo *et al.*, 2003). Meeske *et al.* (2006) and Pulido *et al.* (2010) both concluded that concentrate supplement rate had no effect on milk protein content, provided cows have sufficient access to high-quality pasture. Therefore, although the concentrate supplement intake was 4kg/cow/day lower for cows in the LC treatment (*Table 1*), the milk protein content remained unchanged as cows were grazing a high-quality pasture.

Lactose and somatic cell count

Milk lactose content (P = 0,08) and somatic cell count (SSC; P = 0,3) were not affected by treatment. The milk lactose content in the current study ranged between 46,50g/kg to 48,60g/kg across cows in both treatments, which is within the expected range for milk lactose content of 47g/kg to 48g/kg (Costa *et al.* 2020). A SCC of ≥ 300 000 cells/mℓ milk is universally considered to indicate inflammation in the udder and prevalence of mastitis (Alhussien and Dang, 2018).

Although the mean SSC for the LC supplementation group was 313 040 cells/mℓ, the standard deviation was 101 340 cells/mℓ. This could be the result of one cow in the respective treatment having an abnormal SCC at the various times of milk sampling.

Milk urea nitrogen

Treatment influenced milk urea nitrogen (MUN) content (P < 0,01) significantly. Cows grazing high-quality pasture have a higher intake of total degradable CP, increasing circulating ammonia levels and MUN levels (Melendez *et al.*, 2000; Rajala-Schultz *et al.*, 2001). The rate and amount of carbohydrate digestion are primary regulators of microbial protein synthesis and therefore also MUN levels (Roseler *et al.*, 1993; Dunham, 1996). Thus, higher levels of CP in the pasture and decreased energy availability of cows on the LC treatment resulted in higher MUN levels in the milk.

Concentration of MUN varies between seasons, parity and breed, but the general accepted range for cows grazing pasture is between 10 and 16mg/dℓ of milk (Dunham, 1996). Thus, although MUN levels are elevated in cows in the LC treatment (9,109mg/dℓ versus 6,561mg/dℓ) it is still well within the acceptable MUN range, indicating that minimum dietary carbohydrate inclusion allows for efficient CP digestion and healthy microbial activity. Any fertility risk by elevated N-uptake (MUN > 16mg/dℓ) should therefore not be problematic when cows are fed lower levels of concentrate supplement (Rajala-Schultz *et al.*, 2001).

Body condition

Body condition is important during lactation. Cows on higher energy feeding levels tend to mobilise less adipose tissue to maintain condition. Thus, it could be expected that more adipose tissue mobilisation allowed cows in the LC treatment to sustain milk yield at similar levels to cows in the HC treatment. However, treatment did not influence BW (P = 0,56) and in fact showed a tendency for a higher BCS of cows in the LC treatment (P = 0,07).

Table 2: Mean (± SD) of herbage offered, herbage leftover, herbage intake, concentrate intake and total dry matter intake as affected by different concentrate supplementation rates.

Parameter*	Treatment[1]		SEM	P-value
	LC	HC		
Herbage offered (kg DM cow/day)	12	8		
Herbage intake (kg DM cow/day)	8,20 ± 1,39	5,23 ± 0,99		
Herbage left over (kg cow/day)	1,84 ± 1,58	2,72 ± 1,07		
RPM (post grazing)	7,47	8,09		
Concentrate intake (kg DM cow/day)	3,6	7,2		
TDMI (kg DM cow/day)	11,8	12,43	0,073	<0,01
MR (kg cow/day)	1,06	0,53	0,130	0,100

[1]LC = cows receiving 4kg concentrate supplement and 12kg DM pasture per day; HC = cows receiving 8kg concentrate supplement and 8kg DM pasture per day.
**RPM = rising plate meter; MR = milk response; TDMI = total dry matter intake, calculated by assuming animals consumed all concentrate offered and by adding the offered concentrate allowance to actual herbage intake, which was calculated by the RPM regression.*

Furthermore, BW change and BCS change were not affected by concentrate supplementation rate (P > 0,05). Cows in the LC treatment did indeed have adequate dietary energy uptake to sustain yield without excessive mobilisation of adipose tissue.

Pasture intake

Although concentrate supplementation rate and herbage offered per treatment differed, the total DM offered was 16kg DM/day for cows in both the LC and HC treatments. However, treatment affected total dry matter intake (TDMI) and cows in the HC treatment had a higher TDMI compared to cows in the LC treatment (P < 0,01; *Table 2*). Increasing concentrate supplementation rate increases SR, therefore decreasing the DMI of pasture (Joubert, 2012; Muller, 2017; Ramsbottom *et al.*, 2015).

Pasture allowance, however, differed between treatments with cows on the LC treatment being allocated more pasture than cows on the HC treatment. It is well known that increased pasture allowance leads to a decreased SR (Robaina *et al.*, 1998; Bargo *et al.*, 2002). Therefore, although concentrate supplementation increased from the LC to HC treatment, the pasture allowance decreased, resulting in both treatments only consuming ± 68% of total herbage offered. The increase in TDMI is therefore related to higher concentrate supplementation offered between the treatments. An RPM reading between 10 and 12 indicates well utilised pasture (Irvine *et al.*, 2010).

Thus, the low post-grazing RPM reading recorded (*Table 2*) indicates that pasture might have been overgrazed and could possibly explain the overutilisation of herbage offered for both treatments. The RPM regression used was for total predicted pasture yield and could have led to smaller pasture allowances than what was predicted per treatment group.

Conclusion

Milk yield, milk fat content, milk fat yield, SCC, BW and BCS were not affected by the treatment in the winter trials. MUN was affected by concentrate supplementation rate in the winter trial, but remained within the acceptable MUN range. There was no benefit of increased milk yield or milk composition during the winter trial for cows receiving 8kg/cow/day.

As such, feeding lower rates of concentrate supplement (4kg/cow/ day) and increasing the pasture allowance could be a viable method to ensure better margins over feed cost and possibly increase profitability per hectare.

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Producers remain the heroes in any great silage story

By Susan Marais

If the silage-making process was equated to a story the producer would be the hero and oxygen would be the enemy. "Oxygen is the enemy and producers need to get it out and keep it out if they want to make good silage," said Dr Paolo Fantinati, head of technical services EMEA at Chr. Hansen, during an Envarto farmers' day held at the Bella Rochelle Wedding Venue near Heidelberg, Gauteng in February this year.

"Plants and microbes are alive and therefore they need oxygen. This is something we cannot avoid. However, the less time it takes for a producer to make his silage and seal the bunker, the better," Fantinati said. "Oxygen that is left over in silage must be consumed as soon as possible to ensure that the anaerobic microbes can do their job. These microbes cannot grow where oxygen is present."

The two periods in silage-making that require extra attention are during the initial stages (harvesting and silo filling) and at the end when the silage is opened and reopened, Fantinati added. "Even a product that looks to be high quality can contain mould and yeast. These micro-organisms can thrive as long as oxygen is present. This is why producers should do everything in their power to expel oxygen as soon as possible."

The problem with poorly made silage is that moulds and yeasts can proliferate quite quickly and some of them can release mycotoxins that negatively affect the animal, weakening its immune system and giving rise to diseases such as mastitis in cows. This is why it is important that silage is made as quickly as possible and that it should not be left unattended when opened.

Help during uncertainty

Dr Hannes Viljoen, managing director of Envarto, said the future of plant and animal health is currently uncertain. "The challenges posed by the prevalence of antibiotic resistance in animals and humans are a problem that can be addressed through innovation and scientific research."

Viljoen added that the sustainability of the animal production sector depends on this research. "End consumers are struggling with issues such as disease and population growth. Therefore, it is critical that producers, nutritionists and veterinarians take hands in order to find solutions to the challenges producers face daily."

The speakers for the day (from the left) were Hestia Pienaar, agronomist at Agri Technovation, Dr Paolo Fantinati, head of technical services EMEA at Chr. Hansen, Dr Hannes Viljoen, managing director of Envarto, Dr Ida Linde, livestock scientist at Envarto, Willie Rossouw, national sales manager at Envarto, and Dr Bruno Cappellozza, product manager for beef and dairy DFM at Chr. Hansen.

Numerous producers from Nigel attended the Envarto farmers' day. Here are (from the left) Jaco Barnard, Martin Pistorius, Daan du Toit, JP Warrant and Hentri van Niekerk.


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Agri Technovation agronomist, Hestia Pienaar, said while most things in agriculture are beyond the control of producers, they can control agronomical factors with the help of technology. With the support of agronomists and technological advances, modern-day producers can get a clear indication of exactly what their soil looks like on any given day.

"Nowadays software programs can help farmers to determine their soil's compaction depth. Through these programs producers are not only able to save on fuel – they can also ensure minimal disturbance of the soil."

Pienaar added that it was important that soil is classed before the producer decides what to plant. "Nowadays it has become a trend for farmers to plant nut trees at the outposts of their farms. However, it often happens that those areas really aren't suitable for the specific type of tree that is planted," Pienaar said, adding that this disregard for scientific knowledge comes at a high price.

"When producers adhere to technical know-how, they won't plant the wrong cultivar and they will save a lot of money when it comes to inputs, as they won't for example use more fertiliser than is necessary." Along with the rising cost of inputs it has become more important than ever that producers know where their high- and low-potential soil lies.

How probiotics can help our heroes

Dr Bruno Cappellozza, manager of beef and dairy products at Chr. Hansen, said it is crucial that producers ensure they make high-quality silage else their animals would pay the price. "Healthier feed will ensure healthier cows, which will lead to better production."

This is where probiotics came in. "We still need to keep studying and learning about humans' and animals' gut systems, but it is becoming clear that the gut composition can support health, and probiotics can also be a support tool in this regard. Probiotics, which are live bacteria, can help the animal by inhibiting potentially harmful bacteria, supporting the health of the herd."

Cappellozza said that scientific research has indicated that for every one day a calf is sick during the pre-weaning period, she may produce up to 202,5kg less milk per lactation. A nutritional alternative that supports the health of the herd may therefore also support the productive performance of the animals.

Dr Ida Linde, livestock scientist at Envarto, recently completed her doctoral studies in livestock production, studying the effect of Chr. Hansen's probiotic, Bovacillus, on various South African livestock. "My research showed that there was definitely a health benefit to using probiotics," Linde said, adding that there is a clear correlation between gut and overall health of the animal.

"In one of the trials (a milk goat trial) the farmer could observe that the animals which were given the probiotic had visibly whiter coats. Their hair was also much softer to the touch than the control group."

Heidelberg producer, De Wet Venter, and Werner van der Merwe, representative of Silostop.

Several of AFGRI's technical advisors were also in attendance. Here are (from the left) Leon Botha, Henco Human, Chris Cilliers, Adriaan Steenkamp and Dieter Fleischmann, AFGRI Animal Feeds' national sales and marketing director.

Distribute labour wisely

With regard to the role of labour in successful silage-making, Fantinati said it was also important to deploy the best workers or drivers at the silage bunker, rather than in the field. "Producers will usually decide to have their most skilled drivers on the field, but that person should actually be the one compacting the silage. The most trustworthy person should be there, because he or she should be able to drive the tractor at no more than 4km/hour, compressing no more than 10cm of silage at a time."

It is also crucial that producers avoid building round mountains of silage, and rather try to create a concave shape. "My advice would be to first try and make the silage as flat as possible and then start making it more concave towards the ends."

For more information, visit *www.envarto.co.za.*

DSM breaks new ground with 4th generation phytase

By Elmarie Helberg, Plaas Media

Recently, DSM put innovation into action with the launch of their new fourth-generation phytase, HiPhorius™ which assists in reducing feed cost while improving animal performance.

In his address during the event Raymond du Plessis, performance solutions and precision services manager at DSM, said: "With raw material prices on the rise, we are bringing a product to the market that's got higher efficacy in phosphorous release, therefore resulting in reduced feed cost. Moreover, it improves performance in broilers, layers, swine and fish, bringing more profitability to farming enterprises."

Carrie Walk, principal scientist: global innovation team at DSM, says phytase has a direct effect on the release of phosphorus and myo-inositol in animals, as well as an indirect effect on amino acids, minerals and energy.

HiPhorius™ enables animals to better utilise the naturally occurring phosphorus in feed while reducing the need for inorganic phosphorus supplementation. The product enables producers to increase efficiency, improve thermostability and it gives them access to digital services, assisting them in reducing the cost of feed.

The product utilises end-to-end digital services for intelligent phytase nutrition, thus optimising value for customers by enabling better decision-making and customised application. The upgraded Phytase Web Matrix Calculator is one of the digital services DSM provides. Along with being a matrix calculator, this tool demonstrates the concept of intelligent phytase nutrition while also providing a sustainability and ROI calculator.

Industry challenges

Speakers on the day of the launch also covered commodities and gave an overview of the poultry industry, demonstrating how HiPhorius™ fits into the drive to improve profitability in agriculture.

Johan Geldenhuys, Pimankus hedging manager and market analyst, provided some feedback on the raw material price expectations in the upcoming months and how it will affect the entire sector. He touched on the Russia-Ukraine war, the South African political situation, Eskom and the demand from China. He also mentioned that the cabinet reshuffle did not impress the markets and the GDP was worse than expected given that load shedding affects all sectors.

Dr Abongile Balarane, Egg Organisation general manager at the South African Poultry Association, also gave perspective on the poultry market. With a gross turnover of R11,44 billion at producer level, eggs retain their position as the fourth largest animal product sector in agriculture in South Africa, after poultry meat (R50,96 billion), beef (R43,01 billion) and milk (R21,17 billion).

The total value at retail level was estimated to be R26,27 billion for 2022. Approximately 723 million dozen eggs were sold during the year through various channels. The average price for layer feed in 2022 increased by 19,8% to R5 712/ton, which followed an 18,8% increase the previous year.

Poultry industry challenges

According to Balarane, in January 2021, South Africa's national flock size was estimated at 28 million birds. However, between April 2021 and September 2022, around 2,8 million birds were culled due to highly pathogenic avian influenza (HPAI).

Together with yellow maize and soya bean prices having risen by 33 and 36% due to the ongoing Russia-Ukraine war, fuel prices that have risen 14% in 2022 to date, the electricity tariff that has increased by 9,6% since January 2022 and Eskom's intention to increase tariffs by 32% in 2023 – all these factors put additional strain on the industry.

"These issues harm South Africa's egg producers. The cost of feed, fuel, electricity and labour alone constitute around 80% of the variable costs for the egg producer. With the ongoing uncertainty regarding HPAI, local egg producers remain in a very difficult situation."

Balarane predicts 2023 to be turbulent for the country's producers, putting the nation's food security under threat. "Consumers are likely to be confronted with egg shortages and higher retail prices due to the current feed, electricity and fuel challenges."

According to Raymond du Plessis, performance solutions and precision services manager at DSM sub-Saharan Africa, farming enterprises have become increasingly marginal, driving the need for products that produce more with less.

Visit *www.dsm.com* for more information.