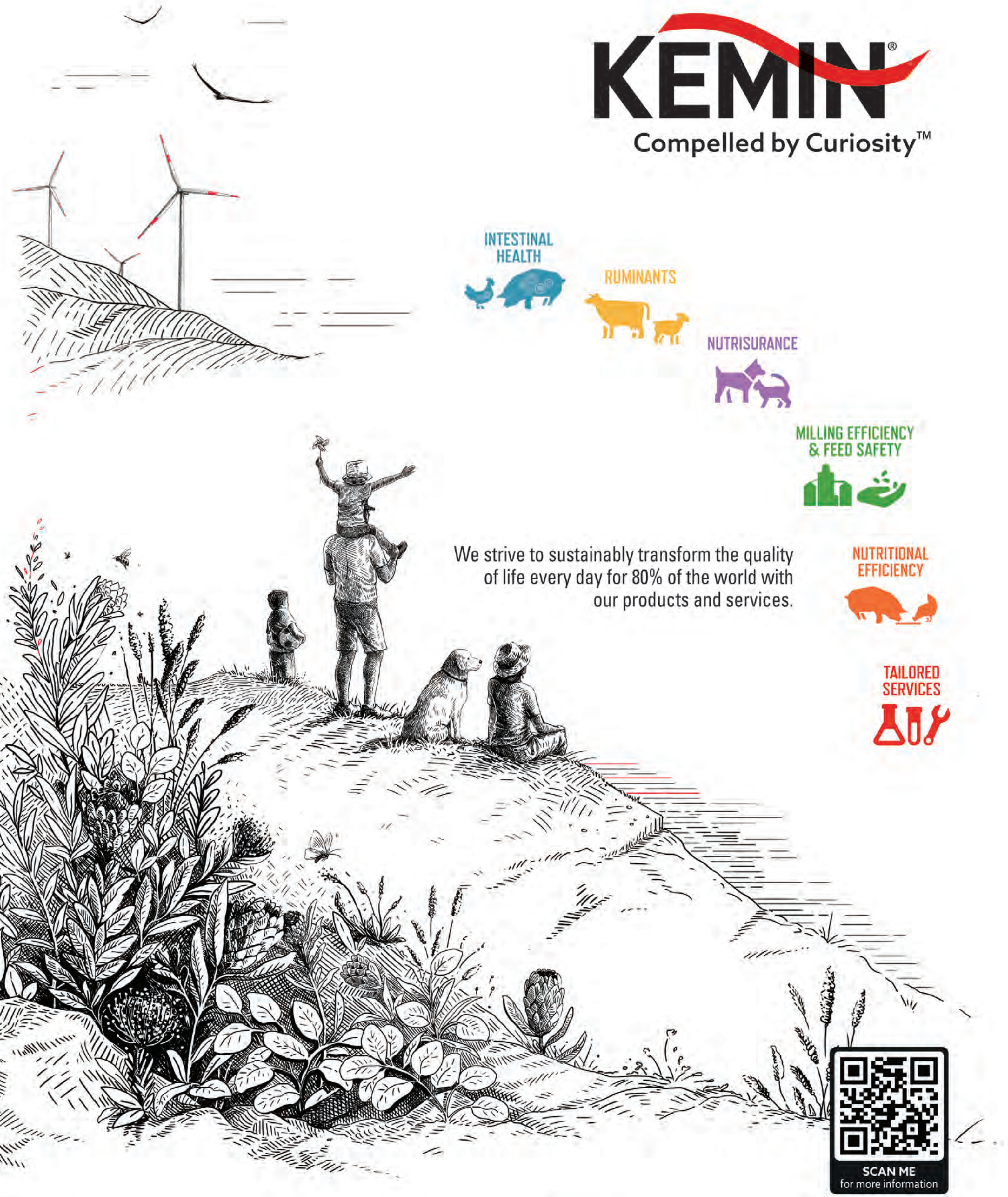
Jul - Sep 2024
Vol 33 No 3
ISSN 2308-0647

Matrix

AFMA
SAFE FEED FOR SAFE FOOD
Animal Feed Manufacturers Association

Quarterly magazine of the Animal Feed Manufacturers Association

Feed Miller Short Course • AMR workshop • Green soya beans • Aflatoxin levels
Solid-state fermentation • Ethoxyquin • Calf diarrhoea • Diurnal emission
Writer's Cup: Fulvic acid | Green seaweed


KEMIN®
Compelled by Curiosity™
INTESTINAL HEALTH
RUMINANTS
NUTRISURANCE
MILLING EFFICIENCY & FEED SAFETY
We strive to sustainably transform the quality of life every day for 80% of the world with our products and services.
NUTRITIONAL EFFICIENCY
TAILORED SERVICES
SCAN ME
for more information


30 jaar/years
bester
Progress is a mindset
Celebrating 30 years of specialisation
Bester Feed & Grain specialises in the supply chain management of feed commodities.
We supply the following:
• Maize
• Soya Products (Meal & Fullfat)
• Sunflower Meal
• Vitamins & Minerals
• Urea & Phosphates
• Amino Acids
• Chop & Bran
• Fishmeal
Contact us
+27 (0)21 809 2500 | info@bester.co.za
www.bester.co.za

The role of feed safety in food safety

By Liesl Breytenbach, executive director, AFMA

The world celebrated Food Safety Day on 7 June. Various World Health Organization (WHO) member states supported this day to draw attention to food safety incidents around the globe, and inspire action to help prevent, detect, and manage foodborne risks and their dire impact on public health. This year's theme focussed on 'preparing for the unexpected' and countries around the world geared up to educate the public on issues of concern, mobilise political will and resources to address the problems, and reinforce best practices.

With an estimated 600 million cases of foodborne illnesses and approximately 420 000 deaths associated with eating contaminated food annually, unsafe food is a global threat to human health, economies, and food security. Children under five years of age carry 40% of the foodborne disease burden, with 125 000 deaths reported every year.

Every food chain link matters

A food incident can happen due to accidents, inadequate controls, food fraud, or natural events, and requires dedicated efforts from policymakers, food safety authorities, producers, and food and feed business operators. "Every link in the food chain matters" was the aptly named theme of a local Food Safety Summit held in Johannesburg in June in support of the global initiative. I was privileged to participate in the programme and share best practices in feed safety and its role in food safety.

Food safety has a critical role in assuring that food stays safe at every stage of the food chain – from production, harvest and slaughtering, processing, storage, and distribution to the consumers' table. Food safety incidents around the world have caused consumers to be increasingly concerned about the safety of their food supply. Hence, they are looking for assurances that safety measures are in place throughout the food chain.

Significant changes in consumption patterns within the global food system can mainly be attributed to increased urbanisation and income. The Organisation for Economic Co-operation and Development (OECD) and Food and Agriculture Organization (FAO) *Agricultural Outlook* predicts that the global average consumption of meat will increase by 2,5% over the next ten years, amounting to an increase of 0,7kg/capita/year. This will lead to an overall growth in intensive livestock production, especially in developing countries, that will require an additional amount of animal feed to be produced.

The challenge is not only to meet the growing demand for feed but to ensure that it is wholesome and safe for the animal, as well as free of undesirable or toxic substances that could compromise the quality and safety of animal-derived foods.

Feed safety is a prerequisite

Feed is an integral part of the food chain, and its safety has been recognised as a shared value and responsibility. The main purpose of animal feed is to provide nutrition to support the production of meat, milk, and eggs. Unfortunately, feed can also be a source of contaminants that may adversely affect the health and production of farm animals, as well as the safety of animal-derived foods. Feed production must therefore be subjected, like food production, to quality assurance of an integrated food safety system.

Hazards may be introduced with source materials or via carryover or contamination of products during handling, storage, and transportation. Biological, chemical, and physical contaminants present at significant levels, as well as essential nutrients above recommended levels in feeds, are likely to harm livestock or people consuming animal-derived foods. Examples of known hazards in feed include pesticide and veterinary drug residues, dioxins, mycotoxins, heavy metals, zoonotic bacterial pathogens, and prions.

Additionally, innovations and novel ingredients such as agro-industrial by-products, insects, food processing by-products, and food waste all pose new threats, and it is not always clear on how to deal with the risks associated with it.

Industry codes of conduct

Each stakeholder in the agri-food value chain is responsible for ensuring that their link is not a particular source of contamination, and co-operation between these organisations and individuals through the use of industry-specific codes of conduct and food safety management systems to address hazard control, must be encouraged.

Consumer confidence in agri-foods will be enhanced with the knowledge that the agri-food value chain understands the importance of sharing the responsibility of product safety and meeting the requirements of products supplied to the subsequent step in the food chain. The grains and oilseeds passport system is a practical example where producers, silo owners, and millers must agree on implementing a shared system of standards and compliance to contribute effectively towards the management of hazards in grains and oilseeds that enter the feed and food chain.

Other examples of industry codes and best practices exist independently within the agri-foods chain, such as the Animal Feed Manufacturers (AFMA) Code of Conduct, South African Pork Producers' (Sappo) Pork 360, Agbiz Grain food safety system, and veterinary authority certifications. However, these systems need to be integrated to enhance their overall impact on agri-food safety, and ensure consistent and vigilant oversight.

Food safety is a shared responsibility between governments, producers, and processors. Specific focus is also needed on how these assurances are communicated to the consumer to encourage their trust and understanding regarding the provision of safe food. Misinformation in the media regarding animal-derived foods is rife, and it will take a collective effort to combat this with accurate information and build consumer confidence in milk, meat, and egg products.

On the cover:
Chem Nutri Analytical
+27 11 316 8800
info@chemnutri.co.za
www.chemnutri.co.za
Cedar Lake Industrial Park, c/o M57 and Porcelain Road, Olifantsfontein, Gauteng, RSA

CONTENTS

EDITORIAL COMMITTEE

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

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

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

Deputy editor: Jayne du Plooy
jayne@plaasmedia.co.za

News editor: Elmarie Helberg
elmarie@plaasmedia.co.za

Design & layout: Inge Gieros
inge@plaasmedia.co.za

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

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

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

Printed and bound by:
Ndabase Printing Solutions
+27 12 343 6291

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

NEWS & VIEWS

Important dates on the AFMA calendar

7 August
AFMA Golf Day, Centurion Country Club, Pretoria

5 to 6 September
AFMA Annual General Meeting, Cathedral Peak Hotel, KwaZulu-Natal

31 October
AFMA Symposium, CSIR, Pretoria

Russia clinches more grain control

Major Western merchants including Cargill Inc and Viterra pulled back from Russia last year after government pressure to make way for local firms. Now, even the country's top private trader is finding it tough to operate amid a spat with the state. That puts the market in the hands of fewer companies, some that have or had links to the Kremlin.

The consolidation picked up after president Vladimir Putin invaded Ukraine and left four firms responsible for three-quarters of grain exports from Russia's Black Sea terminals, giving Moscow more influence over wheat supplies which is essential to taming global food inflation. "Russia's desires to control the commodity world are real, and their influence on grains is growing," said Dan Basse, president of Chicago-based consultant, AgResource.

Cargill, Viterra and Louis Dreyfus Co stopped sourcing grain in Russia for export last year. They were previously among the top ten exporters.

While Western traders still buy cargoes from Russian ports, getting information on things like crop volumes and conditions, stockpiles and exports is harder since they curbed business there. That could become a bigger worry as harvest setbacks leave Russia with less wheat to export. The International Grains Council expects Russia's wheat output to fall about 6% this year.

A key question is how Russia's grain consolidation will impact the world market. It is already trying to implement an unofficial minimum price for its crops and stronger control of the grain sector will make it easier for the government to influence supplies.

The top four Russian traders now control 75% of exports from Russia's Black Sea terminals, up from 45% six years ago, according to Dmitry Rylko, director of Moscow-based consultant IKAR. – *Yahoo! Finance*

KAL Group grows profit

The KAL Group weathered harsh economic conditions in the six months to March 2024 with an increase in earnings and dividends to shareholders, as well as a significant debt reduction. This paved the way for cautious optimism by the group for the coming six-month period.

Profit before taxation increased by 9,7% to R458,5 million (2023: R417,8 million) while gross profit grew by a healthy 8,7% to R1,65 billion (2023: R1,52 billion). Like-for-like expenses grew by only 6,4% and despite high interest rates, net interest earned increased. Headline earnings per share increased by 7,3% to 408,74c/share while recurring headline earnings per share grew by 7,1% to 408,74c/share. An interim dividend of 54c/share (2023: 50c) was declared, an increase of 8%.

The group's continued commitment to reducing gearing levels resulted in net interest-bearing debt reduced by an effective R360 million compared to the same period last year with net interest-bearing debt to equity reducing to 56,5% (2023: 73,8%). – *KAL Group*

First yellow maize imports in five years

The Crop Estimates Committee (CEC) recently estimated the South African maize crop to be at 13,4 million tonnes – down 19% from the year before.

While the global supply of white maize is limited and comes at a price, yellow maize is freely available to import and equally important to combat rising prices. It serves as the primary input for feed rations to among others the broiler industry, which provides the cheapest animal protein for many low-income households.

The Western Cape has a strong feed manufacturing and broiler industry. No maize is produced in the Western Cape but has to be transported from the Northern Cape or the southwestern Free State. Because of high inland transportation costs, it is usually more cost-effective to import yellow maize via the Port of Cape Town.

COFCO, a multinational grain trading company and member of the South African Cereals and Oilseeds Association (Sacota), led the initiative to book the first yellow maize import vessel from Argentina in five years, offloading its cargo of approximately 33 000 tonnes of yellow maize at the end of April. Seaboard Trading is also importing a vessel with a combined cargo of yellow maize, soya bean oilcake, sunflower pellets and soya bean hulls. – *SA Grain*

Natural feed additives could replace antibiotics

Feed additives, such as essential oils and probiotics, can boost broiler growth and health without the use of antibiotics.

"This small-scale study offers preliminary evidence supporting the use of feed additive supplementation as an alternative to antibiotic growth promoters (AGPs) in broiler production, potentially contributing to more sustainable practices. The research focussed on a descriptive analysis of the chicken excreta microbiota following a single experiment involving two feed additives (a probiotic and an essential oil blend)," explained Erika Ganda, assistant professor of food animal microbiomes at Pennsylvania State University, and Ana Fonseca, graduate assistant in Ganda's research group.

The research team found that supplementing the diets of broiler chicks with probiotics over 21 days increased the abundance of good intestinal micro-organisms.

Research into probiotics and essential oils is still in its infancy. More work is needed to better understand how these natural feed additives impact broiler health outcomes, as well as the gastrointestinal tracts of poultry. – *Feed Strategy*

Zim takes lion's share of SA maize exports

Data from the South African Grain Information Service (Sagis) shows that in the 2023/24 marketing year, maize exports amounted to 3,4 million tonnes, down 6% from the previous year.

In the past, South Korea, Japan and Taiwan were the leading markets for South Africa's maize exporters. However, in the 2023/24 marketing year, Zimbabwe took the lion's share of the exports, accounting for 18% of the 3,4 million tonnes of exports.

The surge in exports to Zimbabwe comes after a few years of modest exports to the country because of decent domestic harvest and the restrictions on genetically modified (GM) maize, which the government often used as a barrier to imports in certain seasons. However, the regulations have changed, and Zimbabwe now imports GM maize. – *Agbiz*

China's feed exports rise on weak demand

China is sending record quantities of soya bean meal abroad, as a shrinking number of pigs and weak demand for pork force processors to export their surplus animal feed. China relies on vast amounts of soya beans from South America and the United States (US) which are crushed into meal for livestock and oil for cooking. But cash-strapped shoppers are not spending like they used to, and producers have reduced their herds because prices are too low.

That has left local soya meal prices hovering around three-year lows. Exports, meanwhile, climbed to almost 600 000 tonnes in the first four months of 2024, which is nearly five times the level of the previous year. Destinations include nearby Japan but also far-flung countries like Britain.

The nation's soya bean imports typically climb in the middle of the year. In addition, Chinese crushers will be looking at the effects of heavy rains on the South American crop, as well as rising tensions with the US, as reasons to keep purchases elevated and create a buffer in case supplies dwindle later in the year. – *The Star*

Bühler aims to implement food security projects

Bühler Southern Africa is deeply committed to addressing food security, both a regional and continent-wide concern. "We are actively participating in various discussions, highlighting the importance of this issue and our dedication to finding solutions," commented managing director, Marco Sutter.

As a result, the company has started 2024 in full swing. "Our mission is innovation for a better world. The food and mobility challenges we face demand innovative solutions, technologies, process solutions, and business models.

"We want to contribute to a sustainable world that affords the next generation the same chance to live and develop as today's society. The digitalised world we live in requires higher flexibility and agility to be successful and, as a result, a culture based on self-responsibility and collaboration."

Values are the basics of any company culture, and Sutter highlighted that Bühler Southern Africa cares equally for its customers and colleagues. Sutter affirmed that Bühler is the technology leader in the industry and sets new trends with its advanced solutions. – *Press release, Bühler*

Functional feed boosts sustainable aquaculture

Researchers have developed a novel functional feed ingredient from king oyster mushroom (*Pleurotus eryngii*) root waste and soya bean meal. This co-fermented protein shows promise in improving the growth performance, feed utilisation, immune status, and hepatic and intestinal health of largemouth bass, claims the Chinese team.

Functional feeds, which include bioactive compounds like polysaccharides and protein complexes from mushrooms, are being actively explored for their potential to transform aquaculture into a more sustainable industry.

Various studies have demonstrated the effectiveness of functional soya bean meal fermented by microbes like *Aspergillus*, *Saccharomyces*, *Lactobacillus*, and *Bacillus* in enhancing the growth and immunity of fish. Similarly, *Pleurotus ostreatus* byproduct fermented with *Lactobacillus* and yeast has been shown to improve body weight and immune response in Amur catfish, while fermented mushroom bran hydrolysate boosts the antioxidant capacity of carp.

The king oyster mushroom, already recognised for its health benefits, has been noted for its ability to modify gut microbiota, reduce inflammation in models of chronic colitis, and alleviate oxidative damage in Caco-2 cells. – *Feed Navigator* ❖


Cargill
BAR

Analysis of global animal feed production

By Lucius Phaleng, trade advisor, AFMA

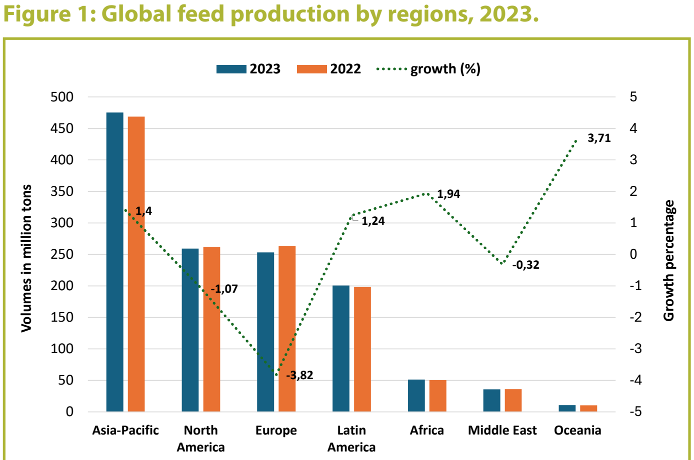
Global feed production saw a slight decline in 2023, totalling 1,29 billion tons – a reduction of 2,6 million tons or 0,2% from the previous year. This minor decrease reflects various factors such as economic instability, climate change impacts, and shifts in farming practices. Since feed production is crucial for livestock farming, which in turn affects the supply of meat and dairy products, this downturn could have significant implications for global food security and market dynamics.

It was indicated that the slower production of animal protein, in response to tight margins experienced by many feed and animal protein companies, also contributed to lower feed demand. Additional influences included inflation-driven consumption changes, high production costs, and geopolitical tensions.

Despite this, the industry has demonstrated resilience and an impressive capacity for recovery. Over the past decade, it has maintained an average annual growth rate of 3%, showcasing its ability to adapt and evolve in the face of challenges. This growth trend has been even more pronounced over the last five years, where the average annual growth rate has accelerated to 3,7%.

This steady growth amid adversity speaks volumes about the industry's capacity for evolution and its commitment to meeting the demands of an ever-changing market landscape.

Figure 1: Global feed production by regions, 2023.



Regional observations

In 2023, feed production rose in most of the world including the Asia-Pacific (1,4%), Latin America (1,24%), Africa (1,94%), and Oceania (3,71%) regions, while it fell in Europe (-3,82%) and North America (-1,07%). Latin America has consistently grown over the past decade, driven by its monogastric export market, and expanding aqua and pet food markets. That growth was led by the broiler sector, with an increase of 2,6%, and the pig sector, with an additional one ton. Aquaculture feed continued its strong growth trend, with an increase of 3,9%.

The Asia-Pacific region experienced a modest increase, despite being significantly affected by animal diseases. Conversely, Europe faced ongoing political and market pressures, particularly in the beef sector. North America saw a decline in feed mills and faced challenges in its beef sector due to drought and high production costs. Africa continued to grow but at a slower rate, with an increase of 1,94% or almost a million tons.

Trends affecting feed production

According to the latest *Alltech Agri-food Outlook*, global feed production is affected by various factors which include the rising costs of feed ingredients, labour, energy, and transportation. High prices for raw materials remain a challenge worldwide. In Europe, factors such as labour costs, policies and standards, and geopolitical events were significant contributors to feed production decline. In Latin America, geopolitical events and supply chain disruptions have been noted.

The spread of animal diseases such as African swine fever (ASF) and highly pathogenic avian influenza (HPAI) poses a significant challenge to the feed industry by affecting animal health and productivity, disrupting supply chains, reducing production efficiency, and necessitating changes in feed formulations. Geopolitical tensions also have a measurable impact on production costs and supply chain stability.

Adverse weather events linked to climate change, such as droughts, floods, frosts, heat waves, and an increase in pests, also affect production by impacting the availability, cost, digestibility, and nutritional value of feed crops and forages. For example, large parts of Europe have experienced water scarcity, worsened by increased competition for water and more frequent water-use restrictions.

Global outlook for 2024

Feed production is anticipated to increase in 2024, driven by improved market conditions as input costs decrease and consumers adapt to uncertainties. Robust production growth is expected in Brazil, with accelerated growth in Southeast Asia, while China and Oceania are likely to see modest increases. Latin America is projected to lead production growth in 2024 but at a slower pace than in 2023. In the United States, reduced livestock numbers are expected to lower feed demand.

Although beef and pork production are predicted to decline, poultry and aquaculture are expected to experience the strongest growth among the species. The 2024 global poultry market outlook is moderately positive, with an anticipated growth of 1,5 to 2%. This growth, while below the long-term average of 2,5% per year, represents a recovery from 2023's 1,1% growth. Most of the growth is expected in Southeast Asia, the Middle East, and Latin America, though at below-average levels.

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


cjp CHEMICALS
Driving Innovation and Excellence
a Bud Group Company
www.cjpchemicals.co.za

Compliance gets the AFMA mark of approval

By Joe Hanekom, managing director, Afri Compliance

The purpose of the Animal Feed Manufacturers Association's (AFMA) code of conduct audit is to verify whether the manufacturing facilities of members and associate members of the association do in fact comply with industry code requirements, which deal with industry-specific areas of importance, and regulatory requirements for the feed sector.

The first AFMA code of conduct certification protocol was developed in 2008 in collaboration with service provider, Afri Compliance, to assist the animal feed industry and feed mills specifically with evaluating their operations to ensure compliance with regulatory and statutory requirements.

AFMA places a very high premium on the integrity and quality of animal feed manufacturing in South Africa. It is, therefore, safe to say that evaluating the manufacturing facilities of AFMA members in terms of their regulatory and operational compliance in the workplace is not only the right thing to do, but it also contributes towards responsible and professional business ethics in the industry.

Process and assessments

The auditing process comprises a complete in-loco audit at the premises of AFMA members and associate members. Afterwards, a report with findings is submitted, and companies are granted sufficient time to rectify areas that require attention. A follow-up is then done to ensure that non-compliant issues are resolved. Once facilities comply with the audit protocol, AFMA issues a certificate of compliance, which remains valid for two years.

The code of conduct assessments aligns with ISO/IEC 17065 requirements, ensuring that internationally accepted processes, procedures, and policies are followed during the auditing process.

The entire AFMA code of conduct auditing process is presented in a user-friendly manner and is designed to be cost-effective, and not infringe on the valuable time of manufacturing facilities. The code of conduct is furthermore structured in a way to be easily understood by members. It embodies the core values and regulatory requirements of the animal feed industry, as well as the ethical obligations in the workplace.

The value proposition

The primary advantage of feed manufacturers complying with the AFMA code of conduct lies in demonstrating transparency and legal compliance within the workplace. This will enhance their credibility as a business.

Another advantage of the AFMA code of conduct is that it plays an important role in promoting ethical business principles, a crucial aspect of today's marketplace. Customers, suppliers, and other stakeholders seek assurance that manufacturing facilities go about their work correctly. In addition, the modern-day consumer wants the assurance that their food is produced safely and responsibly.

The risks associated with regulatory non-compliance or unethical conduct are significant. Demonstrating active efforts to create an ethical and regulatory-compliant operational environment helps AFMA members gain a good reputation within the sector. Trust in their business and the quality and safety of their feed products is enhanced.

By participating in the assessment of the industry code of conduct at feed mills and manufacturing facilities, AFMA members can display proof of their intentions to maintain the safe feed-safe food chain at their workplace. The code of conduct also creates a standard for best practices at feed manufacturing facilities and reduces the risk of any activity that could negatively impact the compliance status of a facility.

Accountability is key

The importance of follow-up assessments to maintain compliance and mitigate non-compliance risks is illustrated in the biennial auditing cycle implemented by AFMA. The structure and scope of the programme reassure members that the code of conduct is audited by an independent, professional assessment body with the required experience, and backed by competent and qualified lead auditors. The fact that the audit is conducted on a biennial basis means the audit costs are spread over a two-year period, which makes it more cost-effective.

It is well known that a code of conduct helps to improve accountability and to establish a compliance culture across an entire industry sector, and by doing the right thing it shows that the feed industry is serious about its ethical stance. The AFMA code of conduct verifies compliance to critical areas of importance that are considered necessary to operate as a manufacturer in the animal feed industry.

Participation in AFMA's code of conduct certification programme showcases members' commitment to good corporate governance. It confirms their role as responsible players in the animal feed industry, emphasising adherence to food safety management systems and compliance with regulatory requirements in the industry.

Successful audit completion distinguishes AFMA members from other feed manufacturing companies. The "safe feed for safe food" mark of approval is awarded to compliant facilities to acknowledge their commitment to the industry standard. This logo can be used on marketing materials, feed products, and trucks to advocate for safe feed practices.

For more information, contact the author at joe@africompliance.co.za.

The speakers, from the left, were Liesl Breytenbach, executive director of AFMA, Sanji Ramdass, production manager at De Heus, Shaun Roux, raw material and logistics controller at De Heus, Lienjie Mogano, project leader at SACNASP, Elzanne Fourie, De Heus technical advisor, August Lingnau, regional sales and product manager KwaZulu-Natal at De Heus, and Sharlene Moodley, De Heus food safety and quality manager. Marianne van der Laarse, managing director of Agrijob, was not available for the photograph.

AFMA reaches out to KZN students

By Liesl Breytenbach and Bee Oelofsen, AFMA

The Animal Feed Manufacturers Association (AFMA) is committed to promoting the animal feed industry and ensuring a sustainable industry supported by the necessary training and skills development opportunities. For this reason, AFMA is passionate about creating knowledge transfer opportunities for animal science students across the country, giving them direct exposure to feed companies and the feed manufacturing industry as a whole.

Currently, AFMA rotates its student outreach activities to reach every relevant tertiary institution at least every second year. This frequency may increase as AFMA endeavours to find ways to reach each university annually.

Perspective on the future

In April this year, AFMA hosted an inspiring student outreach at De Heus in KwaZulu-Natal (KZN). This outreach brought together third-year, fourth-year, and postgraduate animal science students from the University of KwaZulu-Natal, offering them a unique glimpse into the dynamic world of animal feed production. AFMA presented the outreach in partnership with De Heus, Agrijob, and the South African Council for Natural Scientific Professions (SACNASP).

The day started with a warm welcome from the De Heus team. As a global leader in animal nutrition, De Heus emphasised its dedication to innovation and sustainability. Presentations were made by their raw material logistics controller, food safety and quality manager, technical manager, production manager, and technical advisor. This introduction painted a vivid picture of De Heus as an industry leader, as well as a potential future employer where a passion for animal nutrition can truly flourish.

Agrijob was next to take the floor, providing invaluable insights into job market trends and essential skills needed for career success. The students received tips on resumé building, interview techniques, and networking strategies, giving them a clear roadmap for their transition from academia to the professional world.

The final presentation came from SACNASP, the regulatory body for natural science professionals in South Africa, explaining the process of becoming a registered natural scientist, and highlighting the ethical standards and professional advantages that come with accreditation. SACNASP's emphasis on professional development and lifelong learning motivated students to strive for excellence in their future careers and to register early on at SACNASP as a candidate natural scientist, which will improve their marketability.

The practical side

A highlight of the day was undoubtedly the guided tour of the De Heus feed mill. For many students, seeing the inner workings of the feed production process was eye-opening and they were astonished by the machinery and processes ensuring the production of high-quality animal feed.

Throughout the day, students had numerous opportunities to ask questions, interact with speakers, and network. This was more than just an information session; it was a celebration of potential and opportunity, inspiring a new generation of animal scientists to pursue their dreams within the agricultural sector.

For more information, contact the authors at email liesl@afma.co.za or events@afma.co.za.

AFMA Feed Miller Short Course goes from strength to strength

By Susan Marais, Plaas Media

The Animal Feed Manufacturers Association (AFMA) and Ernst Nef of Nef Feed Milling Consulting once again collaborated to present the AFMA Feed Miller Short Course at the Conclave Country Lodge in Gauteng in May.

This year, attendees performed substantially better than last year, with 70% (seven out of every ten attendees) passing. Last year only 60% were able to pass. In addition, one attendee, Marius Aspeling from Nutri Feeds, achieved a perfect score on the written examination held on the final day. Nef remarked that this was the first time a South African participant reached this exceptional achievement.

Nef, a retired former executive director of the Swiss Institute of Feed Technology, observed that South Africa's feed milling industry has definitely benefitted from the course. New attendees this year came armed with knowledge gained from colleagues who had previously attended the course.

Broad coverage

A range of topics relating to animal feed manufacturing were covered during the course. These topics included aspiration systems, batch mixing, liquid addition, hygienising and compacting, expansion, drying and cooling, and mechanical conveying.

Nef said this was his eighth time facilitating the course in South Africa. The first one was held in 2010 and initially presented every two years. Disruptions such as the Covid-19 pandemic led to delays in workshop presentations. Since 2022 the course has been presented annually.

"Initially, the course attracted around 30 participants, but the numbers have picked up," Nef said. "Last year 56 people participated and this year 51 attended, which is a good number. I have also seen the racial profile of attendees changing. I haven't yet analysed this year's racial profile, but last year 50% of the students were people of colour, which is very good."

A clear improvement in feed mill companies' intrinsic knowledge has also become apparent to Nef over the years. "I've observed that companies have become more knowledgeable because of the value the students they sent gained from the course."

The SA feed mill landscape

Over the years Nef has visited several South African feed mills. He believes that many of them are run exceptionally well, while others face efficiency challenges. He does not believe that this differs greatly from what one would encounter elsewhere in the world.

"The technology used in the Far East, Middle East, African and South American countries is generally aligned with European standards, although the

A total of 51 students participated in this year's AFMA Feed Miller Short Course in Pretoria.

Marius Aspeling received a certificate from Ernst Nef of Nef Feed Milling Consulting for achieving the highest mark.

After writing their exam, attendees had the opportunity to blow off some steam with a fun box-cart building competition sponsored by R-Biopharm.

European feed milling sector is declining due to issues such as high production costs and environmental challenges."

AFMA and Nef hope that their efforts will enable the South African feed milling industry to circumvent a similar fate. "Nowadays everyone aims to produce the maximum amount of feed of the best quality at the lowest price. Hence, plant efficiency is critical," Nef noted. "It is important to save energy and minimise production costs while producing good quality products and adhering to legislative requirements, such as preventing cross contamination and ensuring feed homogeneity and segregation."

Course objectives

Liesl Breytenbach, executive director of AFMA, emphasised the purpose of the course, namely to enhance feed mill efficiency. "It is all about learning. It is an intense, in-depth course focussed on all the processes that apply to a feed mill. We expect that the knowledge gained from this course will be taken back to feed mills and assist in enhancing their operational efficiencies."

Traditionally, the course targeted facility and production managers, electrical and mechanical engineers, and maintenance personnel. However, AFMA has since started marketing it to nutritionists as well.

"We encourage good communication between nutritionists and production teams because over the years it has become apparent that good co-operation between these groups is critical for long-term success," Breytenbach said.

The course primarily attracts employees from AFMA's member companies. These companies will usually identify individuals they believe will benefit from the course content. While the course is open to anyone, Breytenbach will not advise a novice to enrol. "If you do not have any feed mill experience, you will struggle to keep up with the pace and technical detail of the course. However, we have had people in the past who moved to a feed mill from a bread-milling environment, and they were able to keep up. That is because there is a basic overlap between the two industries."

However, she believes the standout feature of this course is the nine days students spent learning from Nef. "He is a walking encyclopaedia. If there is something at your feed mill you can't quite figure out and want to know more about it, you have full access to him and are free to ask all your questions directly."

The course has become very popular and several international participants enrolled for the South African course. This year, feed millers from Zimbabwe, Cameroon and Saudi Arabia joined the programme. The course was sponsored by Swiss-based multinational plant equipment manufacturer, the Bühler Group, feed mill automation system supplier, Automill, and one of Europe's leading suppliers of laboratory equipment and biotechnology, R-Biopharm. R-Biopharm is also AFMA's newest member.

Participant feedback

As this year's top student, Aspeling said he found the course to be extremely valuable. Rather than merely observing the machinery at his feed mill, he now understands why it operates the way it does. "It is great to have in-depth knowledge of what is going on during the different steps, and it will help me to improve our processes. If we could push our mixers to produce 12t/h instead of the current 10t, it will benefit our feed mill and company in general."

Aspeling completed his BSc Agric degree at the University of the Free State in 2018 and has been with Nutri Feeds since his internship.

Vimbainashe Saburi Zhungu from National Foods Limited in Harare, Zimbabwe said the knowledge she gained from the course is invaluable. "For me, the highlight was the section on aspiration systems. There are a lot of ways in which we can improve our systems back home and I am excited to take this knowledge back and make the necessary improvements at our facility."

Another Nutri Feeds employee, Cameron Noland, said for him the discussion pertaining to size reduction and conditioning stood out the most. Noland works for Nutri Feeds in Viljoenskroon.

For more information on next year's course, contact Bee Oelofsen at events@afma.co.za.

The AFMA-SAAHA AMR workshop speakers and attendees were, from the left, Prof Shabbir Simjee, Elanco's chief medical officer and global regulatory and technical senior advisor – microbiology and antimicrobials, Liesl Breytenbach, AFMA's executive director, Dr Ruth Lancaster, pharmaceutical policy specialist from the National Department of Health, and Margaret Churchill, executive director of SAAHA.

Workshop for unity against antimicrobial resistance

By Susan Marais, Plaas Media

The Animal Feed Manufacturers Association (AFMA) and the South African Animal Health Association (SAAHA) hosted an antimicrobial resistance (AMR) workshop in Pretoria in April, with the goal of fostering common ground and shared understanding among stakeholders regarding the challenges that AMR poses.

"There is a huge disconnect between what the various stakeholders are doing, and our intention is for this workshop to be the start of getting everybody on the same page," said Margaret Churchill, SAAHA's executive director, adding that policymakers must find a realistic middle ground between what one would like to do to minimise AMR, while keeping ground-level realities in mind.

Prof Shabbir Simjee, Elanco's chief medical officer and global regulatory and technical senior advisor – microbiology and antimicrobials, highlighted that South Africa is not alone in grappling with this issue. Globally, authoritative bodies such as the World Health Organization (WHO), the United States Food and Drug Administration, the World Organisation for Animal Health, and the European Commission lack consensus on fundamental AMR matters.

While the WHO provides guidance to countries, it lacks legal enforcement capabilities. Co-operation between countries is essential to address this challenge.

Centralised One Health desk

One of the main issues highlighted during the workshop is the apparent disconnect between South Africa's government departments – none of which know what the others are doing. To overcome this issue, Dr Ruth Lancaster, a pharmacist from the National Department of Health (DOH), said she hoped that there could one day be a centralised One Health desk functioning at government level.

Dr Lancaster's dream was born from her work on South Africa's national plan on AMR. While putting this plan together in 2014, the DOH primarily focussed on human health. However, after learning that there was an official veterinary strategy in place as well, the DOH incorporated this and amended the document to create the national AMR strategy for 2017 to 2024. As the strategy is only in place until this year, the DOH is currently revising it, and Dr Lancaster invited all stakeholders to provide their input to enable them to put together a relevant, updated strategy.

While South Africa's first AMR surveillance report, which was aligned with WHO definitions, was published in 2018, Dr Lancaster said they needed better data, a process they are currently working on perfecting. "We are basing our information on data obtained from the South African Revenue Service (SARS) and the SAAHA."

As all antimicrobials currently sold in South Africa are imported, the information from SARS seemed relatively dependable. However, the SAAHA and SARS data did not align and the DOH needed to ascertain where the missing antimicrobials were being used.

It is important to look critically at legislative and policy reform, but any amendments would need to strengthen the country's health system. Research and communication are key to achieving these goals. "In the end we want improved outcomes for both human and animal patients. When thinking about this, do keep in mind that you might one day be that patient," Dr Lancaster said.

Industry view of AMR

Various primary agricultural industry role-players discussed their industry's perspective regarding AMR. Dr Peter Evans, CEO of the South African Pork Producers' Organisation (Sappo), highlighted the successes of the industry's Pork 360 programme and continued work to learn more about AMR. Sappo annually spends 10% of its statutory levy income on AMR work. Examples of research projects include studying AMR prevalence and the mitigation of AMR on Gauteng pig farms, and assessing AMR's impact on pig gut health.

Dr Shahn Bisschop, CEO of Avimune, observed a decline in antimicrobial use in the poultry sector due to improved management practices. However, antibiotics remain essential in the industry. Herbal alternatives available in the South African market are not yet effective enough to replace antibiotics. "Most of these products are nothing more than pizza toppings," Dr Bisschop said, adding that antibiotics could not simply be replaced by herbs. "Overall gut health needs to be managed better."

The speakers during the first session of the day were Brett Roosendaal, AFMA board member and convener of the medicated feed subcommittee, Prof Shabbir Simjee, and Margaret Churchill.

Accountability is key

Dr Theo Kotzé, veterinary consultant at the Milk Producers' Organisation (MPO), emphasised the importance of antimicrobials in dairy animal production and health. "One of the key issues here is accountability. Who is accountable for antimicrobials in milk?" The dairy industry, he said, has a good baseline due to Milk SA's advocacy of responsible AMR use. However, they are seeing an uptick in AMR – for example, South African scientific studies have reported a rise in AMR in most pathogens responsible for mastitis.

Dr Hein Nel, manager of veterinary services and auditing at the Food Safety Agency, highlighted that South Africa's game meat sector is mostly export driven – significant volumes of game meat are exported to the European Union annually.

The speakers during the second session were Dr Theo Kotzé, veterinary consultant at the Milk Producers' Organisation, Brett Roosendaal, Dr Peter Evans, CEO of the South African Pork Producers' Organisation, Dr Adriaan Olivier, industry veterinarian at the South African Ostrich Business Chamber, and Dr Hein Nel, manager of veterinary services and auditing at the Food Safety Agency.

While most game graze natural veld, there is a risk of infection if foreign objects (such as bullets, needles and darts) are found lodged in an animal carcass.

To minimise residues, the industry has implemented several mitigation measures. This includes farm registration, registration of professional hunting teams, independent meat inspection and residue sampling, a national chemical residue monitoring and control programme, and export certification.

However, the local game market remains largely unregulated. "As far as export products are concerned, the risk is low. It has been years since we found any samples that tested positive for antibiotics." Dr Nel's assessment does not include game bred for tourism, as it falls outside his jurisdiction.

The ostrich industry and AMR

Dr Adriaan Olivier, industry veterinarian at the South African Ostrich Business Chamber, discussed the ostrich industry's dependence on trade and trust as the key to building trade relationships. "All of our products are fashion-based, but the fashion industry is very finicky."

Dr Olivier said trade becomes strained if everybody is not on the same page. The fact that industry, government, and trade partners could sit together and find common ground helped the industry immensely. They have identified a single laboratory in Europe capable of handling all trade-related testing. "However, this requires participation from all involved."

Currently, 324 South African producers are eligible to export ostrich products. "A veterinarian visits each one regularly and annual animal welfare audits are conducted to keep trade relationships intact."

Antibiotics in moderation

Dr Catriona Lyle, chairperson of the South African Equine Veterinary Association's procedures and policies committee, said there is no national data source available indicating the level of AMR among the estimated 325 000 horses in South Africa. In practice, however, veterinarians are seeing a rise in AMR. A major concern is the fact that there is only a limited number of registered products available to the equine industry.

Dr Lyle emphasised the need to protect the few available products by eliminating the inappropriate use of antibiotics. "Veterinarians should stop using antibiotics for non-infectious conditions such as asthma. If a horse has asthma, it has asthma. It should also not be used as a surgical prophylaxis."

She added that veterinarians need to trust their own judgement and ought not to rely on antibiotics so much. "A moment of self-reflection is needed. Vets should always ask if antibiotics are truly necessary before prescribing it."

However, horse owners are not always able or willing to spend additional money on diagnosis, which is another reason for the overuse of antibiotics.

Antibiotics and animal welfare

According to Prof Simjee, it is important to note that similarly to doctors' Hippocratic oath, veterinarians also promised to provide the best possible care for their patients. Therefore, if there is an antibiotic available to save an animal's life, the veterinarian would use it.

However, as AMR is rising globally, the chances of not having a treatment available could pose a real danger, which is why correct and responsible use is crucial. Prof Simjee said because the return on investment on antibiotics is limited, there is no real incentive to invest in antimicrobial research and development – a company's patent is only valid for around ten years after development, which provides for limited time in which to generate a profit before the patent expires.

Dr Catriona Lyle, chairperson of the South African Equine Veterinary Association's procedures and policies committee.

For more information, contact Bonita Cilliers at technical@afma.co.za or Margaret Churchill at margaret@saaha.co.za.

Feed mill operator training Part 1: Theory

By Wimpie Groenewald, membership liaison officer, AFMA

The scope of the AFMA Livestock Feed Milling Operator e-learning programme is to prepare and equip the learner with knowledge and practical experience to function at the operator level in a livestock feed milling environment.

This article serves to provide information regarding the minimum admission requirements for enrolment in the programme and provides an outline of the content of the theoretical modules.

Minimum admission requirements

- Basic literacy and numeracy skills.
- Computer literacy.
- Dedicated learning space at the workplace.
- Access to a computer and Internet at work and home.
- National Qualifications Framework (NQF) level 3.

Content of theoretical modules

Module 1: The feed milling environment

The module provides an induction or orientation programme for people employed in the livestock feed milling industry.

- **KT1.1 – Introduction to the animal feed industry:** A basic overview of the global and South African feed industry.
- **KT1.2 – Introduction to feed milling operations:** An introduction to the various stages in the production of mixed animal feeds.
- **KT1.3 – Productive work at feed mills:** Work ethics and productive work practices.
- **KT1.4 – Occupational health and safety in the feed milling environment:** Occupational health and safety orientation and safety signs, symbols and personal protective equipment.
- **KT1.5 – Quality studies:** An orientation on quality assurance concepts and practices in the livestock feed milling environment.

Module 2: Material handling equipment

The module provides a general orientation on material handling equipment. A basic description of a range of equipment commonly used in a livestock feed milling facility to handle material.

- **KT2.1 – Material conveying equipment** (elevators, conveyors).
- **KT2.2 – Material distribution** (spouting slides, diverters).
- **KT2.3 – Material cleaning equipment** (sieves, magnets).
- **KT2.4 – Aspiration systems** (dust filters).
- **KT2.5 – Mixed feed bagging systems.**
- **KT2.6 – Bulk material out-loading systems.**

Module 3: Receiving, storage and warehousing

The module focusses on the receiving and storage of raw materials and ingredients.

- **KT3.1 – Bulk material receiving:** Receiving materials delivered in bulk (mostly via road transport), such as maize.
- **KT3.2 – Bulk storage facilities:** After receiving the raw materials, the second major operation at a feed mill is storing the raw materials in such a way that there will be no losses in the quality and quantity of these materials. This topic explores the different facilities commonly used for bulk material storage.
- **KT3.3 – Silo storage risks:** This topic explores two important risks employees are exposed to at the feed receiving and bulk storage areas. Entrapment is the first risk area explored and dust explosions are the second.
- **KT3.4 – Warehousing:** The warehouse offers services to the livestock feed mill by ensuring that a range of stock items are properly stored and available for the production of mixed feeds when required.

Module 4: Mixed feed production

The focus of Module 4 is on the production of mixed livestock feeds through batching, dosing, mixing and particle size reduction.

- **KT4.1 – Process introduction and overview:** A general description of the layout and design options for a mixed feed production system.
- **KT4.2 – Batching and dosing:** Batching and dosing process and equipment.
- **KT4.3 – Mixing:** The equipment and processes related to the mixing of materials.
- **KT4.4 – Liquid addition:** The equipment and process related to the addition of liquids during mixed feed production.
- **KT4.5 – Particle size reduction:** Particle size reduction equipment and processes.
- **WIL4.1 – Experiential learning:** Mixed feed production operations.

Module 5: Feed processing

The module focusses on feed pelleting equipment and processes at a feed mill. Some information is also provided on expansion and extrusion.

- **KT5.1 – Pelleting process overview:** A general description of the pelleting process, equipment used and stages in a pelletising line.
- **KT5.2 – Pelleting technology:** The main components commonly associated with the pellet press are discussed.
- **KT5.3 – Pelleting operations:** General guidelines are provided on the operation of a pellet press.
- **KT5.4 – Post-pelleting treatments:** The treatments applied to pelleted feeds are explained.
- **KT5.5 – Expansion and extrusion:** The expansion and extrusion equipment and processes are briefly introduced.
- **WIL5.1 – Experiential learning:** Feed processing operations.

Module 6: Feed science

The module includes some biology and animal nutrition principles, as well as components used in the production of mixed feeds.

- **KT6.1 – Animal nutrition:** The focus of the topic is on the biology of animal nutrition and digestive processes.
- **KT6.2 – Raw materials and additives:** The materials commonly included in the production of mixed livestock feeds are discussed.
- **KT6.3 – Common terminology:** A link to a publication titled *Common terms used in animal feed and nutrition* is provided. This is an excellent resource that describes the terminology commonly associated with animal nutrition.

Module 7: Operational team leadership

Operational team leadership explores the supervisory roles of a team leader. This is introduced as team leadership concepts, teamwork and team discipline.

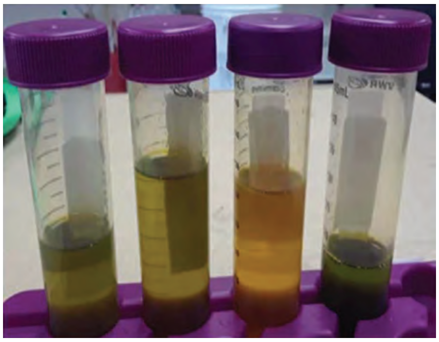
- **KT7.1 – Team leadership:** The team leader as the person who interacts with the employees daily and who holds a position of authority, is introduced by exploring roles, ethics, teamwork, instructions and controlling standards of performance.
- **KT7.2 – Team discipline:** Workfloor discipline, conflict and handling of conflict are presented in this topic.
- **KT7.3 – Team dynamics:** In this topic, the learner is introduced to the role and characteristics of groups and teams in organisations in terms of their influence on the attainment of organisational goals.
- **WIL7.1 – Experiential learning:** Team leadership.

Learners can register for the full course (six months) or select individual modules as short courses.

For more information or to register for this course, contact AFMA at admin@afma.co.za or 012 663 9097.

Green or drought-stressed soya beans: Effects on nutrition and quality

Compiled by Feed First



Oil extracted from harvested, immature soya beans shows the green colour from chlorophyll compared to the desired oil from mature soya beans. (Photograph: Kenneth Hellvang)

Soya beans are one of the most important legume crops in the world due to the number of industries that can benefit from it, including the animal feed industry. The maturity stage of soya beans significantly affects their nutritional composition and quality.

When soya beans have not reached their full maturity by harvest time, often the beans are still green-coloured. The green colour in a soya bean indicates that chlorophyll is still present in the bean. Chlorophyll plays a role in providing energy and nutrients for the development of soya bean seeds.

As the seeds mature, chlorophyll levels decrease, allowing other pigments, such as carotenoids, to become more visible. This change in pigment composition is associated with the transition from green to yellow-brown in soya bean pods as they mature.

Drought stress

Drought stress can lead to delays in soya bean maturation, increasing the time it takes for pods to reach full maturity. As a result, soya bean plants may take longer to produce dry matter and complete the maturation process, leading to delayed pod filling and seed development.

Drought stress can induce premature maturation in soya bean plants, causing leaves to change colour, wilt, and drop prematurely. As a result, soya bean plants may prematurely abort flowers and pods, leading to fewer seeds and yield under drought conditions.

Concerns exist regarding the nutritional quality of soya oilcake produced from immature green or drought-stressed soya beans and the impact of the soya oilcake on broiler production.

Nutritional composition

Significant differences were found in the nutrient composition of immature green, drought-stressed soya beans, and mature soya beans with regard to oil, protein, and fibre (*Table 1*).

Immature green soya beans generally have a lower oil content compared to mature soya beans. The oil content in immature soya beans may range from approximately 5 to 8%, while mature soya beans typically contain around 15 to 20% oil. Drought-stressed soya beans have a lower oil content (8 to 12%) due to the decrease in the concentration of digestible carbohydrates such as glucose, fructose, and sucrose under drought conditions.

Immature green soya beans often have a higher protein content compared to mature soya beans. The protein content tends to decrease as soya beans mature and enter the seed-filling stage. Immature soya beans may contain protein levels ranging from 35 to 45%, whereas mature soya beans generally contain around 30 to 40% protein.

Due to a lower yield and a decreased rate of seed filling, the synthesis of protein in drought-stressed soya beans will be slower and the protein levels will be slightly higher than in mature soya beans. Seed-filling processes and the accumulation of reserves in the developing and maturing seeds are highly sensitive to environmental changes.

Sucrose and fibre content

Immature soya beans may contain higher levels of sucrose to support seed development, whereas mature soya beans have lower sucrose levels as sugars are converted into starches during seed maturation. Drought-stressed soya beans will potentially have lower sucrose levels due to the decreased rate of seed filling and therefore lower concentration of digestible carbohydrates.

Immature green soya beans tend to have a lower fibre content compared

Table 1: Nutrient values of normal, immature green, and drought-stressed green soya beans.

	Normal	Immature green	Drought-stressed green
Protein %	30 – 40	35 – 45	37 – 44
Fibre %	8 – 10	5 – 8	10 – 15
Oil %	15 – 20	8 – 12	5 – 8

to mature soya beans due to the stage of development, whereas mature soya beans have a higher fibre content as the seed coat becomes more fibrous during maturation.

With the information in *Table 1* in mind, the quality of soya oilcake will be affected. Some literature state that when chlorophyll is still present in soya beans it may be present in the soya bean oil, giving it a green tint.

Trypsin inhibitor

Trypsin inhibitors are proteins naturally occurring in soya beans that interfere with the activity of the trypsin enzyme. Studies have shown that the concentration of trypsin inhibitors tends to decrease as soya beans mature. This decrease is due to physiological changes that occur during seed development, including the breakdown of proteins and the activation of protease inhibitors.

As soya beans mature, they undergo processes that reduce the levels of anti-nutritional factors, including trypsin inhibitors, to support seed germination and growth. When soya beans age prematurely due to drought stress, the rate at which proteins are broken down may decrease and result in higher-than-expected trypsin inhibitor levels.

Impact on broiler performance

The inclusion of soya bean oilcake from immature green or drought-stressed soya beans in broiler diets can have varying effects on growth, feed efficiency, and carcass characteristics. Reduced growth and poor performance may be observed when soya oilcake is included at high levels in the feed due to higher trypsin inhibitor levels seen in immature green and drought-stressed soya beans. The quality of soya oilcake and soya oil may differ due to the nutritional composition of the soya beans.

Conclusion

Drought stress can have a major effect on soya bean maturation, leading to delayed development, reduced seed size and yield, premature ageing, and changes in seed composition. While the concentration of trypsin inhibitors generally decreases with soya bean maturation, factors such as genetic variation and environmental conditions, e.g. drought stress, can influence the levels of trypsin inhibitors in soya beans.

The use of soya oilcake and oil produced from immature green or drought-stressed soya beans should be used with caution in broiler diets.❖

Aflatoxin levels in poultry feed: A comparison of mash and pellet forms

By Seyed Soheil Ghaemmaghami, Hasan Rouhanipour and Seyed Davood Sharifi

Mycotoxins are secondary metabolites produced by various genera of fungi, and their presence in crops, processed food, and feed poses a significant global risk due to their highly toxic nature. Among the mycotoxins, aflatoxins are considered one of the most hazardous groups, capable of causing substantial contamination, as recognised by the World Health Organization (WHO, 2011; Omotayo *et al*., 2019).

In addition, aflatoxins have been classified by the International Agency for Research on Cancer (IARC) as highly problematic carcinogens capable of causing liver damage in humans (IARC, 1987; Yilmaz *et al*., 2017). Specifically, aflatoxin B_1 (AFB_1) has been identified by the IARC as a type-A human liver carcinogen (IARC, 1987; WHO, 2011).

Aflatoxins can be produced by various species of *Aspergillus*, including *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nomius*. These fungi commonly contaminate cereals throughout their growth, harvest, storage, transport, and processing stages (Bryden, 2007). Among these species, *A. flavus* is a prevalent pathogen in animal feed and can exist in both toxigenic and nontoxigenic strains, potentially present in poultry feed.

Prevalence and safety concerns

Studies have indicated that approximately 98% of the ingredients used in animal diets contain aflatoxins (including B_1, B_2, G_1, and G_2), with AFB_1 being the most prevalent. Maize, in particular, is highly susceptible to fungal growth and mycotoxin production (Rodrigues *et al*., 2012; Ariyo *et al*., 2013). Aflatoxin contamination is a major concern to human health as AFB_1 could be passed on to humans from poultry products. Research has indicated that AFB_1 exists in a wide range of feed, depending on the region and climate (Labuda and Tanvinova, 2006).

Aflatoxin-producing fungi exhibit varying responses under different conditions, which can be influenced by storage, sampling techniques, geographical regions, seasonal climate changes, and temperature variations. The moisture content of feedstuffs has been identified as one of the most significant predisposing factors for contamination (Kana *et al*., 2013; Abdallah *et al*., 2015). In poultry, it is crucial to measure the concentrations of different types of aflatoxins (B_1, B_2, G_1, and G_2) and total aflatoxin levels in feedstuffs, as these serve as critical indicators of health (Wu, 2015).

Research conducted in recent decades has provided evidence of the detrimental effects of aflatoxins on poultry performance. Chronic aflatoxin poisoning in poultry has been observed to weaken the immune system, and these metabolic compounds possess toxic, carcinogenic and mutagenic properties (Kumar *et al*., 2009; Sirajudeen *et al*., 2011). However, only a few attempts have been made to evaluate the presence of *Aspergillus* species and identify different types of aflatoxins in poultry feed.

Furthermore, maize and soya bean meal are the primary ingredients in Iranian-made poultry feed, with a significant portion being imported from other countries. There are variations in the initial safety of feed ingredients based on their country of origin, as well as differences in the potential for contamination, transportation conditions, and storage practices. Although these ingredients may be susceptible to fungal contamination, the extent of damage can also vary throughout the feed preparation process.

This study aimed to assess the frequency, levels, and natural occurrence of aflatoxins (B_1, B_2, G_1, and G_2) as well as total aflatoxins, in maize, soya bean meal, and poultry finished feed (in both mash and pellet forms).

Results

The results regarding the frequency of *Aspergillus* isolates from ingredients and finished poultry feed were recorded. Among the feed ingredients, maize exhibited a higher frequency of toxigenic isolates compared to soya bean meal (64,2 vs 12,5%, respectively). The pellet form demonstrated a higher frequency of toxigenic isolates compared to mash feed (38,1 vs 33,3%, respectively). Notably, maize samples had the highest proportion of aflatoxigenic isolates, accounting for the majority of the total isolates (64,2%), followed by pelleted feed (38,1%) and mash feed (33,3%), while soya bean meal displayed the lowest level (12,5%).

The analysis of aflatoxins in maize samples revealed that approximately 9,09% of the maize samples (n=22) were found to be contaminated. No contaminants of other

Table 1: Aflatoxins contamination in mash feed samples (n=22).

Aflatoxins	N[1] (%)[2]	Min[3] – max[4] (ppb)[5]	AF contaminated samples higher than standard level (%)[6]
B_1	2 (9,09)	ND[7] – 0,42	0
B_2	1 (4,54)	ND – 0,33	0
G_1	3 (13,36)	ND – 0,69	0
G_2	9 (40,91)	ND – 1,87	0
Total[8]	12 (54,54)	ND – 3,31	0

[1]Number of contaminated samples. [2]Frequency of contaminated samples (aflatoxins infected samples x 100/total sample of mash feed). [3]Min: minimum. [4]Max: maximum. [5]ppb: parts per billion. [6]Frequency of contaminated samples that contain AF levels higher than the maximum tolerance of AF in the European Union. [7]Not-detectable. [8]Sum of all types of aflatoxins B_1, B_2, G_1, and G_2.

Table 2: Aflatoxins contamination in pellet feed samples (n=19).

Aflatoxins	N[1] (%)[2]	Min[3] – max[4] (ppb)[5]	AF contaminated samples higher than standard level (%)[6]
B_1	5 (26,32)	ND[7] – 0,97	0
B_2	2 (10,53)	ND – 0,62	0
G_1	3 (15,79)	ND – 0,98	0
G_2	9 (47,36)	ND – 3,01	0
Total[8]	11 (57,89)	ND – 5,58	0

[1]Number of contaminated samples. [2]Frequency of contaminated samples (aflatoxins infected samples x 100/total sample of pellet feed). [3]Min: minimum. [4]Max: maximum. [5]ppb: parts per billion. [6]Frequency of contaminated samples that contain AF levels higher than the maximum tolerance of AF in the European Union. [7]Not-detectable. [8]Sum of all types of aflatoxins B_1, B_2, G_1, and G_2.

aflatoxins (B_2, G_1, and G_2) were detected in the samples. The concentration of aflatoxins in the studied samples did not exceed the standard limits set for maize aflatoxin in the European Union (EU) (EU, 1993; European Food Safety Authority [EFSA] 2014a, EFSA, 2014b). The analysis of aflatoxins in soya bean meal samples revealed concentrations of 0,61 parts per billion (ppb) for AFB_1, 0,71 ppb for aflatoxin G1 (AFG_1), and 1,05 ppb for aflatoxin G_2 (AFG_2). Aflatoxin B2 was not detected in the soya bean samples.

The frequency of contaminated samples for total aflatoxins was approximately 40,9%. Interestingly, the frequency of contaminated samples for AFG_2 was higher than that of AFB_1 and AFG_1, with percentages of 36,36% compared to 13,63% for each. The concentration of aflatoxins in the soya bean samples of this study did not exceed the standard limits set for maize aflatoxin allowance by the EU (2002; EFSA, 2004a,b).

Aflatoxin contamination levels

Different types of aflatoxins (B_1, B_2, G_1, and G_2) were detected in the finished feed samples in the form of mash (*Table 1*). The maximum level of total aflatoxin in the mash feed samples was found to be 3,31 ppb. Interestingly, the samples contaminated with AFG_2 had higher levels compared to those contaminated with other aflatoxins in the mash feed samples. The frequency of contaminated samples with total aflatoxins in the mash feed was approximately 54,54%. However, the aflatoxin concentrations in the mash feed samples did not exceed the permitted limit set by the EU (EFSA, 2004a,b; EU, 2009).

Different types of aflatoxins (B_1, B_2, G_1, and G_2) were detected in the finished feed samples in pellet form (*Table 2*). The concentration (3,01 ppb) and frequency (47,36%) of samples contaminated with AFG_2 were found to be higher than those contaminated with other aflatoxins in the pelleted feed samples. The frequency of samples contaminated with total aflatoxins was 57,89%. The maximum level of total aflatoxin in the pellet feed samples was 5,58 ppb. Importantly, the concentration of aflatoxins in the pellet feed samples did not exceed the standard levels set by the EU (1993, 2002, 2009; EFSA, 2004a,b).

The frequency of sample contamination with any toxin increased in the finished feed (mash and pellet) compared to maize and soya bean meal (*Figure 1*). As shown in *Figure 1*, the percentages of total aflatoxin contamination in maize, soya bean meal, mash feed, and pellet feed samples were 9,09, 40,91, 54,54, and 57,89%, respectively. Additionally, the contamination of maize, soya bean meal, mash feed, and pellet feed samples with AFB_1 was found to be 9,09, 13,63, 9,09 and 26,32%, respectively.

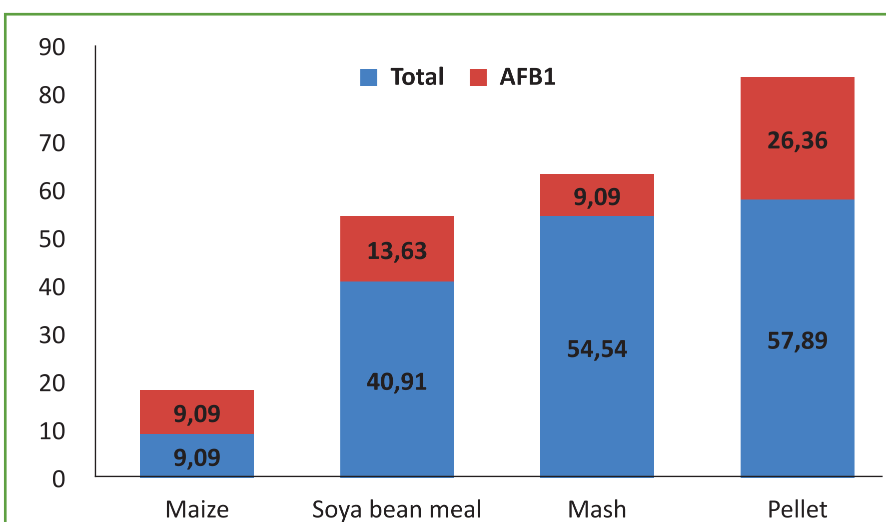
When aggregating the data to obtain overall statistics for all aflatoxins, the results indicate that maize had the lowest presence of aflatoxins (B_1, B_2, G_1, G_2, and total) compared to other feed samples. The frequency of contamination with AFB_1 in pellet feed was higher than in maize, soya bean meal, and the mash form (*Figure 2*).

Discussion

Aflatoxins, among the mycotoxins, are widely recognised for their association with various health and disease risks in poultry and livestock studies. The presence of aflatoxins in poultry feed and feed ingredients is a global concern due to their negative impact on poultry performance and the potential transfer of aflatoxin residues into the human food chain.

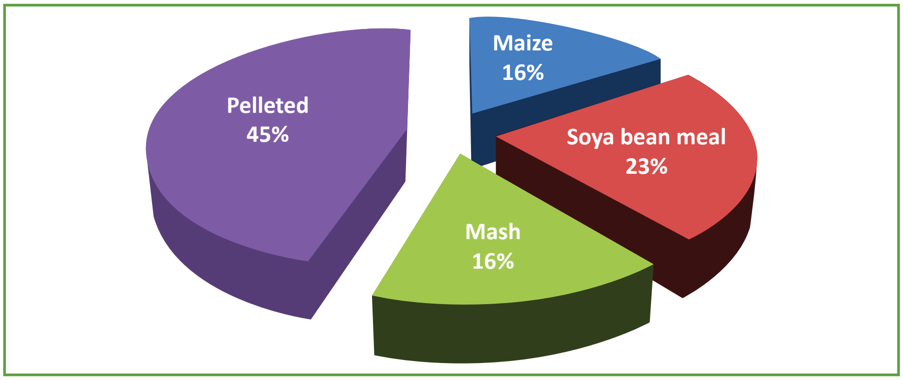
The *Codex Alimentarius* Commission, which plays a central role in the Joint FAO/WHO Food Standards Programme, has established guidelines regarding the maximum permissible levels of aflatoxins (B_1, B_2, G_1, G_2, and M_1) in feedstuffs and complementary feed. According to the *Codex Alimentarius* Commission's guidelines, the maximum acceptable limit for aflatoxins in these products is set at 20 ppb (Kotinagu *et al*., 2015). Many countries have implemented regulations to establish maximum permissible levels of AFBs in food and feed products as a means to mitigate this potential hazard.

Figure 1: The ratio of the percentage of the aflatoxins B1/total aflatoxins in poultry feed.



Farming the rumen with VistaPre-T improves digestion, decreases emissions and increases productivity
VistaPre-T boosts the rumen's natural ability to digest forage allowing for smarter use. That not only lowers feed costs but lowers emissions by up to 15%*. Less pressure on the pocket and less pressure on the environment. It's win-win. Contact us to learn more and to help the food chain make better use of forage. Find out more at www.abvista.com/products/Vistapre-t or contact dean@avipharm.co.za, or emea@ abvista.com. You can also contact Dean at Avipharm Feeds (PTY) Ltd Tel +27 33 3427041/2

Figure 2: The ratio of percentage AFB_1 contamination in poultry feed.



In this study, it was found that the contamination of maize with *A. flavus* was the highest among the sampled feed ingredients. However, interestingly, the levels of aflatoxins (specifically AFB_1, AFB_2, AFG_1, AFG_2, and total aflatoxins) were the lowest among the tested feed samples. Furthermore, the study observed that the concentration and frequency of samples contaminated with AFB_1 were higher compared to samples contaminated with AFB_2, regardless of the type of feed sample (such as maize, soya bean meal, mash, or pellet).

Influence of specific conditions

The levels of toxins in poultry feed samples can be influenced by various natural conditions. Several factors contribute to these variations, including the quantity and composition of proteins and fats in the ingredients, as well as their proportions in the final feed formulation.

Probst *et al*. (2011) conducted a study that revealed that nontoxigenic isolates of *A. flavus*, along with the presence of other fungi, have the potential to influence the natural production of aflatoxins in food and feed. The study further demonstrated that the application of different chemicals resulted in the reduction of fungal toxins or the elimination of aflatoxins, particularly through the use of mycotoxin binders or preservatives in incomplete feed.

The findings of the study demonstrated that the ability of fungi to produce AFB_1 does not necessarily correlate with the level of toxin present. Therefore, the presence of *A. flavus* fungi in poultry feed does not always indicate the presence of aflatoxin in the substrate. The production of aflatoxin depends on specific conditions required for fungal growth and toxin production.

It is essential to carefully screen high-risk ingredients, such as maize and other non-processed ingredients, due to the diverse range of fungal contaminations observed in different types and volumes of poultry feed worldwide. The study observed that finished poultry feed in pelleted form was contaminated, likely due to the widespread distribution of *A. flavus* spores in the environment.

Temperature plays a crucial role in fungal growth and the contamination of feed ingredients with fungal toxins. Fungi tend to thrive in warmer and more humid conditions. After the production and storage of final feeds, fungal growth can occur due to increased nutrient availability. It is important to note that while heating feed to boiling point for at least 30 minutes can eliminate living *Aspergillus* organisms, it does not eliminate spores that can germinate later.

During the transfer of pellets from the pelletiser machine to the cooling machine, exposure to saprophytic *Aspergillus* in the environment can lead to growth during storage. Malfunctions in the equipment can also impact the feed preparation process, potentially affecting the overall quality (Ghaemmaghami *et al*., 2020).

Comparing studies

The findings of our study revealed that maize, soya bean meal, and finished poultry feed exhibited varying degrees of contamination with natural aflatoxins. These results align with similar findings reported by researchers from other countries, such as Charoenpornsook (2006) and Fraga *et al*. (2007), who also observed comparable levels of aflatoxin in certain feed samples. Jelinek *et al*. (1989) conducted a study where they observed variations in the levels of aflatoxin in maize across different locations and years.

In the present study, the contamination caused by aflatoxins did not exceed 5,58µg/kg in any of the samples analysed. In a study conducted by López Gría *et al*. (2010), levels of aflatoxin B_1, B_2, G_1, and G_2 were measured in animal feed samples. Among the 19 samples tested, it was found that two of them had aflatoxin G_2 levels exceeding the standard limit. However, the levels of other aflatoxins were present in negligible amounts in the tested samples.

Conclusions

This study indicates that maize, as an unprocessed ingredient in poultry feed, warrants additional evaluations, particularly when conditions are not optimal for preserving the feed. The assessment showed that maize had the highest percentage of *Aspergillus* spp. mould contamination, while pellet feed had the highest aflatoxin B_1 concentration levels. These findings highlight the importance of considering both fungal contamination and aflatoxin concentration levels when evaluating the risk associated with different feed ingredients, enabling effective management and mitigation of aflatoxin risks in poultry feed production.

The prevention and reduction of *A. flavus* and other aflatoxigenic fungi such as *A. parasiticus* as well as other *Aspergillus section flavi* are highly important in ensuring the quality control of poultry feed. Therefore, the findings suggest that finished feed, especially in pellet form, carries a higher risk of aflatoxin contamination compared to the individual ingredients in poultry feed. This increased risk is particularly evident when suboptimal conditions are present for controlling fungal populations during the manufacturing and storage processes.

Special attention should be given to the quality control measures applied to finished feed to minimise the potential for aflatoxin production and ensure the safety of poultry feed.

This article has been condensed for publication in *AFMA Matrix*. For the full article, send an email to sdsharifi@ut.ac.ir or visit *www.doi.org/10.1016/j. psj.2023.103254.*

Prospects and application of solid-state fermentation in animal feed production: A review

By Garba Betchem, Abdul Razak Monto, Feng Lu, Laura Flavorta Billong and Haile Ma

Solid-state fermentation (SSF) as a substitute for submerged fermentation (SMF) has gained lots of interest in the past decades, primarily due to its low cost and the ability to mimic the natural habitat of various micro-organisms. SSF can be defined as a process that involves the growth of micro-organisms in the absence or little free water within a solid medium.

It has been widely used in several fields to produce enzymes, biofuels, food, feed, and secondary metabolites (antibodies, immune drugs) (Arora *et al*., 2018), with a significant disadvantage being the difficulty in controlling certain parameters such as agitation which hinders its application in industries. Though the recent development and design of new bioreactors is a promising solution to these considerable shortcomings such as agitation and large-scale production.

Application in animal feed

Nevertheless, various *in vitro* studies have also proven SSF to be a promising technology for enriching food products' nutritional and antioxidant properties (mainly cereals, legumes, and animal feed). Several cereal crop residues such as wheat straw, paddy straw, and maize stover are used as biofeed and mostly contain lignin. One of the numerous applications of SSF is in animal feed production, which increases the nutritional properties of feed (Sun *et al*., 2023).

Feed production has gained interest due to several global opportunities and challenges. There is a worldwide demand for animal feed, and it is expected to increase to 70% by 2050 due to population growth, increased income, and industrialisation (Alexandratos and Bruinsma, 2012; Boland *et al*., 2013).

In addition, animal welfare, environmental pollution minimisation, use of novel ingredients, and ingredients unsuitable for human consumption concerning production efficiency are significant challenges facing the feed industry (Babinszky *et al*., 2019). Due to the high demand for high nutritional feeds, several methods are employed to produce quality feeds, one of which is SSF which has been demonstrated to be a suitable alternative in animal feed production.

Moreover, some studies have reported solid fermented feed (SFF) to be a suitable alternative to feed additives such as antibiotics. This has been possible because of two main reasons: First, structural cell walls break down due to the colonisation of the micro-organism; second, the release of specific metabolites (cellulase, protease, xylanase and phytase) secreted by micro-organisms in the fermentation process (Cano *et al*., 2021).

Agro-industrial waste such as soya bean meal, rice bran, wheat bran, maize meal, groundnut husk meal, and flaxseed has intensively been used in animal feed production and the production of feed additives. Moreover, there has been increasing research on new agro-industrial waste for feed production and feed additives such as olive cake, tea dregs, ginkgo leaves, brewers spent grain, and okara (Chebaibi *et al*., 2019; Jiang *et al*., 2019; Ong and Lee, 2021; Wang *et al*., 2018).

The main research methods used to improve SSF end-products can be divided into two groups: First is the mutation of micro-organisms and the optimisation of fermentation parameters; the other involves genetic recombination and metabolic engineering (Cao *et al*., 2018). Genetic engineering technology has made pronounced achievements in microbial breeding by employing advanced molecular genetic manipulation techniques; the safety of the strains is still questioned due to the introduction of foreign genes (Szyjka *et al*., 2017).

The conventional random mutagenesis processes using physical and chemical mutagens are still the most straightforward and cost-effective techniques for improving strains (Câmara *et al*., 2019). Some mutagens, such as ultraviolet (UV), gamma radiation, atmospheric and room temperature plasma (ARTP), ethyl methane sulfonate (EMS), and 1-methyl-2-nitro-1-nitrosoguanidine (NTG) (Câmara *et al*., 2019; Gao *et al*., 2020; Montanari *et al*., 2019; Shu *et al*., 2020) have been applied in SSF to boost and improve the usage of SFF for animal nutrition.

It is widely acknowledged that SSF has enormous potential, but its application in the agro-industrial sector is less

Table 1: Characteristics of solid-state fermentation.

Characteristics	Examples	References
High crude protein content	Inoculated mixed feed (maize, soya bean meal) with *Aspergillus niger* following two-stage fermentation had a 4,8% increase in crude protein.	(Changyou *et al*., 2016)
High organic acid content	Organic acid production reached a maximum of 123g/kg dry weight of maize cob during semi-solid fermentation.	(Mai *et al*., 2016)
High enzyme production	*Bacillus* species cultivated for five days on wheat bran were reported to have a high enzyme production as high as 6 900 U/g.	(Qureshi *et al*., 2016)
High amino acid content	Lactic acid bacteria (LAB) solid-state fermentation of wheat bran significantly increased the content of amino acids.	(Jiang *et al*., 2021 a)
Low anti-nutritional factors (ANFs)	*Aspergillus oryzae* and *Bacillus subtilis* afforded more excellent enhancement in the nutritional profile of okara and brewers spent grain.	(Ong and Lee, 2021)
Low hemicellulose	Two-stage fermentation using *Bacillus subtilis* followed by *Enterococcus faecium* was performed on maize and soya bean meal, and results showed a decrease in hemicellulose from 10,15 to 4,75%	(Changyou *et al*., 2017 b)
High soluble protein content	The SSF process of *Bacillus subtilis* lwo on chickpeas increased the protease activity, resulting in the release of soluble proteins.	(Li and Wang, 2021)

developed than SMF due to large-scale production. However, as pointed out, animal feed may be best produced in SSF, as in soya bean meal feed production for animals. This can be observed in its unique products containing high protein content, enzymes, essential amino acids, and secondary metabolites.

Furthermore, SFF can be characterised based on its intended use, such as (1) fermented feed additives which have functional features (e.g., fermented therapeutic plants which promote animal immunity) (Ahmed *et al*., 2016; Yin *et al*., 2018), and (2) fermented feed components that replace proteins or energy sources, reduce the anti-nutritional factors and improve feed efficiency.

However, despite the critical corpus of research done in SSF, studies have yet to describe its application in the manufacture of animal feed; this review carefully curated recent research on the topic, which is exceptionally important to the sustainable growth of animal production. Besides, it provides a view of the main employed strategies in animal feeding and recent patents and innovations in this sector.

Factors affecting SSF

Biological factors

These factors are associated with living things or organisms' biology, metabolism, and reproduction. These determine the behaviour of the particular species in a specific way and are independent of each other.

Type and strain of micro-organism

The choice of micro-organisms is an essential factor in the SSF process. The most used micro-organisms for SSF are bacteria, fungi, and yeast. Each of them has a peculiar fermentation process; among these three micro-organisms, fungi are the most suitable for SSF due to their hyphal growth and physiological, biochemical, and enzymological properties (Kar *et al*., 2010; Prado *et al*., 2016). However, bacteria have some advantages over fungi due to their rapid growth, their biomass and their metabolites which can easily be measured.

Different organisms affect SSF processes differently as the end products differ from others. This can be observed in *Aspergillus* species where *Aspergillus niger* fermented rapeseed meal had a high decline in anti-nutritional substrates, thereby having high crude protein and amino acid. In contrast, *Aspergillus wentii* could not decrease the anti-nutritional factors significantly in palm kernel meal (Muangkeow and Chinajariyawong, 2013; Changyou *et al*., 2016). Moreover, some bacteria strains like *Bacillus licheniformis* mainly produce alkaline protease, whereas *Bacillus subtilis* are known for their ability to produce neutral protease (Li and Wang, 2021).

Physicochemical factors

These factors are associated with the physicochemical expressions occurring in an SSF system. They affect most of the mechanisms involved in SSF, such as heat transfer. All these factors are not independent of each other. As such, it is crucial to determine the degree of influence a factor has on its counterpart. Moreover, we also need to consider some physicochemical factors that may not directly affect the system but affect the biological factor. Thus, researchers have embarked on optimising SSF parameters so as to obtain high-value fermented end products.

Substrate

A substrate is a solid matrix containing a certain amount of water activity that can favour the growth of a particular micro-organism. The choice of substrate is very crucial for the SSF process. A substrate is a matrix for the growth of micro-organisms and a source of carbon, nitrogen and nutrients. There exist numerous substrates, e.g. biofuel co-products which include distiller grains and sugarcane bagasse, agro-industrial wastes which include oilseed meal, soya bean hulls, and sugar beetroot pulp, and crop residues such as wheat straw and maize stover, as well as discarded fruit and vegetables that have been used in animal nutrition (Sun *et al*., 2024).

It has been reported that fermentation products can be highly variable and appear to depend on the nature and characteristics of the substrates used (Canibe and Jensen, 2012). Moreover, since different micro-organisms require different nutrients for optimal growth, some supplements are often added to a substrate to favour the development of a particular organism; such supplements are zinc, phosphorus, calcium, magnesium, and iodide (Farinas, 2015).

One of the reasons why substrate is a significant factor in the SSF process was investigated by some researchers on the growth of *Aspergillus oryzae*. A study found that low water activity and osmosis made fungi produce defence metabolites such as glycerol, erythritol, and arabitol, which are helpful as bio-products (Ruijter *et al*., 2004).

Physio-chemical properties of SFF

The general characteristics of SFF are similar to that of SSF. That said, SFF has a higher nutritional value than non-fermented feed. Several studies have shown the increase in crude protein, peptide content, low starch, and high amino acids of various fermented agro-industrial waste (Betchem *et al*., 2023; Jiang *et al*., 2021b; Changyou *et al*., 2017b; Changyou, *et al*., 2016).

Nevertheless, SFF characteristics depend solely on the SSF process. Therefore, there can be no specific value to characterise the physical and chemical aspects of SFF. Though, the following observations can be made for SFF. *Table 1* shows some general characteristics of SFF.

Fermented feed in animal nutrition

The use of SFF in animal nutrition has been acknowledged as more beneficial to animals than liquid fermented feed. One major advantage of SFF is its ability to improve animal digestion (Yang *et al*., 2021). During the fermentation process, the microbial activity helps break down complex carbohydrates, cellulose, proteins, and lignin into simpler forms that can be easily digested by the animals.

SSF converts traditional feed into improved feed utilisation and nutrient absorption, leading to better growth performance and feed conversion efficiency in animals. Furthermore, SSF improves the palatability of feed, by reducing the presence of antinutritional factors such as phytates which give a bitter taste when combined with another protein complex. Hence, the reduction of antinutritional factors makes SFF more attractive to animals, reducing the risk of feed wastage.

Henceforth, it is essential to identify the recent advancements in producing high-quality feed and propose techniques that can help improve animal nutrition through SSF. Solid fermented feed is mainly provided to animals without adding other substances or additives (Yuan *et al*., 2017). Agro-industrial waste is often fed directly to animals after fermentation, but they are also used as a substrate to produce feed additives such as enzymes (Leite *et al*., 2021).

Changes in SFF nutritional value

Over the last few decades, there has been a significant increase in the utilisation of SFF for livestock. Solid fermented feed has high nutritional value and provides several health benefits for livestock. However, the nutritional value of SFF has changed over the years due to several factors such as changes in feed formulation, the use of different microbial strains, and advances in fermentation technology.

One of the most significant changes in the nutritional value of SFF is the increase in the crude protein content. This increase can be attributed to the use of high-protein feed ingredients, such as soya bean meal, and the use of certain microbial strains that are known to increase protein synthesis during fermentation. Research studies suggest that the use of specific microbial strains, such as *Bacillus subtilis* or *Lactobacillus plantarum*, can lead to significant increases in crude protein content (Lu *et al*., 2022; Zhao *et al*., 2017). Similarly, the digestibility of SFF has also improved over the years. This is mainly due to improvements in fermentation technology.

As stated, SSF improves the nutritional aspect of agro-industrial waste in different ways depending on the target end-product. It is true that SSF changes the nutritional value of feed raw materials, although in such feeds there are a number of changes that are often much more important than the increase in protein levels. The decrease in the fibre and non-starch polysaccharide content, which limits the digestibility of nutrients is another aspect which improves the nutritional content of SFF (Yang *et al*., 2021). Additionally, the reduction in the level of anti-nutritional substances such as glucosinolates, phytic acid and sinapine plays an important role in the nutritional value of SFF.

Each micro-organism affects the end-product of SSF in its path. Bacteria, especially *Bacillus* species, have been widely used on protein-rich agro-industrial waste (Lu *et al*., 2022), whereas fungi have been widely used on starch-rich substrate (Lin *et al*., 2018). Nevertheless, their effects are not limited to a particular substrate but depend on the desired yield. Moreover, the changes in the nutritional aspect of SFF have been attributed to the factors affecting SSF, a study attributed the change in antioxidant activity, protein and lipid to temperatures, pH and fermentation (Nguyen *et al*., 2022).

Effects of SFF on animals

Solid-state fermentation over the past decades has received enormous attention in the animal nutrition sector. Recently, researchers have conducted several studies (*in vivo* and *in vitro*) to explore the applications of SSF in animal nutrition (Das *et al*., 2022; Jiang *et al*., 2021 b; Shi *et al*., 2017; Zhang *et al*., 2022).

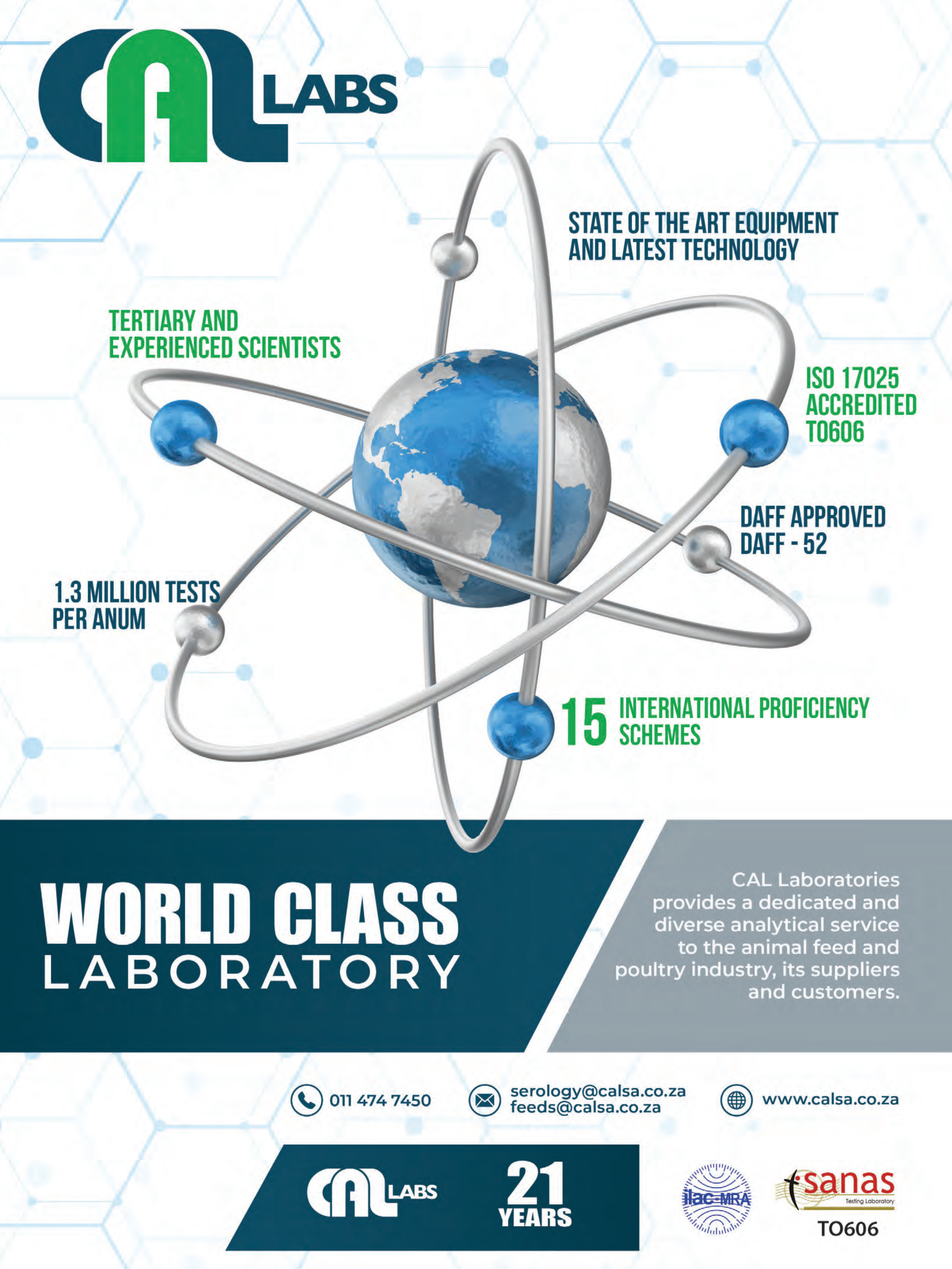
Solid-state fermentation has been defined as a raw feed ingredient or commercial feed in which macromolecular substances and anti-nutritional factors are converted into more efficient and non-toxic nutrients by the metabolic activities of micro-organisms. Moreover, due to the beneficial characteristics of SFF, it is, therefore, safe to use them to feed animals to meet the high demand of consumers for safe products. Consequently, it is crucial to explore the effects of SFF on animals.

Broilers

The feed of broilers consists primarily of grains (maize, soya bean, barley, oats, rice, rye, sorghum) and protein supplements, and its production contributes up to 70% of the total production cost in commercial poultry. Feed ingredients such as wheat, soya bean, barley, and rapeseed contain considerable amounts of non-starch polysaccharides that cannot be easily digested by poultry due to the lack of endogenous hydrolysing enzymes. However, using fermented agro-industrial by-products to replace maize and wheat soya bean-based diets will help minimise production costs and improve feed quality.

Solid fermented feed has been proven to increase the nutritional factors of feeds. This is achieved by improving the solubility of essential amino acids (Borresen *et al*., 2012), increasing the digestibility of various nutrients such as organic matter, nitrogen, amino acids, fibre and calcium (Akinola *et al*., 2015; Ashayerizadeh *et al*., 2018).

Moreover, the dietary addition of solid fermented canola meal improved feed


CAL LABS
STATE OF THE ART EQUIPMENT
AND LATEST TECHNOLOGY
TERTIARY AND
EXPERIENCED SCIENTISTS
ISO 17025
ACCREDITED
T0606
DAFF APPROVED
DAFF - 52
1.3 MILLION TESTS
PER ANUM
15 INTERNATIONAL PROFICIENCY
SCHEMES
WORLD CLASS
LABORATORY
CAL Laboratories provides a dedicated and diverse analytical service to the animal feed and poultry industry, its suppliers and customers.
011 474 7450
serology@calsa.co.za
feeds@calsa.co.za
www.calsa.co.za
CAL LABS
21 YEARS
ilac-MRA
sanas
Testing Laboratory
TO606

intake, calcium digestibility, and retention of nutritional factors but negatively affected bodyweight gain and feed conversion ratio in broilers (Olukomaiya *et al*., 2021). Different studies showed the positive effect on the growth performance of broiler chickens fed with fermented rapeseed meal (Ashayerizadeh *et al*., 2018; Elbaz *et al*., 2023). Additionally, broilers fed a canola diet with enzyme supplementation had an improved feed conversion ratio compared to the SSF canola meal diet at the end of 21 days (Olukomaiya *et al*., 2021).

Solid-state fermentation using *C. crassa* decreased crude fibre, and increased crude protein and amino acid contents of some agro-industrial by-products such as cassava pulp, banana bark, and rice bran which have proven to be potential feed ingredients (probiotics) as they improve the growth and immune system of broilers (Sugiharto *et al*., 2018).

Pigs

Researchers have been focussed on feeding pigs with liquid-fermented feed, but recently, significant focus has been given to feeding pigs with solid-fermented feed. Maize as an energy source and soya bean meal as a plant protein source are the most common feed ingredients in pig nutrition worldwide. Digestive utilisation energy of most pigs varies from 70 to 90%, and the rest (10 to 30%) is excreted in the urine, and faeces are lost as body heat and gases in the gut of pigs (Noblet and Henry, 1993). Since animals cannot use all of the energy and nutritional factors contained in feed grains, hence a need for SFF to maximise energy and nutritional factors utilisation.

Considering the beneficial impacts of SSF on feed safety, nutrient bioavailability, pig growth performance, and meat quality, fermented feed has been regarded as a novel substitute for antimicrobial growth promoters in pigs. Most studies showed a positive impact on protein digestibility in pigs (Hao *et al*., 2020; Wang *et al*., 2018); nevertheless, the effect depends much on the micro-organisms used and the fermentation parameters. In a meta-analysis performed by Xu *et al*. (2019), SSF improved the crude protein of pig feeds with significant heterogeneity (SMD [95% CI] 1,209 [0,501, 1,917], I2 = 86,50%, PQ < 0,001).

Nevertheless, in the same meta-analysis by Xu *et al*. (2019) fermented feed had no significant effects on the growth performance and nutrient digestibility in finishing pigs compared with the essential diet. In the subgroup analyses, fermented ingredients increased the growth performance of weaned piglets and growing pigs, and fermented additives stimulated the growth of pigs at all stages. Including wheat fermented with either *Lactobacillus plantarum* or *Lactobacillus buchneri* in the nutrition of piglet diets also increased the ileal digestibility of starch (Koo *et al*., 2018). Furthermore, including spontaneously fermented barley or wheat in growing pig diets improved the ileal starch digestibility of the diet (Jørgensen *et al*., 2010).

Ruminants

There is an increasing need to optimise the usage of unconventional feed ingredients for ruminants to guarantee sustainable use of resources. Agricultural waste products such as rice straw, soya bean, and wheat straw have great potential to be used as ruminant feed. Solid-state fermentation can produce feeds for ruminants, providing a higher population of yeasts to enhance ruminal fermentation.

In addition, the presence of lignin in these unconventional feeds hinders their efficient usage as a ruminant feed (Moore and Jung, 2001). Therefore, the application of SSF to break lignin and make available nutritional ingredients in feed is essential for feed production. Several methods have been used in the past decades to break the lignin complex using different techniques such as physical and chemical treatment of feeds (Hendriks and Zeeman, 2009).

Due to the high demand for safe and environmentally friendly animal feeds, SSF has been given enormous consideration due to its numerous advantages. The application of SSF for protein improvement of lignocellulosic residues has received significant attention due to its direct applicability to the fermented product for ruminant feeding purposes. A recent study demonstrated the beneficial effects of fermented soya bean meal containing rumen-degradable protein on cattle's milk performance (Fessenden *et al*., 2020).

Another *in vitro* study showed that the replacement of fermented apple bagasse with lucerne hay in an *in vitro* rumen habitat resulted in beneficial changes to living yeast colonies and lactic acid concentration, while not affecting other fermentative and microbial parameters of the *in vitro* rumen environment (Castillo *et al*., 2015). Another study also showed the significant effect of adding fermented yellow wine lees into the diet of cows, improving lactation performance, reducing diet costs, and increasing dairy farming income (Yao *et al*., 2020).

Fungal fermentation of various substrates for ruminant feeding has been demonstrated to be able to degrade more than 50% of the lignin content of rice straw, oil palm frond, and sugarcane bagasse and 59 to 78% of the lignin content of wheat straw (Tuyen *et al*., 2013; Kuijk *et al*., 2015). The application of SSF for ruminants has been mainly focussed on the degradation of the lignin content of ruminant feeds.

Conclusion

The potential of SSF is significant, which is why further study is being conducted. The progress in technologies, such as the production of bioreactors, has greatly enhanced solid fermentation. The utilisation of physical processes such as ultrasonic and magnetic fields during SSF has enhanced the overall output yield. However, further investigation is required to explore the implementation of these physical processes in fermentation. Hence, additional investigation is necessary to further examine the influence of these physical mechanisms on microbes throughout the fermentation process, rather than solely prior to it.

Furthermore, agro-industrial residues are a potential substrate for producing enzymes, peptides and improving their nutritional content by solid-state fermentation, which can be used to improve the welfare of animals. However, there is a need to explore all the different applications of SSF to maximise the low cost of animal feed production and enzyme synthesis.

This article was condensed for publication in *AFMA Matrix*. For the full article or references, send an email to garbabetchem1@gmail.com or mhl@ujs.edu.cn, or visit *www.sciendo.com/article/10.2478/aoas-2024-0029.*

Anton Paar's latest e-book: A guide to food safety and quality

In the ever-evolving field of food science, the safety and quality of food products are of the utmost importance. From the initial concept to the final product, food scientists conduct rigorous testing and analysis to meet regulatory standards and consumer expectations.

Anton Paar's latest e-book serves as an essential resource for professionals engaged in research and development and quality control within the food industry.

Comprehensive food testing

Food testing is a fundamental aspect of the food industry, ensuring that products are safe for consumption and meet quality standards. This process involves a wide range of analyses to detect and quantify various attributes and contaminants, identifying potential hazards and verifying that products are free from harmful elements while maintaining desired quality levels.

Laboratory and in-process testing

Food testing occurs in both laboratory settings and during the production process. Laboratory testing offers controlled conditions for detailed analyses, while in-process testing ensures ongoing compliance and quality during production.

In-line monitoring of parameters such as density, concentration, and viscosity is integral to modern food and feed production. It ensures quality, consistency, and safety while enhancing efficiency and reducing waste.

Laboratory extruders provide insights into the food material behaviour during production, enabling smooth scale-up to full production capacity.

Physical and chemical analyses

Physical and chemical analyses are vital throughout the food production lifecycle:

- **R&D:** Ensures that new product formulations meet safety, stability, and quality standards.
- **Production:** Guides testing of raw materials, processing, quality assurance, packaging, and storage.

Examples of chemical analyses include polarimetry, Raman spectroscopy, refractometry and more. Physical analyses involve texture measurement, colourimetry, rheology, and density determination.

Role in HACCP programmes

Hazard analysis and critical control points (HACCP) programmes rely on physical and chemical testing to monitor and verify safety and quality at critical control points, from raw material inspection to final product assessment.

Read our e-book at *www.bit.ly/45qXwB8* to find out more!

About Anton Paar

Founded in 1922 in Graz, Austria by Anton Paar, the company is the world market leader in the measurement of density and concentration, the determination of dissolved carbon dioxide, and in the fields of rheometry and viscometry. Anton Paar's customers include most of the major beer and softdrink manufacturers worldwide, companies active in the food, chemicals, petroleum, and pharmaceutical industries, as well as leading academic groups.

For many decades, Anton Paar has combined precise mechanical production with the latest achievements in the fields of research and development. In recent years, Anton Paar GmbH invested up to 20% of its annual turnover in research and development. The company offers analytical solutions that are produced within the 11 producing sites (in Europe and the United States).

Since 2003, the Charitable Santner Foundation has been the owner of Anton Paar. It is dedicated exclusively and directly to charitable purposes.

Located in South Africa, Anton Paar Southern Africa (Pty) provides sales, application support and service to users across Southern Africa.

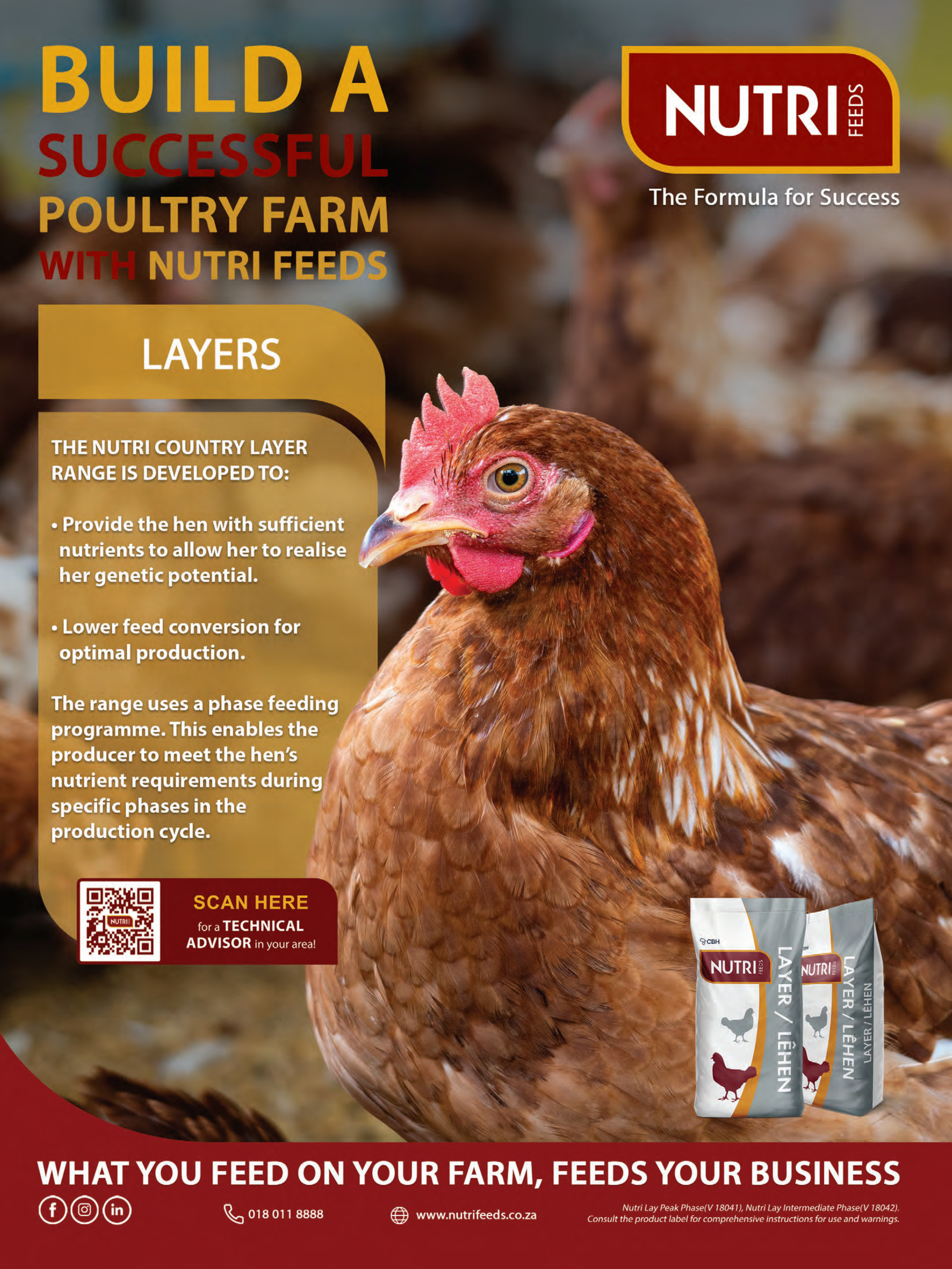
Strategic growth

Besides focussing on research and development, Anton Paar's growth is also based on strategic acquisitions. In 2007, the company acquired Dr Kernchen GmbH near Hannover (Germany, special field optical metrology, Anton Paar OptoTec) followed by Petrotest Group (Germany, special field measurement technology for the petroleum industry, Anton Paar ProveTec) in 2012, and the addition of CSM Instruments SA in Switzerland in 2014 (special field surface analysis, Anton Paar TriTec).

In 2016, Anton Paar acquired a product line of Raman benchtop devices from BaySpec Inc. (US, CA) and licensed the technology for Raman handheld instruments from SciAps Inc. (US, MA). In 2017, Anton Paar integrated technology for particle size analysis bought from Cilas (France) into the product portfolio.

With the acquisition of Quantachrome Instruments (US) in February 2018 and AXO Dresden in February 2019, Anton Paar further expanded its range of measurement technology in the characterisation of pores and porous materials and X-ray optics. With the purchase of Brabender GmbH & Co. KG (Germany, 2023, Anton Paar TorqueTec), the portfolio was expanded with special measuring solutions for the characterisation of kneading properties and lab extrusion.

For more information, contact Poifo Dipholo at poifo.dipholo@anton-paar.com or Serena Govender at serena.govender@anton-paar.com. Alternatively, visit *www.bit.ly/4b2LE9H* or call 010 443 0950.


BUILD A
SUCCESSFUL
POULTRY FARM
WITH NUTRI FEEDS
NUTRI FEEDS
The Formula for Success
LAYERS
THE NUTRI COUNTRY LAYER RANGE IS DEVELOPED TO:
• Provide the hen with sufficient nutrients to allow her to realise her genetic potential.
• Lower feed conversion for optimal production.
The range uses a phase feeding programme. This enables the producer to meet the hen's nutrient requirements during specific phases in the production cycle.
SCAN HERE
for a TECHNICAL ADVISOR in your area!
CBH
NUTRI FEEDS
LAYER / LÊHEN
WHAT YOU FEED ON YOUR FARM, FEEDS YOUR BUSINESS
018 011 8888
www.nutrifeeds.co.za
Nutri Lay Peak Phase(V 18041), Nutri Lay Intermediate Phase(V 18042).
Consult the product label for comprehensive instructions for use and warnings.

Nutri Feeds puts their eggs in the Dr Böhmer School basket

By Lynette Louw

The Dr Böhmer School is a Bloemfontein high school with approximately 550 pupils aged 12 to 19, mostly from disadvantaged backgrounds. Because most of the children have learning disabilities, the school has a very practical approach to education, providing a learning environment in which pupils can acquire functional and technical skills such as hairdressing, welding, and food production. These subjects assist in equipping them to become less dependent on others for their care, and to apply for jobs where they can put their skills to work.

A unique project

Pupils were recently offered the opportunity to enhance their skills, when feed manufacturer and AFMA member, Nutri Feeds, decided to invest their time and effort in assisting the school with feed for their egg production unit. Nutri Feeds, a subsidiary of Country Bird Holdings, is a major player in the South African poultry industry.

"In 2021, the Dr Böhmer School introduced agriculture to its curriculum," explains Santa Ferreira, the national marketing manager at Nutri Feeds. "However, because the school is urban-based and not rural, they do not have the luxury of keeping larger livestock – this is partly because of space and partly because of municipal regulations. Hence, they purchased 90 point-of-lay hens which provided them with the opportunity to run a livestock enterprise which would teach and encourage self-sustainability. Nutri Feeds was contacted to make feed recommendations for the unit."

With one of their four feed factories located in Bloemfontein, it simply made sense for Nutri Feeds to become involved on a larger scale, says Ferreira. This took on the form of technical support, feed recommendations, and of course poultry feed. "From our side we ensure that the chickens receive the correct rations, and that the housing has sufficient airflow and light. We also provide advice regarding the correct handling of poultry, and correct ways of providing feed and water."

Becoming egg-sufficient

The project has since gone from strength to strength and the school now has 320 layer hens. "In fact, just recently I was told that the school has purchased another 100 young layers. They currently collect an average of 180 eggs per day from the hens."

The school is able to provide eggs to its hostel as well as their home economics class where pupils are taught to cook. Surplus eggs are sold for an extra income.

From the left are teachers Johan Nel and LW Viljoen with Henco Christie and MJ Jonker of Nutri Feeds.


Pupils and staff of the school constructed these A-frame layer hen cages.

The pupils involved in the project have become young egg producers of note – they care for and feed the birds, clean the housing, and collect the eggs daily. They even participated in the initial construction of the hen house. Principal Kallie Viljoen is very pleased with their progress and says they hope to increase the scale of the operation in time.

"There is the old saying that if you teach a man to fish, you feed him for life. In this case, we simply gave our feed and support, but it went a very long way. Besides making a difference in these children's lives, we stand in awe of the manner in which they have taken ownership of this project," says Ferreira.

Investing in the future

Besides their continued involvement in the school project, Nutri Feeds is also serious about equipping young emerging farmers with the skills needed to successfully produce chickens on a commercial scale.

During the recent instalment of Nampo at Bothaville in May, the company announced their intention to present a series of workshops to inform and upskill emerging farmers. "It is in our country's interest that we upskill and mentor emerging farmers, and ensure that they become the commercial producers of the future. Further details on these workshops will be made available in due course."

For more information, email Santa Ferreira at sferreira@cbh.africa.

Farmwise offers a comprehensive brokerage service with a diverse client base which includes producers, consumers and speculators from a wide geographical area.

This enables us to keep an 'ear to the ground' at all times. We have a low client to broker ratio, which ensures prompt and efficient service.

The company has developed an internet based trading system that enables our clients to monitor the market in real time, as well as placing electronic orders.

Farmwise was one of the founding members of the AMD Division of SAFEX,so we have stood the test of time.

T +27 11787-3666
info@farmwise.co.za
PO Box 3660, Randburg, 2125
www.farmwise.co.za

Farmwise grains

Specialised services offered by Farmwise:

- **Trading on the South African Futures Exchange (SAFEX)**
  We have a highly skilled team of professionals that provide market-based solutions to all participants.
- **Currency futures trading**
  Farmwise is a member of the Yield-X division of the JSE. Allow us to hedge your currency risk.
- **Spot trading**
  This market encompasses a wide variety of feed grains and other specialty products. To ensure successful trading in this environment in-depth knowledge of the marketplace, the counter parties involved and the risks inherent to these activities are required.
- **In-silo grain financing** provides our clients the financial flexibility to make considered marketing decisions. Cash flow constraints should not force a market participant into a marketing decision and Farmwise provides the where withal to ensure this.
- **Regular workshops and in-house training,** ensures that staff and clients remain on the cutting edge of all new developments in the marketplace.

Dr Hannalien Meyer: Mycotoxin myth buster


Dr Hannalien Meyer.

By Elmarie Smit, Plaas Media

Mycotoxins occur worldwide in major grains, and producers, traders and processors are all challenged with preventing serious health problems among people and animals. Little was known about the multi-mycotoxin occurrence in maize and wheat produced commercially in South Africa. But behind the scenes, analytical chemist, Dr Hannalien Meyer, quietly conducted the first comprehensive study that reports on the multi-mycotoxin occurrence in South African-produced wheat and maize crops.

She has also been a member of the Animal Feed Manufacturers Association (AFMA)'s technical committee since 2015 and the convenor of the mycotoxin subcomittee since 2018, the year in which this subcommittee revised AFMA's code of practice for the control of mycotoxins in the production of animal feed in South Africa.

An analytical mind

Dr Meyer received a PhD in chemistry in April this year on the chemical analysis and survey of mycotoxins in South African-produced grain, with emphasis on those produced by *Stenocarpella maydis* in maize. The main objectives of this study were to gain insight into the prevalence of grain-related mycotoxins in South African-produced wheat and maize, and to better understand the potential risks associated with toxins produced by *S. maydis* infection in maize.

Dr Meyer has 43 years' experience in different analytical laboratories. Her focus is the development and validation of analytical methods using different chromatographic and mass spectrometric techniques such as GC with selective detectors, GCMS, HPLC and LC-MS/MS to determine pesticide residues, veterinary drugs, mycotoxins, and other contaminants in food and feed matrices and environmental samples.

She has been a technical signatory in South African National Accreditation System-accredited testing facilities for more than 20 years and presented several laboratory hands-on training programmes to analysts from different African countries. She also received the Chromatographer of the Year Award in 2014 from ChromSA for her contributions to chromatography in South Africa.

Mycotoxins under the microscope

Dr Meyer's PhD looks into the occurrence of the different mycotoxin groups in wheat and maize (white and yellow) commercially produced in all the production regions of South Africa over four consecutive production seasons. The absence of aflatoxins in the 1 400 maize samples collected at harvest offers valuable scientific-based information for food safety and food security in South Africa.

The concentration ranges, regional variations, and seasonal trends of deoxynivalenol (DON), fumonisins (FUM), and zearalenone (ZEA) in white and yellow maize reported for all the production regions created an increased awareness among producers, traders, and food and feed processors regarding the possible exposure of South African consumers and animals to these toxic grain-based mycotoxins in food and feed.

Diplodiatoxin was found for the first time in white and yellow maize in a follow-up, four-year survey conducted from 2019 to 2022. Maize contaminated with diplodiatoxin with no visible *S. maydis* infection was widespread in six of South Africa's seven production regions.

The study made a positive contribution to the grain value chain to ensure food and feed safety. With the confirmation of DON and FUM in South African maize, the country's mycotoxin regulations were expanded in 2016. Maximum allowable levels for FB1 and FB2 in maize and DON in cereal grains intended for further processing, as well as maximum levels in related processed food products ready for human consumption, were published. The season and regional variations in the occurrence of the different mycotoxins emphasised the importance of ongoing monitoring along the grain value chain.

A fundamental role at the SAGL

Dr Meyer joined the Southern African Grain Laboratory (SAGL) as a technical specialist in 2012, focussing on the development and implementation of new analytical methods and techniques for chemical analyses, mycotoxin analysis in food and feed, vitamins in fortified food products, and sugars and amino acid analysis. At the SAGL she developed and validated an LC-MS/MS method for the simultaneous quantitative analyses of 14 different mycotoxins (regulated and emerging mycotoxins) in wheat and maize.

The establishment of an accredited test facility for multi-mycotoxin analyses, with a capacity to analyse a large number of samples every season, enabled the long-term monitoring of multi-mycotoxins. The samples were collected at grain storage facilities when consignments were delivered at harvest.

The SAGL established another long-term multi-mycotoxin monitoring project with funding from the Maize Trust and the participation of feed manufacturers affiliated to AFMA and feed millers affiliated to the National Chamber of Milling. Representative maize samples are collected at the pre-processing stage in the value chain. The results of this long-term monitoring (since 2015) of regulated mycotoxins in white and yellow maize at the pre-processing stage confirmed the seasonal variations in the occurrence and concentrations of DON, FUM, ZEA, and the metabolite 15-ADON.

The annual survey results offer the AFMA feed mills insight into the mycotoxin levels of raw maize processed at feed processing plants in South Africa, and assist them in developing sound practices to reduce mycotoxin contamination along the value chain.

For enquiries, contact the SAGL on 012 807 4019 or visit *www.sagl.co.za.*

Ethoxyquin attenuates enteric oxidative stress and inflammation in heat-stressed broilers

By Abdelmotaleb Elokil, Shijun Li, Wei Chen, Omar Farid, Khaled Abouelezz, Khairy Zohair, Farid Nassar, Esteftah El-komy, Soha Farag and Mahmoud Elattrouny

Intestinal oxidative stress in broilers is produced by chronic heat stress (HS) and has a negative impact on poultry performance as it induces intestinal inflammation and promotes the invasion of gram-negative bacteria, such as bacterial lipopolysaccharide (LPS). Therefore, dietary inclusion of the antioxidant compound, ethoxyquin (EQ), could improve enteric antioxidant capacity, immune responses, and the epithelial barrier, and maintain the symbiotic gut microbiota community.

To investigate the effects of EQ supplementation on alleviating enteric oxidative stress in heat-stressed broilers, 200 one-day-old male Ross 308 broilers were randomly assigned to four groups (n = 50 chicks/group; n = 10 chicks/replicate) and fed a basal diet supplemented with 0 (CT), 50 (EQ-50), 100 (EQ-100), and 200 (EQ-200) mg EQ/kg^{-1} for five weeks. The chicks were raised in floor pens inside the broiler farm at a temperature and humidity index (THI) of 29 from d 21 to d 35.

Results

Effect of dietary supplementation of EQ on growth performance and relative organ index

The results presented in *Table 1* show that the growth performance of heat-stressed Ross 308 male broiler chicks was affected by dietary EQ levels. The results reveal that supplementing broiler diets with 50, 100, and 200mg EQ/kg had significant linear and quadratic effects on FBW, ADG, and survival.

The average FBW and ADG were affected by EQ dietary inclusion, which was significantly increased in the EQ-50 group compared to the other groups. The highest average FBW (2 318,5g) was observed in the EQ-50 group (P < 0,05, linear or quadratic; P < 0,05). In addition, ADG significantly increased (P < 0,01, linear or quadratic P < 0,05) in chicks in the EQ-50 group compared to the other groups. The average FCR showed a significant response (P < 0,05) to the EQ substitution.

There were statistically significant differences (P < 0,05, linear or quadratic P < 0,05) in the average carcass yield and relative organ weights, including those of the liver, gizzard, and heart between the groups. The highest average (linear or quadratic, P < 0,05) of carcass yield (1 785,05g) and index weights for heart (0,63%) was observed in the EQ-100 group. In addition, the highest average (P < 0,05) of index weights for liver (2,43%), and gizzard (2,01%) were measured in the EQ-200, and CT groups, respectively.

Effect of EQ on hepatic antioxidant activities and total adenylate

The results reveal significant differences among the groups in the liver concentration of malondialdehyde (MDA), which was significantly lower (P < 0,05, linear P < 0,05) in the EQ-50 group than in the other groups. A significant increase in the liver activity of nitric oxide (NO) (P < 0,05, linear or quadratic P < 0,05) and catalase (CAT) (P < 0,05, quadratic P < 0,05) was observed in the EQ-50 group compared to the other groups.

The highest activity of superoxide dismutase (SOD) (P < 0,05, linear or quadratic P < 0,05) and concentration of adenosine triphosphate (ATP) (P < 0,05, linear or quadratic P < 0,05) in the liver tissue were measured in the EQ-100 group. In contrast, the liver concentrations of adenosine diphosphate (ADP) (P < 0,05) and adenosine monophosphate (AMP) (P < 0,05, quadratic P < 0,05) were significantly increased in the CT and EQ-200 groups, respectively. The concentration of the digestive enzymes significantly increased (P < 0,05) the lipase and amylase levels in the mucosal tissue of the EQ-50 group.

Effect of EQ on serum immunity and cytokine activities

The results reveal significant differences in the serum concentrations of immunoglobulin A (IgA) (P < 0,05), immunoglobulin G (IgG) (P < 0,05, linear P < 0,05), and immunoglobulin M (IgM) (P < 0,01, quadratic P < 0,05), which increased in the EQ-100, EQ-50 and EQ-200 groups, respectively.

In addition, serum concentrations of ALT and AST were significantly higher (P < 0,05) in the CT and EQ-200 groups than in the other groups. The highest average serum concentrations of interleukin 10 (IL-10) and tumour necrosis factor-alpha (TNF-α) (linear P < 0,05) were observed in the EQ-100 group. There was an increase in the serum content of interleukin 6 (IL-6) and transforming growth factor-beta (TGF-β) (P < 0,05, linear P < 0,05) in the EQ-50 group compared with the other groups.

Effect of EQ on essential amino acid profile in MLD basis on dry matter

There was a significant increase in the concentrations of aspartic acid (P < 0,05, quadratic P < 0,05), alanine (P < 0,05, linear or quadratic P < 0,05), and threonine (P < 0,05, linear or quadratic P < 0,05) in the CT group compared to those in the other groups. Similarly, the highest *musculus longissimus dorsi* (MLD) of leucine (P < 0,05, linear P < 0,05) and glutamic acid (P < 0,05) were observed in the EQ-50 group.

The highest concentrations of valine (P < 0,05), methionine (P < 0,05), and arginine (P < 0,01, linear P < 0,01, and quadratic P < 0,05) were observed in the EQ-100 group. In addition, the MLD concentrations of lysine, isoleucine, and glycine were significantly higher (P < 0,05) in the EQ-200 group than those in the other groups. However, there were no significant differences in the MLD concentrations of histidine and tyrosine between groups (*Table 2*).

Effect of EQ on cytokines gene-related anti-inflammatory and growth factors

The expression of glutathione peroxidase 4 (GPx 4) (P < 0,01, linear P < 0,05), insulin-like growth factor 1 (IGF-1) (P < 0,01), and IL-6 (P < 0,01) were significantly upregulated in the liver tissue of the EQ-50 group compared to the other groups.

Table 1: Effect of dietary supplementation of ethoxyquin (EQ) on the growth performance of heat-stressed broilers.

	EQ supplementation					P-value[3]		
Variables[1]	**CT**	**EQ-50**	**EQ-100**	**EQ-200**	**SEM**	P_{ANOVA}	P_{lin}	P_{quad}
IBW (g)	44,9	44,5	44,8	44,9	3,215	0,078	0,114	0,104
FBW (g)	2 207,2[b]	2 318,5[a]	2 282,8[ab]	2 307,3[a]	13,541	0,002	0,003	0,016
ADFI (g/day)	87,37[b]	93,21[a]	91,31[a]	88,77[b]	1,304	0,036	0,528	0,673
ADG (g/day)	61,2[b]	65[a]	63,9[a]	64,7[a]	1,271	0,031	0,021	0,015
FCR (g/g)	1,41[a]	1,44[a]	1,42[a]	1,31[b]	0,042	0,028	0,117	0,109
Survivability (%)	90,13[b]	96,67[a]	90[b]	96,25[a]	2,431	0,041	0,745	0,209

[1]IBW = initial bodyweight at first day of age; FBW = final bodyweight at 35th day of age; ADG = average daily body gain; ADFI = average daily feed intake; FCR = feed conversion ratio (g/g); ADFI (g/day)/ADG (g/day). [2]All data are expressed as mean ± SEM (n = 50 chicks/group). Means in the same row within each classification bearing different letters superscripts are significantly (P ≤ 0,05) different. CT, EQ-50, EQ-100, and EQ-200 = chicks were fed basal diet supplemented with 0mg, 50mg, 100mg and 200mg EQ/kg diet, respectively. [3]The statistical analysis tests the differences between EQ treatments (ANOVA) and the linear (lin) and quadratic (quad) effect of EQ inclusion levels (polynomial contrasts). SEM, pooled standard error of mean.

The expression of GH (P < 0,05) was significantly upregulated in the liver tissue of the EQ-100 group compared with that in the other groups.

In addition, the expression of heat shock protein family A (Hsp70) was significantly (P < 0,05) upregulated in the liver tissue of the EQ-200 group compared to that in the other groups. In contrast, the expression of Il-10 genes was significantly downregulated (P < 0,05, quadratic P < 0,05) in the liver tissue of the EQs groups compared with that of the CT group.

Effect of EQ on gut microbiota community structure

The results of α-diversity measures for caecum microbiota among groups do not show any significant differences, including Chao1, Simpson, Shannon, Pilelou, Observed species, and Goods coverage. The results of β-diversity measures for caecum microbiota among groups show significant differences (P < 0,05) in principal component analysis (PCA), principal co-ordinate analysis (PCoA), and non-metric multidimensional scaling (NMDS) analysis of weighted unique fraction metric or UniFrac distance among groups. The CT group showed a significant correlation in the close cluster by the ANOSIM analysis of PCA, PCoA, and NMDS, far away from the EQs groups of heat-stressed broilers.

At the phylum level, *Firmicutes*, *Proteobacteria*, *Actinobacteriota*, *Bacteroidota*, and *Cyanobacteria* were predominant (top five phyla) in the caecal microbiota. The analysis at the phylum level revealed that the highest average (P < 0,05) relative abundance of the *Firmicutes* phyla was 85,83% and appeared in the EQ-50 group compared to the other groups of the CT (75,18%), EQ-100 (83,97%), and EQ-200 (76,93%).

Our results show that there is a significant increase (P < 0,05) in the relative abundance of *Bacteroidota* phyla in the EQ-50 group compared to the other groups. However, the lowest average (P < 0,05) relative abundance of the *Proteobacteria* phyla from Gram-negative bacteria was 2,39%, which appeared also in the EQ-50 group, compared to the other groups of the CT (13,33%), EQ-100 (12,83%), and EQ-200 (19,78%) groups. The highest average (P < 0,05) relative abundance of the *Cyanobacteria* phyla was 10,61% which was found in the CT group and was twenty times as much as that in the other groups: EQ-50 (0,62%), EQ-100 (0,26%), and EQ-200 (0,38%).

Likewise, the highest average (P < 0,05) relative abundance of the *Actinobacteria* phyla was 9,8% and was found in the CT group, compared to that of the other groups: EQ-50 (2,74%), EQ-100 (6,15%), and EQ-200 (2,77%). In addition, the relative abundance of *Verrucomicrobia* phyla significantly decreased (P < 0,05) in the EQ-50 group compared to that of the other groups.

At the genus level, the relative abundance of *Lactobacillus*, *Ligilactobacillus*, *Limosilactobacillus*, *Pediococcus*, *Blautia*, and *Faecalibacterium* showed a significant increase (P < 0,05) in the EQ-50 group; however, the relative abundance of *Streptococcus*, *Enterococcus*, *Escherichia-Shigella*, *Staphylococcus*, *Clostridia*, and *Psychrobacter* showed a significant increase (P < 0,05) in the CT group in comparison to the other groups.

Effect of EQ on gut microbiota community function

The top 50 most significant (P < 0,05) abundant KEGG orthologous genes were clustered in a heat map, combined with the analysis of microbial function among the groups. All samples from each group formed clusters based on similar microbial compositions. The PCoA plot shows that the samples belonging to the CT group are more similar to each other than those from the EQ groups. The top five KEGG orthologues annotated in the CT group were lipopolysaccharide-binding protein (K05399), engulfment and cell motility protein 1 (K12366), programmed cell death 1 ligand 2 (K06708), Bcl-2-antagonist of cell death (K02158), and engulfment and cell motility protein 3 (K19241).

The top five abundance KEGG orthologues annotated in the EQ-50 group were transport system ATP-binding protein (K01990), GTPase (K03595), branched-chain amino acid aminotransferase (K00826), family transcriptional regulator (K03435), and uridine nucleosidase (K01240). Similarly, the top five KEGG orthologues of translation initiation factor (K02518), translation initiation factor (K03113), tricarboxylic transport membrane protein (K07795), arachidonate lipoxygenase (K00460), and basic amino acid family (K03294) were the most annotated in the EQ-100 group.

The top five KEGG orthologues of the transport system were permease protein (K02033), glycosyltransferase-related protein (K00754), anaerobic regulatory protein (K01420), DNA-repair protein complement (K10847), and RNAbinding transduction-associated protein 3 (K14942)


GO FOR
GOLD
Insta-Pro's proprietary mechanical ExPress® process creates quality, organic & highly efficient feed & food ingredients.
Broilers reach market weight 4.8 days faster when using ExPress® soymeal & Hi-Gel™ corn in diet formulations. Grow 1 additional flock per barn per year.
INSTA-PRO
INTERNATIONAL
GET IN TOUCH
Michael Martin | Sales Director - Africa, Europe, Middle East
mmartin@insta-pro.com | +32.475.626.320


SAVE YOUR INCOME
FROM GOING UP IN SMOKE!
SAVE YOUR INCOME
FROM GOING UP IN SMOKE!
↑ Survival under heat stress
+9 pts
94%
90%
99%
No heat stress
Heat stress
Safmannan®
w. Heat stress
Program
Heat stress
↑ Milk yield under heat stress
(69 ≤ THI ≤ 79)
+1.5 kg/d
36.3
37.8
Control
Actisaf®
Program
Heat stress
phileo-lesaffre.com/heat-stress/poultry
Phileo
by Lesaffre
phileo-lesaffre.com/heat-stress/dairycows
Francois du Toit | 082 471 5034 | f.dutoit@phileo.lesaffre.com
Caitlin Vosloo | 071 401 5983 | c.vosloo@phileo.lesaffre.com
Hylton Buntting | 082 648 8302 | h.buntting@phileo.lesaffre.com

Table 2: Effect of dietary supplementation of Ethoxyquin (EQ) on the essential amino acid profile in MLD basis on dry matter in heat-stressed broilers.

	EQ supplementation[2]					P-value[3]		
Variables[1]	**CT**	**EQ-50**	**EQ-100**	**EQ-200**	**SEM**	P_{ANOVA}	P_{lin}	P_{quad}
Lysine	12,28[a]	8,13[b]	8,95[b]	12,82[a]	1,221	0,034	0,104	0,122
Leucine	8,55[b]	12,35[a]	12,07[a]	9,04[b]	0,975	0,024	0,044	0,081
Isoleucine	5,06[b]	5,51[b]	7,11[a]	7,62[a]	0,502	0,050	0,456	0,172
Valine	5,49	6,44	6,50	5,67	0,921	0,131	0,062	0,117
Methionine	2,15[b]	2,87[a]	2,96[a]	2,18[b]	0,237	0,046	0,064	0,108
Serine	8,93[b]	13,99[a]	13,36[a]	10,01[b]	1,221	0,059	0,419	0,338
Aspartic	25,41[a]	24,93[a]	17,34[b]	19,21[b]	2,028	0,021	0,064	0,048
Glutamic	5,19[b]	7,76[a]	7,51[a]	5,21[b]	0,674	0,026	0,186	0,101
Alanine	8,66[a]	5,27[b]	7,68[a]	5,19[b]	0,746	0,035	0,039	0,022
Arginine	4,04[b]	5,97[a]	6,07[a]	4,51[b]	0,331	0,001	0,001	0,043
Histidine	4,96	5,62	5,82	5,36	0,802	0,172	0,276	0,119
Glycine	4,12[b]	4,35[b]	5,84[a]	6,25[a]	0,527	0,025	0,118	0,202
Tyrosine	3,09	3,66	3,61	3,22	0,398	0,161	0,227	0,531
Threonine	5,94[a]	5,26[a]	5,82[a]	4,31[b]	0,246	0,032	0,004	0,018

[1]Muscle amino acids content per mmol/g tissue. [2]All data are expressed as mean ± SEM (n = 10 chicks/group). Means in the same row within each classification bearing different letters superscripts are significantly (P ≤ 0,05) different. CT, EQ-50, EQ-100, and EQ-200 = chicks were fed basal diet supplemented with 0mg, 50mg, 100mg and 200mg EQ/kg diet, respectively. [3]The statistical analysis tests the differences between EQ treatments (ANOVA) and the linear (lin) and quadratic (quad) effect of EQ inclusion levels (polynomial contrasts). SEM, pooled standard error of mean.

were the most annotated in the EQ-200 group. PCoA analysis revealed significant differences in these KEGG orthologues among the groups.

Discussion

In the present study, the addition of 50, 100, and 200mg EQ/kg diet for five weeks helped prevent OS by enhancing heat tolerance, which induced performance improvements in heat-stressed broilers. This beneficial effect enhances the blood metabolite molecules of hepatic antioxidant enzyme activities and their energy content, promoting serum immunity and cytokine activities associated with heat tolerance.

Our results show a significant increase in the final bodyweight and average daily weight gain in the EQ-50 group, which was caused by promoting mRNA expression of Hsp70, IGF-1, and GH genes in the EQ groups. These results are in agreement with those found by Cabel and Calabotta (1988), who indicated that EQ supplementation at 62,5 and 125ppm resulted in significantly heavier broiler birds at 49 d of age but had no significant effect on feed efficiency.

Significant interactions between the additions of EQ with dl-α-tocopherol acetate to the broiler diet were observed on the blood variables and the activity of glutathione peroxidase in plasma, improving effects on the weight gain and the vitamins profile (Lauridsen *et al*., 1995). Similar to our findings, Ohshima *et al*. (Ohshima *et al*., 1996) reported that plasma cholesterol level was significantly reduced from 209 with the control diet to 157mg dl^{-1} with the diet containing 50mg kg^{-1} EQ (EQ-50).

The inclusion of 2,2% EQ in a commercial canthaxanthin product (CCX) for in ovo feeding of broiler embryos showed an improvement in the oxidation status of chicks, the hatchability, and their growth performance (Araújo *et al*., 2020). Lauridsen *et al*. (Lauridsen *et al*., 1994) found a significant effect of diet supplementation with 150ppm EQ on the antioxidative and oxidative balance in broilers, which improved weight gain, feed conversion, hematocrit, hemolytic properties of erythrocytes, and plasma activities of the enzymes glutamate oxaloacetate transaminase (GOT), creatine kinase (CK), and glutathione peroxidase (GSH-Px).

In laying hens, dietary inclusion of 250mg EQ/kg^{-1} diet improved egg performance traits and feed efficiency and reduced mortality after an outbreak of Newcastle disease (Bartov *et al*., 1991). The primary effect of EQ supplementation (75 ppm) is to enhance the biosynthesis of polyunsaturated fatty acids (PUFA) in broiler livers (Donaldson, 1993), as PUFA deficiency in liver diseases is known to be associated with pathophysiological and clinical significance (Cabré and Gassull, 1996).

Health status

Liver antioxidant activity and total adenylate levels were determined to evaluate the health status of heat-stressed broilers supplemented with EQs. The present study shows that diet supplementation with 50mg EQ/kg^{-1} (EQ-50) can be beneficial for liver antioxidant enzyme activities (GSH, NO, and CAT) and for reducing MDA concentration.

However, the liver concentration of ATP increased in the EQ-100 group, which was caused by the promotion of mRNA expression of Hsp70, SOD2, GPx 4, IGF-1, and GH genes in the EQs groups. For this reason, EQs play a vital role in the biosynthesis and catalytic processes related to cellular antioxidant defence, which regulates candidate roles in the structural and functional enzymes of biological pathways.

The observed increase in antioxidant ability was expected because EQ is a well-known feed antioxidant for both

domestic animals and fish (Dai and Cho, 2022; Abeyta *et al*., 2023). EQ is considered a model inducer of both phase I and II biotransformation enzymes involved in the metabolism of xenobiotics, playing a key role in mediating phase reactions (e.g., oxidation or reduction) and producing more hydrophilic compounds that are catalysed by the CYP (cytochrome P450) enzyme family to enhance the expression of proteins involved in detoxification (Berdikova-Bohne *et al*., 2007).

The digestive enzymes, lipase and amylase, were elevated in the EQ-50 group, which correlated with blood antioxidants and immune efficiency. Thus, the weaning administration of milk replacer plus 350mg EQ/kg^{-1} replacer for 35 d significantly improved the blood activities of GSH-PX, CAT, and T-AOC in dairy Holstein calves (Wei *et al*., 2023).

Gut microbiota

In the present study, it was found that supplementation with 50, 100, and 200mg EQ/kg^{-1} diet for five weeks helped prevent intestinal inflammation by enhancing the enteric antioxidant capacity and cytokine secretion, promoting the symbiotic lifestyle of the gut microbiota community in comparison with the control group. Indeed, gut microbiota is emerging as a promising target for the management or prevention of inflammatory and metabolic disorders, including immune responses, the epithelial barrier, and cell proliferation.

However, host redox signalling dysfunction due to OS prompts the rapid generation of opportunistic bacteria-induced ROS and RNS such as LPS (Neish and Jones, 2014). Hence, the composition of gut microbiota has been used as a potential biomarker for HS in monogastric species (He *et al*., 2021). Optimising host redox signalling through antioxidant supplementation, such as EQ, in heat-stressed broilers may help protect intestinal barrier integrity and symbiotic gut microbiota from OS induced by HS.

Our findings show that a distinct microbial community colonised the gut due to EQ supplementation, which improved intestinal function and antimicrobial pathogenicity compared to the CT group. The relative abundances of *Lactobacillus*, *Ligilactobacillus*, *Limosilactobacillus*, *Pediococcus*, *Blautia*, and *Faecalibacterium* increased in the EQ-50 group. These genera are often used as beneficial probiotic products to ameliorate HS by enhancing intestinal barrier function and improving the gut microbiota (Zhang *et al*., 2017).

Collectively, our results show that the prevalence of intestinal bacterial pathogen phyla (*Proteobacteria*, *Cyanobacteria*, *Actinobacteria*, and *Verrucomicrobia*) in the gut microbiota increased in the CT group of chicks compared to that in the EQ groups. These phyla correlate with the detrimental effects of hyperthermia and OS on the gut epithelium, leading to impaired permeability and susceptibility to infection and inflammation (Mishra and Jha, 2019; He *et al*., 2021).

Likewise, the relative abundance of the intestinal bacterial pathogen genus of *Streptococcus*, *Enterococcus*, *Escherichia-Shigella*, *Staphylococcus*, *Clostridia*, and *Psychrobacter* which were raised in the gut microbiota increased in the CT group of chicks. This is in agreement with previous studies which found that enteric bacterial pathogens induced by chronically heat-stressed broilers may cause disturbances in the bile acid pool, leading to lipid metabolism disorders and decreased growth performance (Zhang *et al*., 2023).

The results of microbial function prediction using second-level KEGG pathway analysis reveals that the top five KEGG orthologues annotated in the CT group are lipopolysaccharide-binding protein, engulfment and cell motility protein 1, programmed cell death 1 ligand 2, Bcl-2-antagonist of cell death, and engulfment and cell motility protein 3. These functions are closely associated with the OS in heat-stressed broilers (Ayo and Ogbuagu, 2021; Biswal *et al*., 2022). In contrast, candidate pathways related to antioxidant defence, such as the transport system ATP-binding protein, GTPase, family transcriptional regulator, and uridine nucleosidase, were highly expressed in the EQ-50 group.

In chicks under heat exposure, there are elevated numbers of several detrimental genera, including *Escherichia*, *Shigella*, and *Clostridium* against advantageous bacteria, such as *Lactobacillus* and *Ruminococcaceae*, leading to the generation of alpha-toxins and contributing to the occurrence of necrotizing enterocolitis (Cao *et al*., 2021). In addition, these genera induce multiple physiological alterations in the microbiota-gut-brain axis of heat-stressed broilers, primarily via bacterially derived metabolites, hormones, and neurotransmitters, which disrupt host metabolic homeostasis, health, and behaviour (Cao *et al*., 2021; Zhang *et al*., 2023).

Recently, a mucosal microbiology approach identified some microbial groups such as *Streptococcus*, *Enterococcus*, *Escherichia-Shigella*, *Staphylococcus*, *Clostridia*, and *Psychrobacter* that are closely associated with promoting intestinal inflammatory bowel diseases and it is associated with pathological infection (Daniel *et al*., 2021).

Conclusion

The improvement in growth performance traits, relative organ index, hepatic antioxidant enzymes, serum immune and cytokine content may be attributed to the prevention of enteric oxidative stress by the abundant antioxidants produced due to the potential ability of EQ supplementation.

Dietary supplementation of 50mg EQ/kg^{-1} for five weeks to heat-stressed broilers enhanced hepatic antioxidant activity (GSH, CAT, and NO) and inhibited MDA production. In addition, it increased the activities of serum IgG, IL-6, and TGF-b, and ultimately enhanced intestinal barrier function leading to the colonisation of a distinct symbiotic microbiota community such as *Lactobacillus*, *Ligilactobacillus*, *Limosilactobacillus*, and *Faecalibacterium*.

Furthermore, EQ promoted the expression of Hsp70, SOD2, GPx 4, IL-6, and IGF-1 cytokine gene-related anti-inflammatory and growth factors in heat-stressed hepatic broilers. Thus, EQ-50 may reduce epithelial cell injury and hyperpermeability, preventing bacterial lipopolysaccharide efflux from the intestinal lumen into the circulatory system and affecting organ systems.

In summary, 50mg EQ kg/diet for five weeks is potentially a suitable feed supplement for attenuating enteric oxidative stress and intestinal inflammation, thus improving productivity in heat-stressed broilers.

This article has been condensed for publication in *AFMA Matrix*. For the full article and a list of references, visit *www.doi.org/10.1016/j.psj.2024.103761* or send an email to cwei010230@163.com.

EVONIK
Leading Beyond Chemistry

Tributyrin supplementation: Benefits to laying hens

By Natasha Davison, business manager, Evonik

Butyric acid is a short-chain fatty acid that serves as the main energy source for colonocytes. Colonocytes are crucial in the absorption of water and electrolytes from the hindgut, and have a high turnover rate every three to four days, which is a highly energy-intensive process. Butyric acid not only encourages the growth and differentiation of epithelial cells, but also acts as a cell mediator, regulating immune modulation, gene expression, and oxidative stress.

In addition, the low pH of butyric acid reduces the pH in the gut, preventing the colonisation of pathogenic bacteria (which inherently prefer higher pH levels) and, as a result, is able to modulate intestinal bacteria (Bedford and Gong, 2018).

Supplementing pure butyric acid into the diets of animals in itself presents a challenge due to the difficult handling properties of the acid. Butyric acid has a strong odour, which is unpleasant and unpalatable to animals. In addition, due to the desired location for absorption being the intestinal area (in particular the colon), rapid absorption in the foregut needs to be prevented.

One way to mitigate both challenges is through the use of protective technologies, such as supplementing the butyric acid in the form of butyrate glycerides, otherwise known as tributyrin.

The benefits of tributyrin

Butyrate glycerides come in various forms, including mono-, di- and tributyrins. ProPhorce™ SR 130 is a tributyrin with three butyric acid molecules bound to a glycerol backbone. The butyrate molecules are only released from the glycerol bonds through the activity of lipase in the small intestine, thereby protecting it from degradation earlier in the gastrointestinal tract.

Tributyrins are the most efficient source of butyrate esters and can contain three times the amount of butyric acid molecules compared to other protected sources, such as coated butyric acid.

Tributyrin in layer nutrition

Layer hens experience several stressful periods which pose digestive and physiological challenges. These are during the pullet-rearing, pre-peak/peak and late-lay phases. In the pullet-rearing phase, the layer is preparing for the high demand of nutrients which is required during peak lay. Therefore, the absorption and utilisation of nutrients need to be optimised during this period to support peak lay.

During the pre-peak and peak-lay periods, there is a sudden and drastic high demand for nutrients, in particular calcium and phosphorus, needed to support increased egg production. The bird may also face some pathogenic challenges during this phase in the form of coccidiosis, *E. coli* and *Clostridia perfringens*. Lastly, in old layers (post 55 weeks of age), intestinal functionalities

Figure 1: The improvement in egg production with the supplementation of ProPhorce™ SR 130 in old layers.

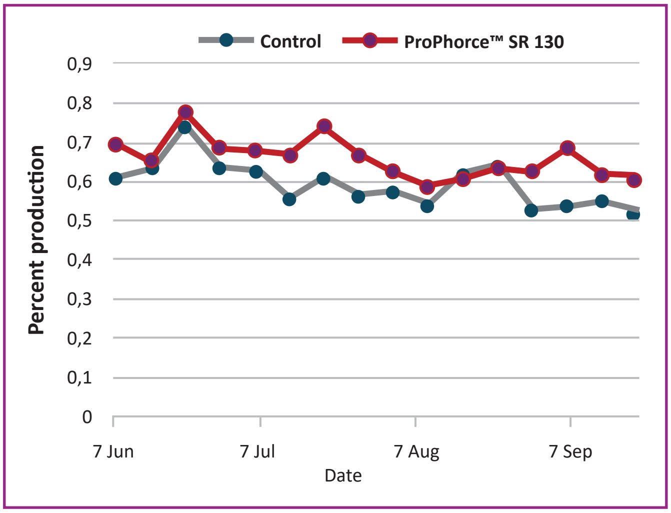


Figure 2: The effect of ProPhorce™ SR 130 supplementation on egg weight in old layers.

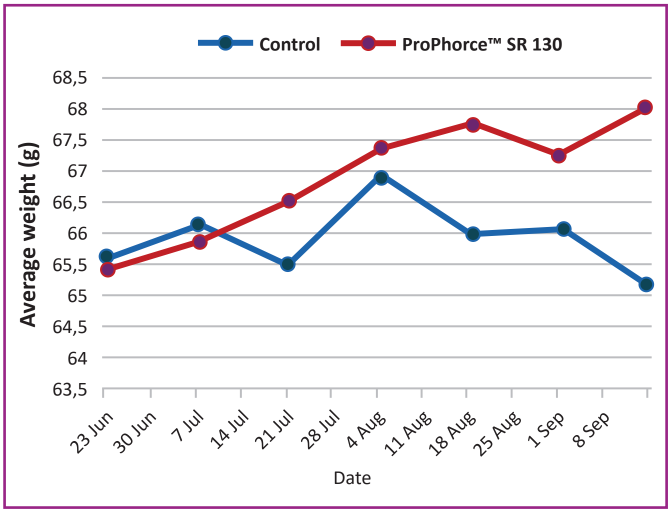
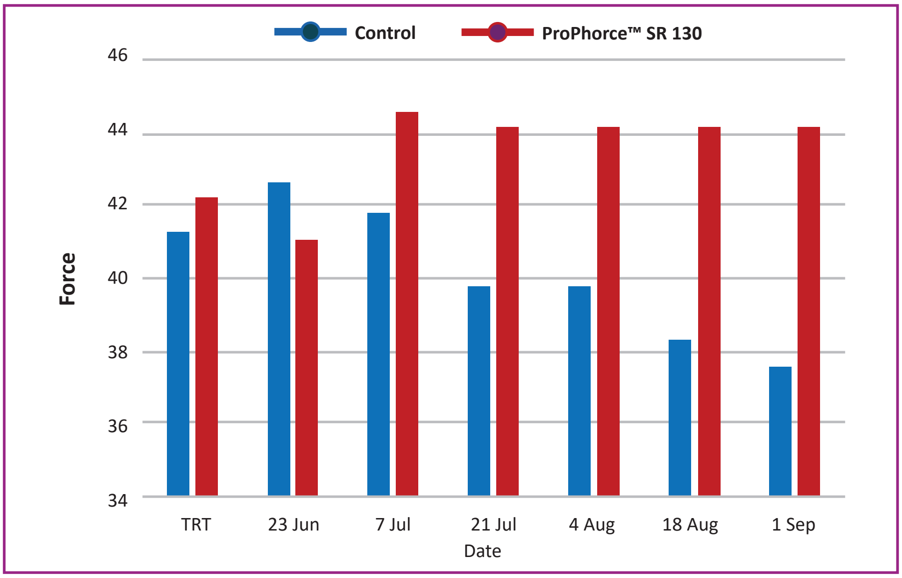


Figure 3: The effect of ProPhorce™ SR 130 supplementation on eggshell quality in old layers.



are declining, thereby impairing the absorption of calcium and phosphorus. Calcium and phosphorus are key nutrients in layer nutrition, influencing egg production rate, and egg quality and size; this may result in a lower egg production rate and poor eggshell quality during late lay.

Therefore, the intestinal physiology needs to operate at a very high level during all three of these phases to withstand this increased level of stress.

Supplementation trial

A trial was conducted in old laying hens (86 weeks of age) to assess the impact of ProPhorce™ SR 130 supplementation on egg production, egg weight and eggshell quality. ProPhorce™ SR 130 was able to increase egg production when compared to the control birds, as demonstrated in *Figure 1*. In addition, egg weight and eggshell quality (as demonstrated through an increase in eggshell strength) were improved (*Figure 2* and *3*), respectively.

These results consistently demonstrate an improvement in performance with the supplementation of ProPhorce™ SR 130 in layer diets to support the birds through the increased physiological and digestive challenges of intensive production.

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

Soya bean replacement by alternative protein sources in pig nutrition and its effect on meat quality

By Silvia Parrini, Chiara Aquilani, Carolina Pugliese, Riccardo Bozzi and Francesco Sirtori

In livestock systems, proteins feeds are one of the most expensive and limiting feed ingredients in diet formulations. The production and supply of feeds are critical steps due to the environmental impact they are connected to, such as land use change, land occupation, and energy and water use.

By 2050, the world population will reach more than nine billion people, and consequently, agricultural production will need to increase by 50% to meet food demand, whereas the arable land per person is expected to decrease. Simultaneously, the improvement of living standards in developing countries will determine an increase in global demand for sustainable animal protein.

Poultry and swine require ~42% of the total feed production and supply 20% of animal proteins, while ruminants use about 19% of feed to produce 20% of animal protein. Considering the organic pig sector, the difficulty in meeting demand for protein appears even more evident. Further, some Protected Designation of Origin (PDO) products of relevant interest for European consumers impose specific rules for animal diets, including limitations on the supply of protein resources.

This causes a dependency on imports of non-genetically modified raw feeds that are more expensive than other resources. Therefore, research on the possibilities of replacing soya bean (SB) in the nutrition of monogastric animals, particularly pigs, poultry and waterfowl, has been performed, also with attention to local or available feed resources for food production.

Materials and methods

An extensive literature screening was performed to evaluate the effects of replacing SB with alternative protein sources on slaughtering and meat quality traits of pork. The review process considered research findings published from 2012 to the present and was limited to the *Sus scrofa* species.

The protein sources for feeding piglets and growing pigs can be classified by their origin, local availability, sustainability, environmental impact, and level of innovation (i.e., conventional or studied for decades or innovative). When evaluating the potential replacement of SB, it is necessary to consider the physical and chemical characteristics of alternative resources, the level of essential amino acids, and the presence of anti-nutritional factors, as well as how they are converted by animals' physiology into final products.

In many cases, different protein sources were used in combination, and in some cases also, amino acid addition was applied. The use of diets containing combinations of multiple protein sources to overcome their singular limits has been reported by different authors.

Legume alternatives to soya bean

Legumes, and in particular dry seeds of species belonging to the *Fabaceae* family, have been used in feed formulations for pigs to complement cereals thanks to their chemical and physical characteristics. However, integration of essential amino acids is usually necessary to achieve balanced diets. Legumes show high protein content and low fat, but their nutritional composition is very different among species and dependent on variety, location, growing conditions and management where they are cultivated.

Nevertheless, legume use in animal feeding is also limited by the presence of antinutritional components that may decrease growth rates, feed intake and feed utilisation. Antinutritional compounds encompass alkaloids, flavonoids, glycosides, isoflavones, phenols, phytosterols, phytic acid, protease inhibitors, saponins, and tannins. Non-processed and/or processed legume seeds have the highest potential to replace SB, avoiding the presence of GMO in feed mixtures for monogastric animals. They are also a cheap energy and amino acid source in cereal-based diets.

In the swine sector, most of the studies in recent years considered a singular source or in association with other raw materials (legumes and other vegetable resources) to replace soya bean meal (SBM) completely or partially.

The main legume resources used as alternatives to SB in pigs' diets formulation are:

- Pea (*Pisum sativum* L.) (P).
- Fava bean (*Vicia faba* L.) (FB).
- Lupin seed (*Lupinus* L.).
- Other legume species such as vetch (*Vicia sativa* and *Vicia narbonensis*), red peas (*Lathyrus cicera* and *L. sativus*), and ervil (*Vicia ervilia*) are used less in pig diets due to their poor palatability and antinutritional factors.
- Tropical forage legumes such as psophocarpus (*Psophocarpus scandens*), stylosanthes (*Stylosanthes guianensis*) and vigna (*Vigna unguiculata*); and leaf meals such as stylo (*Stylosanthes guianensis*) associated with porcupine joint vetch (*Aeschynomene histrix*).
- Concentrates (pulp and juice) of other green plants such as grass and forage legumes e.g. white clover (*Trifolium repens* L.), red clover (*Trifolium pratense* L.), lucerne (*Medicago sativa* L.) and perennial ryegrass (*Lolium perenne*).

Oilseed by-products

Defatted by-products, such as meals, cakes, and expellers, are obtained from oil-bearing plant species in widespread cultivation for oil production. These by-products, such as those from linseed (*Linum usitatissimum*) and sesame (*Sesamum indicum*), remain rich in crude proteins and could be used as ingredients for animal nutrition.

Rapeseed meal (RM) intended for animal feed is derived from the press cake that remains post oil extraction. Its protein content is about 35% dry matter (DM), but with higher fibre content than SB (ADF ~19%, NDF ~26% DM). Rapeseed is high in its content of both sulphur-containing amino acids and phosphorus, even if its use in animal feeding is limited by the presence of antinutritional factors such as glucosinolates, tannins and phenols. RM has been included in feeding mixtures for pigs, up to 15% of the total diet. Recently, the canola meal (CM) from a variety of rapeseed was tested as a feed ingredient due to its low content of erucic acid and glucosinolate.

In the studied period, research on oilseed by-products for pig feed was mainly focussed on RM. Works in the literature reported that RM was integrated at the same level as an alternative protein source, such as legume seeds, while some trials used it as the main source for replacing SB. The addition of RM involved a decrease in feed intake due to the presence of glucosinolate, which reduced the tastiness of the diet.

Sobotka and Fiedorowicz-Szatkowska tested three diets with different inclusions of 00-RM in the growing period (50% substitution with only RM; 50% substitution with RM and FB mixture; 50% substitution with rapeseed and lupin mixture) and a total replacement in the finishing period with respect to the control diet with SBM. They found no significant differences regarding average daily gain (ADG) and feed conversion ratio (FCR), demonstrating the flexibility of using this protein source alone or together with other resources. Further, Hanczakowsk and Świątkiewicz concluded that rapeseed can be a good addition to other legumes to replace SB without having negative results.

Skoufos *et al*. reported that in order to replace 100% of SBM with RM, it is necessary to add synthetic amino acids to the mixture to balance the nutritional quality. In this way, they managed to have no differences in performance compared to the use of SB and with respect to other works in which the addition was not present. However, the results were contradicting.

The inclusion of RM can exceed 20% without compromising growth performance, whereas others have shown that with RM inclusion, a percentage of less than 20% can compromise some performance parameters. These contradictory results may arise from differences in the glucosinolate concentrations due to the use of different varieties of rapeseed. The negative impact of RM found, for example, by Torres-Pitarch *et al*., on the growth performance of pigs, could be linked precisely to the high amount of anti-mutritional factors present in the variety used.

The inclusion of RM as total or partial substitution of SB did not seem to influence the characteristics of the carcass. Sobotka and Fiedorowicz-Szatkowska reported no difference in carcass traits (dressing percentage, backfat thickness, lean content) with inclusion of rapeseed both as a total substitute of SB and in mixtures containing multiple alternative protein sources.

The inclusion of RM also as partial substitution of SB had no significant effect on the main carcass traits such as weight, carcass yield, carcass lean percentage, composition of cuts, and fat thickness. In most cases, the use of RM as an addition to diets with other main protein sources did not show effects on the carcass except for slight variations in values related to fattening.

Plant source alternatives

Fava beans (FB) and pea were the legumes most used as alternatives to SB. However, their effects on meat quality traits were often contrasting. Milczarek *et al*. tested the replacement of 28% SB with two varieties of low-tannin FB. Among the studied traits, only drip loss of meat from pigs fed the Albus FB variety was reduced if compared with the SB-fed group. Additionally, the Amulet FB variety increased the content of polyunsaturated fatty acids (PUFA) in meat when compared with the SB group.

The other traits examined (pH, colour, chemical composition) were unaffected by SB replacement. Similarly, pH, colour, water-holding capacity, and tenderness were not affected by replacing 42% of SB with pea, or 58% with FB. Instead, totally replacing SB with FB or pea brought some differences in colour, as observed by Sirtori *et al*., who reported an increased a* value in the group fed FB. With regard to chemical composition, meat from pigs fed the diet with pea and FB was lower in moisture, whereas crude protein was higher in the pea diet and lower in the FB diet. Sirtori *et al*. observed modifications in meat chemical composition only in the group fed with pea, resulting in higher moisture and fat content compared with the SB-fed group.

Different levels of SB replacement with alternative legumes were tested by Rauw *et al*. and Seoni *et al*. The former investigated the effects of increasing replacement levels of SB with narbon vetch (NV) on meat chemical composition, observing that replacing SB by up to 20% did not affect the moisture, fat and protein content of the meat. Contrarily, at increasing levels of SB replacement with sainfoin, a significant linear increase in pH at three hours post-mortem as well as of drip loss were observed. A tendency of hardness to linearly increase was also observed. Eventually, colour remained unaffected by the SB replacement levels.

Several changes in the FA profile were also observed, pinpointing a linear decreasing trend in saturated fatty acids (SFA), whereas monounsaturated fatty acids (MUFA) quadratically increased as the percentage of SB replacement was raised. Total PUFA was unaffected by dietary modification, as C18:2n-6 levels did not vary with increasing SB replacement levels. However, C20:2n-6 and C22:4n-6 decreased, while C18:3n-3 and C22:5n-3 levels linearly increased accordingly with higher percentages of sainfoin. This led to a significant linear decrease in the Σn-6-to-Σn-3 fatty acid ratio, as well as to an increase in the desaturation index (defined as the C18:1n-9/C18:0) of meat.

Oilseed byproduct alternatives

Only two studies reported the effects on meat quality after replacing SB with oilseed byproducts. Little *et al*. tested 33, 66 or 100% SB replacement with canola meal (CM) from two different varieties (conventional or high protein). None of the investigated parameters (pH, colour, texture, WHC, and chemical composition) were affected by SB replacement except for L*, which linearly decreased as the level of high-protein CM increased.

Total replacement of SB using RM was also investigated. In this case, the dietary treatment had a great impact on the chemical composition of different pork cuts; it decreased the fat content of shoulder and steak, while increasing the same parameter in ham and belly. Additionally, crude protein and moisture varied accordingly with fat content.

Concerning the FA profile, the RM-fed group showed lower contents of SFA and PUFA in steak, whereas MUFA increased. At the level of single FAs, SB replacement by RM affected C16:1, C17:0, C18:1n-9c, C18:2n-6c, and C18:3n-3 contents in steak, as well as C18:3n-3 and C20:2 contents in shoulder. Skoufos *et al*. also investigated the lipid oxidation of ham and steak at four and seven days, observing that SB replacement with RM had no effect on pork stability during storage.

Eventually, six studies investigated the use of different combinations of alternative protein sources to obtain an optimal SB replacement in terms of nutritive value and FA profile of the diet. Total replacement of SB was generally performed in the finisher diet.

Experimental dietary treatments tested by Hanczakowska and Swiatkiewicz, consisting of different legumes (pea, field bean, blue lupin, or yellow lupin) in combination with rapeseed press cake, did not affect WHC and colour. Instead, Fiedorowicz-Szatkowska observed that the combination of low-tannin pea and RM, or high-tannin FB with RM, as SB substitutes did not affect meat chemical composition and PUFA, but increased MUFA contents. SFA was lower in the pea + RM group than in the SB group, while in the FB + RM fed group, it was similar to both.

The research of Zmudzińska confirmed the results of the previous studies; indeed, the SB replacement in the fattener diet with a combination of RM, pea, and yellow lupin did not affect either meat chemical composition or physical traits (pH, colour, and waterholding capacity [WHC]). Partially in agreement with that finding, Sońta, studying different levels (37, 52, 68, 100%) of SB replacement, concluded that the experimental diets did not affect meat physical traits (colour and WHC), but slightly increased meat protein content.

Moreover, in agreement with results reported by Fiedorowicz-Szatkowska, increasing levels of SB replacement, up to total substitution, reduced the SFA content in meat. However, Sońta also observed an increase in PUFA with increasing levels of SB replacement, whereas MUFA was unchanged.

This article was condensed for publication in *AFMA Matrix*. For the full article or references, visit *www.mdpi.com/2076-2615/13/3/494* or send an email to chiara.aquilani@unifi.it.


EXTRACTING MORE VALUE
FROM FEED

More profit: Make the feedase effect work for you

Adapted by Marthie Nickols, Vitam International

Technical developments in animal production efficiency have come a long way but there is still room for improvement. Non-starch polysaccharides (NSPs), which make up 7 to 19% of the plant-based raw materials in poultry and swine diets, pose a challenge to production efficiency. Finding the right combination of enzyme activities to unlock nutrients from a variety of raw materials is essential. To do this, scientists need to understand the complex structures of NSPs.

Arabinoxylan chains, the most prevalent NSP, are key anti-nutritional factors present in plant-based raw materials. Breaking them down enhances nutrient and energy availability. Proper use of exogenous enzymes can reduce diet costs by improving nutrient digestibility, allowing a reduction in the nutrient density of feed. New research has demonstrated that with the correct combination of exogenous enzymes, activating the **feedase** effect can recover up to 3% nutrient dilution, including metabolisable energy and digestible amino acid content, in standard maize and soya diets.

Anti-nutritional effects of NSPs

NSPs trap nutrients, making them unavailable by encapsulating them or increasing digesta viscosity. Both soluble and insoluble NSPs hinder the digestion of starch, amino acids, calcium, phosphorus, and lipids. The impact depends on NSP quantity and properties, which differ among raw materials such as maize and wheat, where maize has more complex arabinoxylan structures. These factors directly influence the efficacy of the enzymatic degradation.

Understanding the indigestible components in feed ingredients will lead to solutions to potentially improve digestibility. Carbohydrases vary in their effects on NSPs due to the high variability in the NSP structures, which is why using the right combination of diverse carbohydrases will lead to enhanced effects.

The feedase effect

Different enzymes are needed to degrade each part of the indigestible dietary fraction. The ability of efficient multi-enzyme solutions to improve global feed digestibility is known as the **feedase** effect. This improves global feed digestibility by breaking down indigestible feed fractions and anti-nutritional factors, releasing more nutrients.

Research shows that the correct combination of arabinofuranosidases (Abf) and xylanases, along with beta-glucanases and cellulases, work synergistically. This synergistic action results in the greater breakdown of arabinoxylan. Abf enzymes debranch arabinoxylans, improving xylanase access to the xylose backbone, enhancing nutrient release and reducing anti-nutritional effects. This increases metabolisable energy and nutrient availability, including amino acids and lipids.

Understanding the combined effect of different enzymes on the same feed allows for more efficient nutrient release, the **feedase** effect.

Getting more from your feed

The efficacy of the **feedase** effect has already been demonstrated in scientific studies and commercial evaluations where several studies showed that FCR and other performance parameters could be recovered after reformulating diets and applying the **feedase** concept with an efficacious enzyme complex to a variety of diets. The **feedase** concept offers a different way to achieve feed cost savings.

Latest research

A new study examined the impact of adding a **feedase** to standard and diluted broiler diets on nutrient digestibility and energy utilisation. Broilers were given one of four dietary treatments from days 12 to 22. The diluted diet showed significantly lower apparent metabolisable energy (AME) content compared to the control.

Adding **feedase** improved energy digestibility, increasing the AME content of the diluted and standard diets by 2,8 and 2,9%, respectively, making the diluted diet's AME comparable to the standard diet. This demonstrates that the **feedase** effect fully compensated for the 3% nutrient dilution. At the ileal level, amino acid digestibility increased by an average of 4,4% with the **feedase**, indicating restored nutrient availability.

Carbohydrases are added to diets to improve feed efficiency, by increasing metabolisable energy. Energy is not a nutrient per se but rather a sum of energy coming from nutrient combustion, including valuable protein. In a world demanding more protein, which is both cheaper and more sustainable, the **feedase** concept could be part of the solution.

As mentioned, it has been shown that with the addition of a **feedase** to a diluted ration, broilers were able to access the same level of AME and amino acids as from the unsupplemented control diet. This enables a feed cost saving by lowering the need to include valuable raw materials and allows for the inclusion of more byproducts.

Conclusion

The **feedase** concept represents a significant advancement in animal nutrition, offering a transformative approach to improving feed efficiency and sustainability. By harnessing the synergistic effects of the correct enzyme combinations, we can unlock essential nutrients from indigestible fractions, enhancing metabolisable energy and overall feed efficacy. This not only reduces feed costs, but also addresses the growing demand for affordable, sustainable protein sources.

Implementing the **feedase** concept optimises gut health, lowers production expenses, and minimises environmental impact, all without compromising the performance of either poultry or swine. Embracing this innovative strategy is a crucial step towards more efficient and sustainable animal production.

"Make the feedase effect work for you" was adapted from an article written by Maamer Jlali, Pierre Cozannet, Marcio Ceccantini and Sofia Zenagui, Adisseo. For more information and references, send an email to Marthie Nickols at marthien@vitam.co.za.

Five potential marine organisms used in aquafeed

By Samaneh Azarpajouh, veterinarian

Aquaculture is one of the fastest-growing food-producing industries worldwide and it is predicted that by 2050, aquaculture production will reach 140 million tonnes. However, the constant growth of aquaculture relies on access to sustainable supplies of protein feedstuff as a fundamental macronutrient in aquafeed.

Due to the sustainability issues attached to the supply of protein from fishmeal, there is a need to develop alternative sustainable protein resources for aquaculture to underpin the increasing demands for aquafeed and to remediate nutrient discharges.

1 Microalgae

Microalgae are considered an alternative source of protein, fatty acids, carotenoids, and antioxidants including polyphenols, sterols, and vitamins A and E for sustainable aquaculture. Microalgae cultivates on non-arable land which minimises water demand. In addition, microalgae convert nutrients into high-quality feed ingredients, uptake carbon dioxide in the environment, and decrease environmental footprint, water pollution and deleterious ecological effects.

Microalgae contain high essential polyunsaturated fatty acids and amino acids; however, there is an upper limit to how large a fraction of the aquafeed can be comprised of microalgae.

Replacing fishmeal with microalgae to up to 30% increases growth and survival rate, improves pigmentation, enhances immunological response and overall health of aquaculture species. The bioactive compounds in microalgae have immune-stimulating properties and anti-parasitic effects. Therefore, replacing fishmeal with microalgae improves the resistance to various types of infection.

2 Fungi

Fungal biomass contains protein, essential amino acids, polyunsaturated fatty acids, fibres, minerals, and vitamins. By-products of fungi can replace fishmeal in aquafeed with beneficial effects on fish and shrimp species.

Marine fungi have antimicrobial properties against fish and shrimp pathogens such as *Lactococcus garvieae*, *Vibrio anguillarum*, *Vibrio harveyi*, *Yersinia ruckeri* and *Vagococcus salmoninarum*. In addition, fungal bioactive molecules have antioxidant and immunostimulation properties which improve the health of aquatic animals, including sea cucumbers, by regulating the host gut-microbiota structure.

Furthermore, mycoproteins are a sustainable protein resource for aquafeed due to their capability to grow in bioreactors with high metabolic rates using different by-products such as carbon and nitrogen sources. Moreover, fungi produce a variety of enzymes to convert different substrates into biomasses rich in polysaccharides with immunostimulant activity.

3 Marine bacteria

Bacteria are beneficial in the managing of aquafeed production costs, to maintain feed performance, and to improve the health of aquaculture species. Single-cell protein sources such as bacteria can be fed to different taxonomic groups of zooplankton used as live food to larvae in aquaculture hatcheries, or directly included in the diet of fish, bivalves, and crustaceans.

Bacteria produce high values of crude protein, essential amino acids, vitamins, phospholipids, and bioactive secondary metabolites. Bacterial single-cell protein-based products are effective growth promoters which can replace fishmeal, and boost immune response and survival of aquaculture species such as salmonids and shrimp.

In addition, marine bacteria such as *Bacillus* strains produce beneficial enzymes such as proteases, carbohydrolases and lipases which improve food degradation, thus enhancing the nutritional value of aquafeed.

4 Macroalgae

Macroalgae or seaweed are multicellular, large-size algae, visible with the naked eye and comprised of protein and secondary metabolites that could benefit farmed fish. In addition, various macroalgae have prophylactic and therapeutic properties.

Furthermore, macroalgae genera such as *Asparagopsis* spp. and *Sargassum* spp. have antimicrobial properties against many infectious agents of fish and shrimp. Besides antimicrobial activity, macroalgae exhibit anti-inflammatory and immune modulation traits in fish. However, application of macroalgae biomass as a source of protein in aquafeed requires key technical innovations to improve nutritional quality of macroalgae protein source.

5 Macroinvertebrates

Macroinvertebrates such as amphipods are rich in protein (approximately 40% of their dry weight) and contain less than 10% carbohydrates and lipids. In addition, amphipods contain well-balanced fatty acid composition, with high levels of favourable polyunsaturated fatty acids such as eicosapentaenoic acid, and docosahexaenoic acid.

Caprellid amphipods can be used as a live aquafeed either collected in field or cultured. Amphipods have adequate nutritional values for applications in aquaculture, but cultivation processes lead to low survival rates or species reproduction.

Concluding remarks

Application of marine organisms such as microalgae, fungi, marine bacteria, macroalgae, and macro-invertebrates as sustainable protein-enriched ingredients in aquafeed improves aquaculture sustainability and efficiency. However, marine organisms contain varied protein and bioactive compounds. Therefore, further research is needed to understand their impact on target aquaculture species.

This article was originally published in *All About Feed*. For the original script, visit *www.allaboutfeed.net.*

Diarrhoea in dairy calves: Interrelations between probiotics, gut microbiota, intestinal barrier and immune response

By Munwar Ali, Chang Xu, Qazal Hina, Aoyun Li and Kun Li

The interplay between gut microbiota and host health has attracted significant interest in the animal science community. Maintaining gut microbiota homeostasis by supplementing probiotics to treat clinical conditions such as calf diarrhoea is an emerging area of research nowadays because of increased concerns regarding antimicrobial resistance (AMR) and drug residues in animal products.

Probiotics reduce the incidence of calf diarrhoea by increasing the gut microbiota diversity and richness with more commensal bacteria such as *Lactobacillus* and *Bifidobacterium* that produce antimicrobial compounds, as well as modulating the immune response by increasing cytokines, interleukin-2 (IL-2), IL-4, IL-6, IL-10, and reducing tumour necrosis factor-α (TNF-α), by increasing production of antibodies, especially immunoglobulin E (IgE) and IgG, differentiating naive Th lymphocytes (Tho) into Th1, hence stimulating innate immunity and priming the adaptive immune response.

Specific probiotic strains of bacteria and yeast (*Saccharomyces cerevisiae*) derived probiotics maintain the integrity of the intestinal barrier.

In this review, data is organised to address the role of probiotics in treating calf diarrhoea by modulating gut microbiota and stimulating an immune response against key pathogens, to present animal and veterinary scientists and nutritionists with a new concept to treat infectious diseases from the perspective of the gut microbiota, increasing animal health, performance, and welfare.

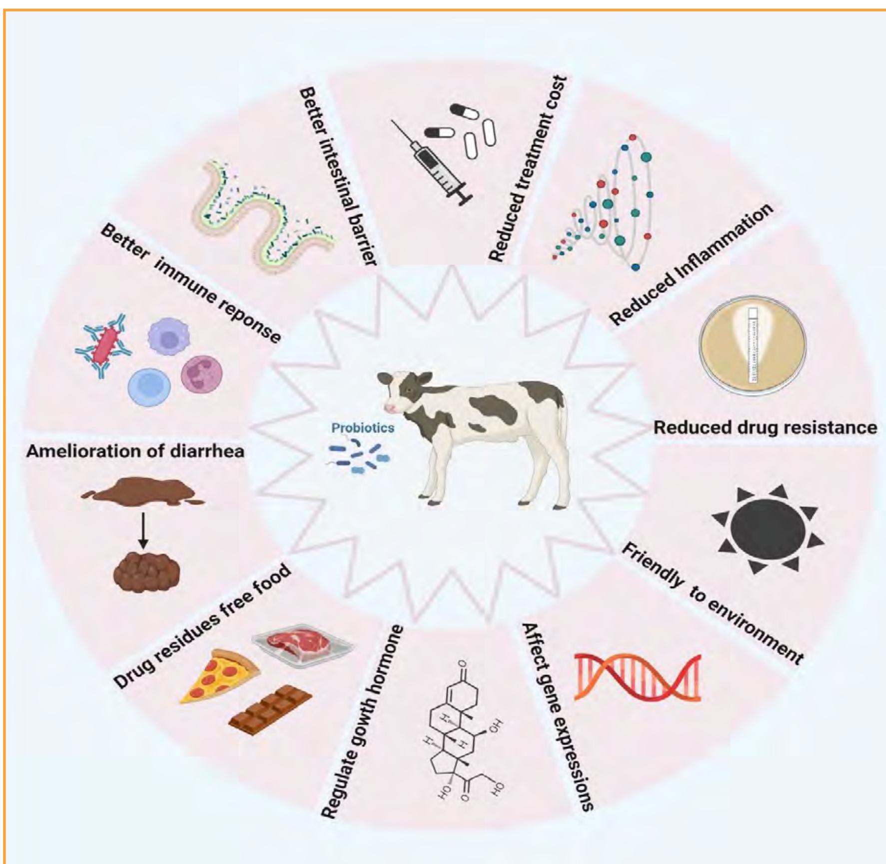
Shift to probiotics

In the United States, oxytetracycline, amoxicillin, neomycin, streptomycin, sulfachloropyridazine, chlortetracycline, and sulfamethazine are all approved for the treatment of diarrhoea in calves (Constable 2004). These antimicrobials are used as therapeutic agents in approximately 75% of calves suffering from diarrhoea during the pre-weaning period.

Due to this dependence on these antimicrobials, pathogens such as *Salmonella* and *Escherichia coli* (*E. coli*) are no longer sensitive to these antimicrobials (Malmuthuge *et al*. 2015, Scott *et al*. 2018). Additionally, irrational antimicrobial usage can weaken the host's immune system, allowing opportunistic pathogens, such as coronavirus, to attack and colonise the gut (Bartels *et al*. 2010). Antibiotics not only kill the pathogens but also kill the commensal gut microflora, resulting in gut microbiota dysbiosis (Oultram *et al*. 2015, Van Vleck Pereira *et al*. 2016), So, the discovery of potential antibiotic substitutes is a necessity.

Homeostasis of the gut microbiome is important for maintaining the functions of the gastrointestinal tract (GIT) in neonatal calves (Júnior and Bittar 2021). Probiotics and their metabolites maintain gut

Figure 1: Potential effects of probiotic supplementation on neonatal calf health.



microbiome homeostasis by modifying the gut microbial environment through cross-feeding interactions, competing for nutrients and receptor binding in intestinal epithelial cells, decreasing pH, inhibiting the development of pathogenic bacteria by producing antibacterial compounds such as bacteriocins, as well as by modulating the enteroendocrine system, nervous system (vagal afferent fibres), and immune response.

By interacting with TLRs, probiotics enhance the integrity of the intestinal barrier and reduce inflammation (Lebeer *et al*. 2018, Plaza-Diaz *et al*. 2019). Various probiotics produce a variety of bactericidal and bacteriostatic compounds, including hydrogen peroxide, bacteriocins, antibiotics, diacetyl, and organic acids (Cholewińska *et al*. 2020) (*Figure 1*).

Pathogen inhibitor

Considering the bacteria-bacterial correlations in calves, it is established that there is a negative correlation between pathogenic *E. coli*/*Shigella* and *Lactobacillus* (Fan *et al*. 2021). Hence, probiotics can inhibit the development and release of pathogenic microbes due to their antagonistic impact, alleviating clinical symptoms of a disease (Chaves *et al*. 2017).

For example, as probiotics *Lactobacilli* and *Bifidobacteria* have been documented to inhibit the growth of a large number of diarrheagenic pathogens, including *Listeria monocytogenes* (*L. monocytogenes*), *Helicobacter pylori* (*H. pylori*), *Salmonella*, *E. coli*, and rotavirus (Chenoll *et al*. 2011). Also, the use of probiotic *Lact. Rhamnosus* (Lcr35) significantly reduced the adherence of enterotoxigenic *Salmonella*, *Klebsiella pneumoniae* (*K. pneumoniae*), and enteropathogenic *E. coli* (EPEC) to the mucosa of the intestine by blocking their adhering sites (Frizzo *et al*. 2010).

Several *Lactobacillus* spps. (*L. johnsonii*, *L. amylovorus*, *L. animalis*, and *L. reuteri*) in the gut of healthy calves inhibited *Salmonella typhimurium* (*S. typhimurium*) and pathogenic *E. coli* K88 (Fan *et al*. 2021). Furthermore, fermentation products from *Lact. acidophilus* La-5 significantly reduced the capability of enterohaemorrhagic *E. coli* serotype O157:H7 (EHEC O157:H7) to produce extracellular autoinducer-2; so, the colonisation and disease progression by EHEC O157:H7 in the gut can be avoided (Medellin-Pena *et al*. 2007).

The compounds produced by probiotic strains can also inhibit pathogenic fungi present in the GIT (Kwoji *et al*. 2021). For instance, *Lact*. spps. (e.g. *Lact. coryniformis*) produces antifungal compounds such as mevalonolactone, methylhydantoin, SCFAs, and benzoic acid (Prema *et al*. 2010).

Meanwhile, the potential ability of bacterial cell walls to absorb heavy metals (Kinoshita and Protocols 2019) enables some multispecies probiotics to absorb hazardous substances in animals. This broadened the use of probiotics as feed supplements in detoxification therapy and biotechnology (Astolfi *et al*. 2019) (*Figure 1*). Probiotics improve environmental health by decreasing the formation of hazardous chemicals and reducing the ammonia (NH_3) emission by animals (Rhouma *et al*. 2017).

Probiotics as antidiarrheal agents

Salmonella enterica, *E. coli*, *Clostridium perfringens* (*C. perfringens*), *Cryptosporidium parvum* (*C. parvum*), *L. monocytogenes*, bovine viral diarrhoea virus (BVDV), bovine rotavirus (BRV), bovine coronavirus (BCoV), etc. are some common enteric pathogens mainly responsible for diarrhoea in calves (Cho and Yoon 2014).

The bacterial infection progresses quickly from reduced nutrient absorption, diarrhoea, weight loss, and weakness to extreme dehydration and, in some cases, mortality in a short time (>24 hours) (Smith 2009). Actually, after the first infection, impairment of intestinal epithelial barrier function and imbalance of gut microbiome exposes neonatal calves to superinfection by multiple enteric opportunistic pathogens (e.g., *Cryptosporidium*, *Salm*., *Clostridia*, *E. coli*, rotavirus, coronavirus, etc.), leading to sharp increases in mortality rate (Zhang *et al*. 2021).

Early regulation and management of nutrition have long-lasting effects in terms of the growth, development, and health of calves. In young ruminants, during the first two to three months of life, the abomasum and intestine are the primary sites of digestion (Kertz *et al*. 2017). The initial gut colonisers consume the oxygen present in the GIT. Thus, strict anaerobic gut microbiota has developed (Jost *et al*. 2012).

Before weaning, the intestinal microflora is mainly comprised of bacteria, particularly *Bifidobacterium*, *Lactobacillus*, *Streptococcus*, *Peptostreptococcus*, *Corynebacterium*, and *Clostridium* being more abundant among the 167 genera identified in the intestinal tract of the neonatal calf (Fan *et al*. 2020, Kim *et al*. 2021a). As the calf grows and consumes more solid feed than milk, significant changes in gut microflora can be seen (Fonty *et al*. 1987, Malmuthuge *et al*. 2019).

A healthy gut ecology prevents the invasion and colonisation of infectious foreign pathogens (Kim *et al*. 2021a). It stimulates the host immune response through interactions between immune cells and antigens during the early days of life (Fan *et al*. 2020).

Modulating gut microbiota

Complex gut microbial communities and the animal's overall well-being are strongly linked (O'Hara *et al*. 2020). For instance, developing an adequate gut microbiota before weaning (within six to seven weeks of birth) strongly influences the development and health of the calf (Oikonomou *et al*. 2013), enhances the growth, improves bodyweight gain and functionality of the GIT, minimises the risk of diarrhoea, and thus decreases death rates in calves (Priestley *et al*. 2013).

The reduced diversity of microbes in calves with diarrhoea compared to healthy calves suggests that gut microbiota diversity is linked to host health (Oikonomou *et al*. 2013). Consequently, interrelations between calf diarrhoea, gut microbial composition, and microbiota dysbiosis justify the concept of treating calf diarrhoea by modulating gut microbiota (Kim *et al*. 2021a). A decrease in the faecal score in the case of antibiotic-mediated diarrhoea was observed after modulation of the gut microbiota by probiotics administration (Sanders *et al*. 2010).

An MSP approach

Multi-strain/species probiotics (MSP) are more efficient than single-strain probiotics, particularly in managing antibiotic-induced diarrhoea, weight gain, and post-intestinal inflammation and in boosting resistance against microbial infections, both in humans and animals (Renaud *et al*. 2019). When CMP (4g/calf/day) containing

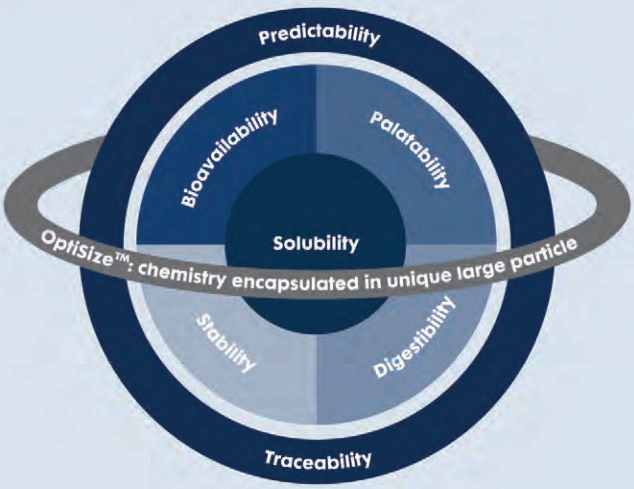
Westside
IntelliBond®
a Selko product

10 YEARS

CELEBRATING 10 YEARS OF INTELLIBOND IN SOUTH AFRICA

A DECADE OF EXCELLENCE

Key characteristics that make IntelliBond® unique



Highly stable, it doesn't destroy rumen bacteria, or tie up essential nutrients in the feed

- Vitamins
- Enzymes
- Fats (lipids)
- Probiotics

Superior handling properties

- Non-hygroscopic, no lumping for easy use, better mixability
- Greater trace mineral concentration makes IntelliBond® a more efficient product to formulate and feed. Less inventory to store and fewer bags to handle

Bioavailability & tolerance

- Superior feed conversion
- Superior weight gain and yield

Productivity/bio-efficacy

- Increased bioavailability versus inorganics, results in the delivery of significantly more trace mineral to the animal
- Equivalent bioavailability to the best performing organic trace minerals
- IntelliBond® trace minerals are approved for use in organic grown livestock

OptiSize™ Particles provide superior handling, precision and uniformity

Uniform-blending

Because of their shape and uniform size, OptiSize particles blend easily throughout the feed and avoid segregation.

Non-reactive

They won't bind with other nutrients or promote oxidation.

Non-hygroscopic

Because particles don't absorb moisture from the air, they won't cake or clump in the mixer or in the bag.

Essentially dust-free

OptiSize particles won't create dust or excessive residue, which makes cleanup easy and reduces carryover risk.

Highly uniform 100–300 micron spheres

Uniformity in both size and shape of the particles makes for easier and more predictable measuring and handling.

Trace minerals formulated with OptiSize Large Particle Technology give you precise, uniform results.

To read more about the key characteristics that make Intellibond unique, follow the QR code.

Westside Enterprises is the sole distributor of the IntelliBond Product Range in Southern Africa.

T +27 21 686 8265
W www.westside.co.za
E info@westside.co.za

Westside

Lact. bavaricus, *Lact. coryniformis*, *Lact. sake*, *Lact. curvatus*, *Lact. casei*, *Lact. rhamnosus*, *Leuconostoc* spps., and *Streptococcus* spps. with an overall viable count of 1,3 x 10^7 cfu g^{-1} for each, was fed, diarrhoea frequency was reduced in the probiotic-treated group (Kekana *et al*. 2020).

Similarly, after exposing calves with EPEC at the age of seven to 21 days, when lactic acid bacteria (43,4 × 10^9 cfu g^{-1}) with the specific isolates consisting of *Pediococcus acidilactici* (*Ped. acidilactici*) (41,2 × 10^9 cfu g^{-1}), *Ent. faecium* (43,4 × 10^7 cfu g^{-1}), *Bifidobacterium bifidum* (*B. bifidum*) (43,4 × 10^7 cfu g^{-1}), *Lact. acidophilus* (43,4 × 10^7 cfu g^{-1}) and *Lact. casei* (43,4 × 10^7 cfu g^{-1}) was administered as a probiotic, diarrhoea in the treated group was resolved 2,4 times quicker compared to the control group (Wu *et al*. 2021b).

The inclusion of MSP lowered the occurrence of diarrhoea during the first month of life, and the overall abundance of *Porphyromonadaceae* was elevated significantly in treated calves compared to the control group (Kim *et al*. 2021b).

Symbiotic complex supplement (SYM), constituting mannan-oligosaccharides (prebiotic), *Lact. acidophilus*, *B. subtilis*, *Saccharomyces cerevisiae* (*S. cerevisiae*), *Ent. faecium*, plus fibro lytic enzymes such as xylanase, hemicellulase, and cellulase, induced a significant reduction in the severity of diarrhoea when fed to calves (Marcondes *et al*. 2016). Similarly, there were lower faecal scores in calves fed 250mg/day MSP (*Lact. sake*, *Lact. salivarius*, and *Lact. casei*) compared to the control group (Stefańska *et al*. 2021). Also, adding *Fecalibacterium prausnitzii* (*F. prausnitzii*) in the first few days of calf life decreased calf mortality by reducing the incidence of diarrhoea (Foditsch *et al*. 2015).

Production of stable levels of lactate in the rumen by probiotics promotes the proliferation of *S. cerevisiae*, which typically amplifies the gut microbes that compete against starch-utilisers, scavenge oxygen, limit the lactate accumulation, control pH, and develop a more beneficial habitat for the activity of fibro lytic microbes (Kembabazi *et al*. 2021).

Adding *S. cerevisiae* (10 × 10^9 cfu g^{-1}) to milk reduced the incidence of diarrhoea by increasing the population of healthy gut microbiota and showed good survival rates along the GIT in calves (Villot *et al*. 2019). Administering MSP and yeast (1g contains *L.B. subtilis* 3 × 10^9 cfu g^{-1}, *S. cerevisiae* 1 × 10^9 cfu g^{-1}, and *L. acidophilus* 3 × 10^9 cfu g^{-1}) after the outbreak of diarrhoea in calves reduced the duration of diarrhoea in the treated group (Renaud *et al*. 2019). In another trial, diarrhoea in treated male calves decreased when fed *S. cerevisiae boulardii* (SCB) in milk (Villot *et al*. 2019).

Intestinal repair and growth

The gut epithelium performs many vital digestive and immune functions, such as absorption of nutrients, facilitation of microbial cross-talk, and acting as a barrier against pathogenic bacterial invasion (Chung *et al*. 2012, Helander and Fändriks 2014, Rautava and Walker 2007).

Probiotics not only reduce diarrhoea but also initiate the repair of the intestinal barrier function after damage. For example, *E. coli Nissle* 1917 (EcN1917) and *Lact. casei* DN-114001 prevents the disruption of the mucosal barrier by EPEC and even restores mucosal integrity in human colorectal adenocarcinoma cells (Caco-2) and transplantable human carcinoma cells (T84 cells) (Sartor and hepatology 2006). The p40 and p75 are two *Lact. rhamnosus* GG-derived proteins that prevent cytokine-induced cell apoptosis by inhibiting the pro-apoptotic p38/mitogen-activated protein kinase (MAPK) and by activating the antiapoptotic protein kinase B (PKB/Akt) in a phosphatidyl inositol-3-kinase-dependent pathway (Yan *et al*. 2007).

Probiotics, by repairing gut epithelium and restoring the integrity of intestinal barriers, improve the growth and development of calves (Karamzadeh-Dehaghani *et al*. 2021a) in terms of increased nutrient absorption, healthy microbiota, and stronger immune response (Biswas *et al*. 2022, Renaud *et al*. 2019).

Immunomodulation

The gut microbiome plays a significant role by stimulating the host immune response. This effect is particularly prominent in newborns, where the early interplay of microbial communities can initiate long-term immune responses and the development of the mucosal barrier (Chung *et al*. 2012, Petersson *et al*. 2011, Sharma *et al*. 1995).

The gut itself provides the localised immune response. Several immune cells such as neutrophils, NKs, DCs, mast cells, lymphocytes (T and B lymphocytes), and macrophages regulate the signalling between the mucosal immune system and gut microbiome to detect and neutralise pathogens (Kwon *et al*. 2010). Research using mice as model animals indicated that gut microflora dysbiosis negatively affects macrophage functions by modulating them into a hyperactive state that results in T-cell dysfunction (Scott *et al*. 2018), also resulting in increased gut permeability that allows entry of infectious agents and hence, increased disease load (Tulstrup *et al*. 2015).

Probiotic bacteria improve digestive capacity, mucosal gut barrier, and immune functions (Uyeno *et al*. 2015), reducing the risk of diarrhoea. Therefore, using probiotics in animal nutrition can decrease disease outbreaks by stimulating immune functions (Chase 2018). Bacterial colonisation in the gut stimulates DCs, which are pivotal in the induction of immune response (Christensen *et al*. 2002). Probiotics modulate the functioning of DCs by enhancing their maturation, upregulating the gene expression for cytokines, and increasing the surface expression of the major histocompatibility complex (MHC) (Ciprandi *et al*. 2004). Activation of DCs by *Lactobacilli* signals has been proved (Mohamadzadeh *et al*. 2005).

A mixture of probiotics consisting of *L. casei*, *B. fragilis*, *B. bifidium*, *L. acidophilus*, *L. reuteri*, *L. rhamnosus* Lcr35, and *Streptococcus thermophilus* (*Strept. thermophilus*), when used, increased TNF, IL-12, p40, IL-1β, IL-12p70 and IL-23 (Kwon *et al*. 2010). After using *Lact. casei* OLL2768, attenuation of the heat-stable ETEC PAMP-induced pro-inflammatory response in bovine intestinal epithelial cells was observed (Takanashi *et al*. 2013).

Probiotic strains in the gut modulated the expression of different genes. The immunomodulatory effect of probiotics was confirmed by comparing the cytokine production between targeted gene-deleted mutants and wild-type strains (Meijerink *et al*. 2010). Soluble protein (p40) derived from *Lact. rhamnosus* was

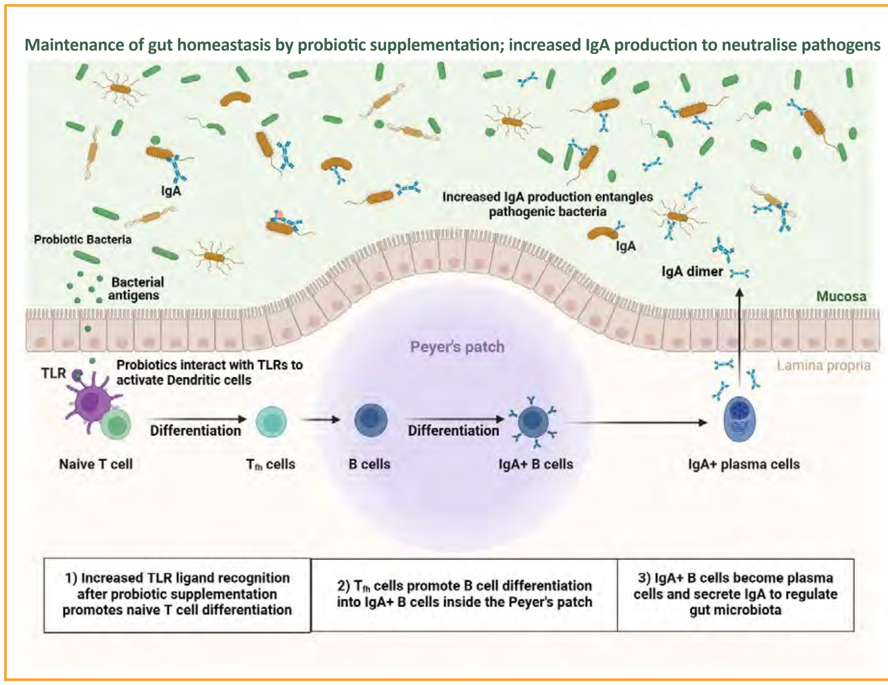
also found to be associated with reduced production of tumour necrosis factor-α (TNF-α), IL-6, interferon-γ, and influenced chemotaxis, suggesting the role of p40 in regulating Th1 immune response and innate immunity (Yan and Polk 2011). *Bacillus fragilis* influences immune response by producing zwitterionic polysaccharides and ameliorating inflammatory symptoms (Mazmanian *et al*. 2005).

Maintaining a healthy gut microbiome through probiotic supplementation also increases the production of mucus, antimicrobial peptides, and IgA, (Bischoff 2011). It was observed that IgG, IgM, and IgA levels were significantly higher in the probiotic-treated group (Wu *et al*. 2021b). Another research study showed that in young calves, *Lactobacillus* spp. when administered, their serum IgG levels increased, indicating a potential role of the host-microbiome interactions in maintaining the calf's health (Al-Saiady and Advances 2010).

Bacillus fragilis supplementation also increased IgA in the host (Cash *et al*. 2006). Secretory IgA (SIgA) entangles pathogens in the intestinal mucus to remove them via peristaltic movement, blocking their interaction with enteric epithelial receptors. Activation of mucosal immune response (e.g., SIgA production) against mucosal antigens (pathogens) depends on the intestinal stimulation by multiple cytokines (IL-5, IL-4, IL-10, and IL-6) and transforming growth factor-β (TGF-β), also, depends upon sampling by Peyer's patch M cells, processing of antigen by antigen-presenting cells (e.g. DCs), that stimulates the differentiation of naive Th lymphocytes (Tho) into Th1, which further stimulates IgE (allergic and parasitic reactions) production by B lymphocytes (Kadowaki 2007).

Finally, mesenteric lymph nodes and gut-associated lymphoid tissues (GALTs), play a role in eliminating the pathogens (Brandtzaeg 2010). These results indicated that intestinal homeostasis by probiotic administration stimulates a stronger immune response against pathogens responsible for intestinal damage and diarrhoea (*Figure 2*).

Figure 2: Probiotic supplementation increases IgA production via activating toll-like receptors (TLRs).



Probiotics activate dendritic cells (DCs) by interacting with TLRs on their surface. This leads to the differentiation of naive T cells to Th cells, which results in the conversion of B lymphocytes into plasma cells and, hence, increased production of IgA. IgA regulates intestinal homeostasis by entangling and killing pathogenic microbes.

Potential probiotic limitations

Irrespective of so many advantages of probiotics, there are some potential limitations. For example, the efficacy of probiotics is strain-dependent, with different strains of the same species showing different results (Sanders *et al*. 2019).

Also, the survivability of probiotics in harsh GIT conditions is a challenge, hindering their effectiveness (Geier *et al*. 2006); variable efficacy in different individuals based on their gut microbiota compositions and other host-specific factors (Gibson *et al*. 2017), safety concerns regarding their usage, especially in immunocompromised individuals (e.g. AIDS patients) (Doron and Gorbach 2006), regulatory issues, quality control, probiotic-drug interactions, contribution in the genetic material of host's microbiota and transfer of antibiotic-resistant genes by probiotics are some potential limitations associated with their usage (Reid *et al*. 2019, Suez *et al*. 2019).

Micro-encapsulation, quantification of strain-dependent effects in the host, and exploration of probiotic and gut microbiota interactions in detail will overcome these limitations (Geier *et al*. 2006).

Conclusions

Probiotics increase the relative abundance of gut-friendly bacteria, stimulate innate immunity and prime adaptive immunity, increase microbial diversity and richness in the gut, reduce the incidence of diarrhoea, improve feed efficiency and weight gain by increasing the integrity of the intestinal barrier, and improve local metabolism. However, these effects are strain-dependent and need further investigation to fully explore the role of the gut microbiome in eliminating the notorious pathogens.

This article was condensed for publication in *AFMA Matrix*. For the full article or references, visit *www.doi.org/10.1016/j.jia.2024.05.022*, or send an email to Aoyun Li at aoyunli@henau.edu.cn or Kun Li at lk3005@njau.edu.cn.

Effect of nitrate supplementation on diurnal emission of enteric methane and nitrous oxide

By Wenji Wang, Mogens Larsen, Martin Riis Weisbjerg, Anne Louise Frydendahl Hellwing and Peter Lund

Methane (CH_4) emission from ruminants results in significant losses of energy (Niu *et al*., 2018) and is an important greenhouse gas (GHG) which contributes to global warming. Previous studies have shown that nitrate has a promising CH_4 mitigation effect in dairy cows (Olijhoek *et al*., 2016; Feng *et al*., 2020; Wang *et al*., 2023).

Unfortunately, higher nitrous oxide (N_2O) production has also been noted when nitrate was applied to reduce CH_4 emission (Petersen *et al*., 2015). A better understanding of the effect of nitrate supplementation on N_2O emission from the rumen is crucial to assess the net global warming mitigation effect of nitrate supplementation in dairy cows, as N_2O might counteract the reduction in carbon dioxide (CO_2) equivalents from the animal as N_2O has a higher global warming potential then CH_4.

The N_2O emission from cows is very low (Petersen *et al*. (2015), and instruments able to measure the low concentrations are expensive. An alternative approach would be to measure the headspace concentration of N_2O and relate it to the CH_4 emission measured in the chamber.

To our knowledge, no previous study has looked into the N_2O emission based on rumen headspace samples. The respiration chamber is considered the golden standard for measuring CH_4 emission from an individual cow (Huhtanen *et al*., 2015), while a more detailed investigation of the diurnal pattern of gas production in the rumen could provide valuable insight into the relationship between nitrate supplementation and CH_4 production in the rumen.

The objective of this study was to investigate the effect of nitrate supplementation on ruminal fermentation and related emissions of CH_4, CO_2, and H_2 on cow level, CH_4, CO_2, and N_2O on rumen level and prediction of N_2O on cow level from rumen concentration. Therefore, we hypothesised that replacing urea with nitrate would cause an increased N_2O production in the rumen at the same time as it reduces CH_4 production.

Methods and materials

Four Danish Holstein dairy cows fitted with ruminal cannulas were used in a 2 × 2 crossover design with two periods of 14 days duration. Cows were fed *ad libitum* with two experimental diets based on either urea or nitrate (8,6g NO_3^-/kg of dry matter [DM]) supplementation.

Samples of ruminal fluid, blood, and rumen headspace gas were collected. Gas exchange was measured in respiration chambers during a 96-hour period. N_2O emission was calculated from the ratio between CH_4 and N_2O in the rumen head space and the measured CH_4 emission.

Results

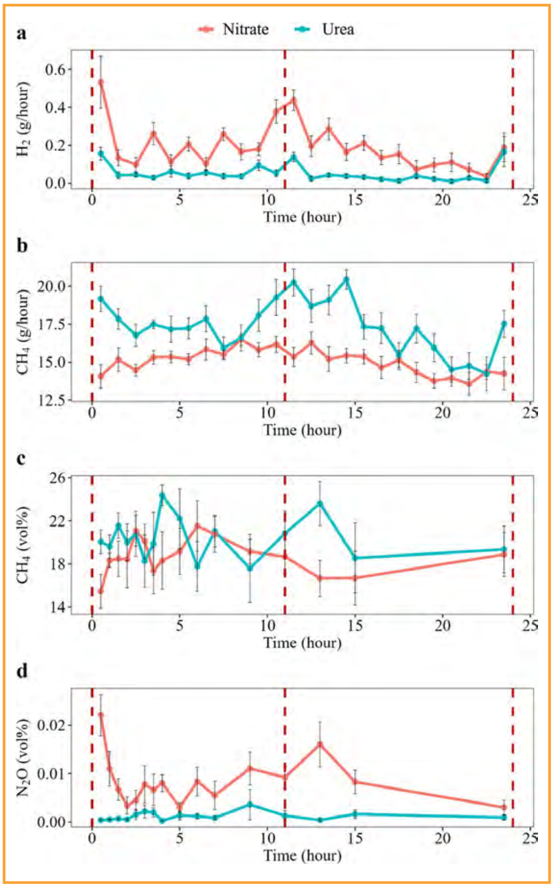
The DMI did not differ between treatments ($P = 0,95$), and neither did the energy corrected milk (ECM) yield nor milk composition ($P \geq 0,05$). The average ECM yield and percentage of fat, protein, and lactose were 27,2 ± 3,9kg/d, 4,54 ± 0,43, 3,91 ± 0,26 and 4,71 ± 0,10%, respectively, for cows receiving urea supplementation, and 27,2 ± 3,9kg/d, 4,51 ± 0,43, 3,94 ± 0,26 and 4,75 ± 0,10%, respectively, for cows receiving nitrate supplementation.

Daily CH_4 production, yield, per kg of fat and protein corrected milk (FPCM) and percentage of gross energy intake (GEI) were 13,3, 13,8, 36,7, and 14,1% lower for nitrate compared with urea supplementation ($P = 0,04$, $P = 0,02$, $P < 0,01$, and $P = 0,02$, respectively). Nitrate supplementation tended to give lower CH_4 intensity compared with urea supplementation ($P = 0,06$). Correspondingly, nitrate supplementation increased daily H_2 production and H_2 yield by 3,65 and 0,16g/kg DMI compared with urea supplementation ($P \leq 0,01$).

Aligned with the results of daily gas emission, cows receiving nitrate supplementation also showed a lower average hourly CH_4 production but higher average hourly H_2 production than cows receiving urea supplementation in the respiration chamber measurement (both $P < 0,01$). In addition, a tendency for an interaction between nitrate supplementation and time on the hourly CH_4 production was noted ($P = 0,06$). Hourly CH_4 production from cows receiving urea supplementation peaked shortly after feeding whereas less fluctuation was observed in cows receiving nitrate supplementation.

Nitrate supplementation increased N_2O production (0,47g/d) five-fold compared with urea supplementation (0,10g/d)

Figure 1: Diurnal pattern in CH_4 and H_2 emission in respiration chambers (g/hour), and CH_4 and N_2O volume proportions in rumen headspace. The x-axis is time related to morning feeding (dashed line indicates feeding). Values are LSM, and the error bars are SE of LSM.



(P < 0,001). The N_2O production is still small, but the greenhouse potential of N_2O compared with CH_4 is 273 vs 27 (IPCC, 2021), thereby counteracting around 7% of the CH_4 reduction obtained by nitrate addition.

Nitrate supplementation

Cows receiving nitrate supplementation showed a lower rumen headspace CH_4 volume proportion (P < 0,01) and greater rumen headspace CO_2 and N_2O volume proportions (P < 0,01) compared with cows receiving urea supplementation. Cows receiving nitrate supplementation showed a persistent lower hourly CH_4 production and persistent higher hourly H_2 production compared with cows receiving urea supplementation over the day (*Figure 1a*).

The ruminal total volatile fatty acid (VFA) concentration and molar proportion of individual VFA did not differ between urea or nitrate supplementation (P > 0,05; *Table 1*), except for a higher valerate proportion with nitrate supplementation (P = 0,05). Ruminal NH_3 concentration, pH, and redox potential did not differ between treatments (P > 0,05).

The concentration of haemoglobin (Hb) and methaemoglobin (MetHb) proportion of total haemoglobin in blood did not differ between treatments (P > 0,05) and averaged 6,71 ± 0,36mmol/ℓ and 2,19 ± 0,06%, respectively, for urea supplementation and 6,8 ± 0,36mmol/ℓ and 2,10 ± 0,06%, respectively, for nitrate supplementation.

Substantial mitigation effects of nitrate supplementation on CH_4 production (13,3%) and CH_4 yield (13,8%) were found in the current study as also reported in previous studies (Olijhoek *et al*., 2016; Wang *et al*., 2023). In theory, supplementation of 8,6g nitrate (NO_3^-) per kg of DM would result in a reduction of 2,22g CH_4/kg DMI if NO_3^- is completely reduced to NH_4^+. The 2,5g/kg DMI reduction in CH_4 yield was equivalent to 113% of the stoichiometric CH_4 reduction potential of nitrate level (8,6g NO_3^-/kg of DM) applied in the current study (Ungerfeld and Kohn, 2006; Maigaard *et al*., 2024).

The CH_4 mitigation efficacy of nitrate supplementation in the current study was higher than the values reported in most previous studies. Likewise, in our recent study, we observed an average CH_4 yield reduction of 137% of theoretical stoichiometric CH_4 reduction across primiparous and multiparous cows with 10g/kg DM supplementation level (Wang *et al*., 2023).

Methane emission can be influenced by diet composition, e.g., forage: concentrate ratio and forage composition (Hristov *et al*., 2022). Therefore, differences in the composition of the diets between studies might also explain differences in observed CH_4 mitigation efficacy of nitrate supplementation. However, there is no evidence that nitrate was completely reduced to ammonia in the rumen although a higher than 100% of CH_4 mitigation potential was found in the current study.

It is suggested that adding nitrate is toxic for rumen archaea as Asanuma *et al*. (2015) found a dramatic decline in the ruminal methanogens abundance in goats receiving nitrate. Therefore, the possible toxicity to methanogens of adding nitrate probably contributes to CH_4 mitigation by reducing methanogen activity.

Chamber vs headspace measures

The increase in H_2 indicated that not all the H_2 redirected from methanogenesis was used in the nitrate reduction process. The increased H_2 might also be ascribed to reduced methanogen activity since the toxic effect of nitrate on methanogens (Asanuma *et al*., 2015), thereby reducing hydrogen consumption. In addition, urea or nitrate supplementation might also induce a variation in feeding behaviour diurnally, especially right after feeding.

The diurnal patterns of CH_4 production (chambers) and CH_4 volume proportion (rumen headspace) reflect each other (*Figure 1b* and *1c*). This alignment probably implies that the methane emission from the hindgut is limited and not large enough to show a substantial impact on the methane emission from ruminal methane production.

There were four time points where cows supplemented with urea showed higher hourly CH_4 production from chamber measurement, but a lower volume proportion of CH_4 from rumen headspace measurement. This discrepancy within some time points might partly originate from pH fluctuations, as pH highly affects the dissolved CO_2 in rumen fluid and thereby CO_2 release to the headspace which again affects the headspace composition (Maigaard *et al*., 2024). Maigaard *et al*. (2024) found that average headspace CH_4 concentrations were highly correlated to CH_4 yield (g/kg DMI) measured in respiration chambers.

We believe headspace sampling combined with chamber measures can be a strong tool to get quantitative production data for minor gases and isotopes not measurable by the chamber instruments (like N_2O in the present study)

Table 1: Ruminal fermentation characteristics in dairy cows with urea or nitrate supplementation.

Item	Treatment (Trt)			P-values		
	Urea	Nitrate	SEM	Trt	Time	Trt x time[1]
Total VFA, mmol/ℓ	125	123	3,58	0,67	0,03	0,85
VFA, mol/100 mol						
Acetate	59,9	59,6	1,04	0,79	0,64	0,79
Propionate	21,4	20,6	0,93	0,20	0,77	0,72
Isobutyrate	0,70	0,67	0,05	0,23	0,02	0,35
Butyrate	13,9	14,7	0,54	0,10	0,40	0,26
Isovalerate	1,41	1,40	0,33	0,91	0,96	0,98
Valerate	1,88	2,11	0,22	0,02	0,44	0,27
Caproate	0,78	0,86	0,07	0,28	0,63	0,32
A:P ratio[2]	2,82	2,93	0,16	0,36	0,82	0,88
NH_3, mmol/ ℓ	5,44	4,50	1,06	0,54	0,68	0,80
Glucose, mmol/ℓ	17,4	18,3	0,68	0,10	0,40	0,26
L-lactate, mmol/ℓ	0,97	1,07	0,09	0,28	0,63	0,32
Ruminol pH	6,28	6,38	0,07	0,29	0,14	0,93
Redox, mV	-216	-221	12,9	0,71	0,68	0,29

[1] Interaction between treatment and time. [2] Acetate: propionate ratio.

or with concentrations below the detection limit in the chamber outflow.

The fluctuating CO_2 dissolving might mask the headspace composition for time points. However, CH_4 concentrations in rumen fluid and headspace both correlated well with CH_4 yield measured in chambers (Maigaard *et al*., 2024), and the headspace ratio to CH_4 of a certain gas should be unaffected by CO_2 fluctuations and is therefore assumed to be proper for the quantification of daily production of such gases when CH_4 is measured in chamber.

An interaction between nitrate supplementation and time on hourly CH_4 production was noted, whereas, neither time nor interaction between nitrate supplementation and time were significant in the rumen headspace data. Moreover, the increase in N_2O production indicates that some of the NO_3^- -N probably ended up in N_2O, which is in agreement with Petersen *et al*. (2015). While an increase in N_2O in the rumen headspace was observed, a full picture of N_2O emissions would require evaluating emissions from manure and urine, which were not measured in the current study.

Ruminal volatile fatty acids

Ruminal VFA proportions were unaffected in the current study, besides a higher valerate proportion and a tendency for increased butyrate proportion for nitrate compared with urea supplementation. It has been reported that nitrate addition could promote the growth of some ruminal cellulolytic bacteria species which also could improve the valerate production in the rumen (Zhao *et a*l., 2015).

The effect of nitrate supplementation on VFA composition varies between different studies. Olijhoek *et al*. (2016) reported a tendency to a linear decrease in propionate proportion and a linear increase in butyrate when feeding three nitrate doses of 5,3, 13,6, and 21,1g/kg of DM. A higher acetate proportion and lower propionate proportion were observed in cows receiving nitrate addition levels of 22,5, 21,5, and 18g/kg of DM, respectively (Guyader *et al*., 2015; Troy *et al*., 2015). One reason for this variation is the sampling time. All studies which have shown a significant effect of nitrate addition on VFA had a sampling time within a few hours after feeding.

However, Olijhoek *et al*. (2016) sampled at different time points evenly spread during the day and only found tendencies for effects. Li *et al*. (2012) did not find any effect of nitrate supplementation on rumen VFA when collecting rumen samples more than six hours after feeding.

The effects of nitrate supplementation on rumen VFA also followed the diurnal patterns of CH_4 emission, rumen pH, and H_2 pressure in the rumen caused by nitrate supplementation (Guyader *et al*., 2015; Olijhoek *et al*., 2016). In addition, the effect of nitrate supplementation also depends on the dose of supplementation as the CH_4 mitigation of nitrate supplementation is a dose-dependent effect (Feng *et al*., 2020).

Rumen headspace gas data was corrected for varying ambient air contamination as the nitrogen concentration in our samples was considerably higher than the values (2 to 4%) reported in a previous study (Moate *et al*., 1997). Therefore, the data in this study was calculated as the volume proportion of the three gases in question instead of concentration. The reason for this contamination was probably that our sampling method did not completely prevent pollution with ambient air.

Conclusion

The use of nitrate as a CH_4 mitigation tool induces a risk of increasing environmental and climate effects from the manure if nitrate is added as a top dressing and not substituted for other N sources in the diet as it then will increase N output, especially in urine. However, further work is required to evaluate such trade-offs. The methane potential of the faeces seems unaffected by nitrate addition (Maigaard *et al*., 2022).

With a dose of 8,6g/kg of DM, nitrate supplementation reduced CH_4 production and yield with the largest effect on production just after feeding, and increased H_2 production and yield compared with urea supplementation. However, nitrate supplementation also increased N_2O production compared with urea supplementation.

This article has been condensed for publication in *AFMA Matrix*. For the full article and a list of references, visit *www.doi.org/10.3168/jdsc.2023-0541* or email Wenji Wang at Wangwj@anivet.au.dk or Peter Lund at Peter.Lund@anivet.au.dk.

Nutribase
Feed supplements for Africa

MEGALAC:
The industry leader in global dairy feed fat nutrition

Forty years after it first rolled off the manufacturing line, Volac Wilmar's flagship rumen-protected fat supplement, Megalac, continues to be a global leader in feed fat nutrition and one of the biggest brands in global agriculture.

According to Dr Richard Kirkland, global technical manager at Volac Wilmar, the long-standing success has been a result of the company remaining at the forefront of feed fat research and the development of proven feeding solutions for dairy producers.

Dr Richard Kirkland (left), global technical manager at Volac Wilmar, with Prof Don Palmquist at the 2023 American Dairy Science Association conference in Ottawa, Canada. Prof Palmquist's lab at Ohio State University in the United States, developed the calcium salt technology which resulted in a rumen-insoluble supplement. This technology was commercialised by Volac for the first production of Megalac in the early 1980s.

"Fat is one of the key macro-nutrients in dairy production, and considerable research on animal requirements has been conducted over several decades. However, it was clear that adding higher levels of oils, or higher fat ingredients, to rations had detrimental effects on fibre digestibility and milk fat," says Dr Kirkland.

The calcium salt technology developed by Prof Don Palmquist's lab at Ohio State University in the United States, combined fatty acids with calcium, producing a rumen-insoluble supplement. Using this technology, Megalac was commercialised by Volac as the first calcium salt rumen-protected feed fat supplement, enabling dairy producers to take advantage of the energy density benefits of fat without disrupting rumen function and fibre digestibility.

Leader in dairy feed fat nutrition

Megalac has become a household name in global dairy production, being fed on dairy farms on every continent and all the major dairy countries.

Growth in the marketplace has been driven by extensive independent research at universities and by Volac Wilmar and its partners, says Dr Kirkland. "A groundbreaking area of research has been the discovery that specific fatty acids influence multiple areas of cow production such as nutrient partitioning, body condition, fertility, milk fat and milk yield."

Oleic acid (C18:1) helps partition nutrients towards body fat stores, reducing body condition loss in the critical early lactation period. This fatty acid also improves total fat digestibility and can enhance fertility through improved egg and embryo development. For improving milk fat production and yield, palmitic acid (C16:0) is particularly beneficial; however, research shows increased production in early lactation through C16:0 supplementation can come at the expense of additional body condition and weight loss, so care must be taken if supplementing with higher levels of C16:0 through early lactation.

The next 40 years

According to Dr Kirkland, Volac Wilmar continues to invest in fat nutrition research, with new areas exploring the benefits of rumen-protected fats on reducing methane production underway.

"Volac Wilmar will continue to invest in understanding the influence of fatty acids in ruminant diets to help producers optimise on-farm production while overcoming new challenges," concludes Dr Kirkland.

For more information, contact Nutribase on 012 348 0116 or visit *www.nutribase.co.za.*

MAXIMISE CAPACITY & STREAMLINE EFFICIENCY WITH PROMTEK

Improving capacity, modernising an older production line design, speeding up materials handling, and reducing energy consumption were some of the enhancements Promtek engineered for a global leader in premix manufacturing. Incorporating Promtek's system into the premix factory was highly beneficial, contributing to improved mixing, reduced downtime, and enhanced accuracy of mixtures. The system generates better reports and ensures robust quality control. Overall, it significantly boosts productivity and efficiency.

HOW PROMTEK BENEFITS PREMIX FACTORIES

Utilizing Promtek provides Premix Factory with a **reliable system**, delivering accurate and valuable reports.

The system operates with **minimal tolerance**, ensuring precision in the production process.

Enhanced security in the end result product instills confidence in sending out **high-quality goods.**

Since implementing Promtek, there has been a substantial **increase in production**, moving from 400M to an impressive 2000MT.

WHY PREMIX FACTORIES CHOOSE PROMTEK

Research and Reputation
Constant Innovation

Expert Support
24/7 Support and Assistance

Positive User Experience
Reliable, User-friendly

Product Handling
Manages Diverse Products

Precision
Highly Efficient

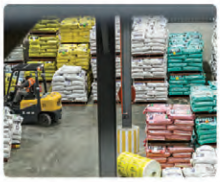
Reliability
Consistent Performance

PROMTEK

Improving Premix Factories' Production Processes

Target versus actual

Promtek sends the actual mixing values to the ERP system. The stock values are updated live showing the real availability. No production losses have to be written off after a stocktake.



Product categorisation

Categorisation improves finished product safety. Promtek allows products to be categorised according to mixing sequence and will schedule production to prevent cross contamination.

Pictures speak a thousand words

Customised contral screen visuals made initial acceptance and training easier for operators. The screen graphics were designed to imitate the real equipment in the factory.

Additional time saving benefits

Bag additions converted to bulk auto dosing by the control system saves time. Operators can now run two mixers instead of one. Employees can spend more time on higher value tasks.

Barcode creation

Promtek automatically creates barcodes from the ERP attached to a raw material pallet. Barcode recognition makes it possible to track and trace lot codes and bin numbers throughout.

Health and Safety

Promtek stores all safety information for ingredients. When adding a specific ingredient a warning may be shown. The system produces a record of safety warnings for the user.

AFMA
SAFE FEED FOR SAFE FOOD
Animal Feed Manufacturers Association

AFMA INTERVARSITY WRITER'S CUP 2024: WINNER ROUND 3 / LITERATURE REVIEW

Fulvic acid in broiler production

By Danielle van der Merwe, Stellenbosch University

An increase in intensive animal production leading to increased stocking density, exposure to disease, increased stress (Tang *et al*., 2023), coupled with increased antibiotic resistance due to the overuse of antibiotics and the ban on antibiotic use in some countries (Abreu *et al*., 2023) have created a need for an alternative additive to antibiotics. An additive is needed that can combat the decline in immunity, productivity, and feed efficiency caused by high stocking densities, intensive feeding, and antibiotic resistance (Feng *et al*., 2022).

Fulvic acid (FA) is a novel feed additive that can potentially fulfil this role. Fulvic acid is one of three categories that fall under the group humic substances (HSs), which are organic acids formed through the decomposition and transformation of microbial materials, plants, and animal residues. Fulvic acid is a weak acid mixture that can be extracted from various sources. It has a small molecular weight and many biologically active molecules (Feng *et al*., 2022).

Action of fulvic acid

Fulvic acid has been used to treat digestive tract diseases and has been known to treat ulcerative carbuncle. It has been shown to have antioxidant, anti-inflammatory, immunomodulatory, antiviral, and antidiabetic properties. Studies have suggested that the addition of FA could improve growth rate, feed conversion ratio, and immunity. Fulvic acid has the potential to regulate productive performance through its regulation of immune activity and influence on gut microbiota and mechanisms that underlie production performance (Feng *et al*., 2022).

Fulvic acid has been seen to have a chelating effect on metals (Xiao *et al*., 2022). For environmentally derived FA, this is a problem because they tend to adsorb and bind heavy metals from the soil or water sources from which they are isolated, becoming toxic when fed to animals.

Humic substances containing FA have, however, in some cases been found to promote the secretion of digestive enzymes such as lysozyme, protease and acid-alkaline phosphate, which promote intestinal absorption, digestion (and therefore mineral availability), and production performance (Tang *et al*., 2023). In contrast, mineral chelation may aid in mineral availability when FA is broken down if beneficial minerals are bound to the FA. This could influence the maintenance of homeostasis, activation of enzymes, vitamin uptake, and blood enzyme activity.

Fulvic acid has also been seen to influence blood metabolites such as glucose, protein, triglyceride, high-density lipoproteins, low-density lipoproteins, and total cholesterol, which influences animal health and meat quality. Colour, water-holding capacity and pH are important for meat quality and have been seen to be affected by the addition of FA (Ozturk *et al*., 2012). There is evidence that FA has the potential to improve meat quality through alteration of the total protein and fat content owing to the lower oxidation and higher antioxidant activity (Hudák *et al*., 2021).

HA and FA inclusion

Studies on HS containing FA as well as humic acids (HAs) are more prevalent than those where FA was used alone. It has been noted that when an HS with a 2,08 HA:FA was used in a broiler trial, an increase in bodyweight, intestinal villi length, and intestinal crypt depth was seen with a lower feed conversion ratio (FCR) and total cholesterol (Domínguez-Negrete *et al*., 2021).

Ozturk *et al*. (2012) used an HS with 2,08 HA:FA with inclusions of 0,5, 1 and 1,5% and determined that the 1,5% inclusion level was best, producing the largest bodyweight gain (BWG), carcass and dressing percentage. There was, however, variation in meat colour characteristics, blood cholesterol levels, performance, carcass, and gut traits. Due to the increase in BWG, nutrient absorption and lower FCR, HSs were deemed an alternative antimicrobial and growth promoter (Ozturk *et al*., 2012).

Hudák *et al*. (2021) found that including HS at 0,7% (minimum 5% FA) increased protein content and lightness of muscle while decreasing fat content and muscle pH. The inclusion of HS led to improved oxidative stability and sensory variables as well as juiciness after storage (Hudák *et al*., 2021).

Including HS in levels of 0,8 and 1% resulted in changes to dry matter, fat, water, and protein concentration with decreases in phosphates and pH in breast and thigh muscle. Meat from the HS groups scored higher in aroma smell point scores, taste and juiciness, decreased lightness and increased redness were seen in the meat, demonstrating that HS inclusion can affect physiochemical and organoleptic characteristics of meat (Semjon *et al*., 2020).

Including only FA

When looking at FA alone, a study using 98% pure FA at 0,2, 0,6 and 1g/kg reported that all the treatment groups had a higher bodyweight than that of the control containing no FA. The 0,6g/kg treatment produced a lower FCR than the control. Treatment groups showed an increase in digestive enzymes such as amylase, lipase and protease, which may have led to the increased bodyweight. In the 0,6 and 1g/kg an increase in water, protein and lipid contents of meat was seen as well as a shift in fatty acid ratios due to antioxidant activity and protease expression (Mao, 2019).

Feng *et al*. (2022) reported a decrease in abdominal fat when FA was included in poultry diets. An increase in immune regulatory cytokines and a decrease in pro-inflammatory factors was seen. This demonstrates the ability of FA to regulate the immune response through the activation of anti-inflammatory and deactivation of pro-inflammatory cytokines (Feng *et al*., 2022).

Fulvic acid could influence the microbiota of the intestine, affecting immune regulation and intestinal homeostasis, which could regulate fat and energy metabolism and/or promote immunity through the reduction of pathogenic bacteria (Feng *et al*., 2022).

Danielle van der Merwe.

Xiao *et al*. (2022) investigated the effect of FA inclusion on biochemical indices, antioxidant parameters, and gut microbiota. Fulvic acid decreased aspartate aminotransferase and total bilirubin, thus protecting cells by reducing free radicals, and increased T-AOC and GSH-Px, which are vital antioxidant enzymes, thus protecting against oxidative damage.

Intestinal flora *Firmicutes*, which digest cellulose aiding in nutrient and energy intake, increased as well as *Megamonas* which produce acetic and propionic acid from fibre to be used in energy metabolism. There was a decrease in pathogenic bacteria such as actinobacteria, proteobacteria and enterobacteria (Xiao *et al*., 2022).

HS as immunostimulant

Inclusion of HS at a 0,8% level resulted in an increase in the ratio of CD4+ to CD8+ lymphocytes and an increase in intestinal mucin 2 (MUC2), indicating an immunostimulant property. Mucin 2 is a gel-forming mucin involved in the mucosal layer (the first surface sight of infection) and is the primary barrier against pathogens and the target for secretory immunoglobulins. A decrease in pathogenic enterobacteria and an increase in beneficial lactic acid bacteria was also reported (Mudroňová *et al*., 2020).

At a 0,15% inclusion of FA, an increase in *Alistipes* was seen, which have a protective effect against many diseases. Evidence was found that FA may be able to contribute to the upregulation of the functional pathway of micro-organism's nitrogen metabolism, thus reducing ammonia output (Tang *et al*., 2023).

Influence on gut microbiota

The effects of FA on gut microbiota are not well documented and the direct effects on pathogenic bacteria such as *Salmonella* are non-existent. A study using Xianju yellow chickens investigated the effect of 1 500mg/kg FA with a 19,28% content on gut microbiota. Alpha diversity, richness and evenness were not affected, but beta diversity, principal component analysis (PCA) and hierarchical clustering analysis differed between the control and the treatment. The first and second components of the PCA explained 42,59 and 17,7% of the variation in the degree of hierarchal clustering, respectively.

There was a significant difference in gut microbiota from phylum to genus in the treatment group compared to the control. There was an increase in abundance of *Lachnoclostridium* and unclassified *Rikenellaceae* which are associated with inflammation, immunity and intestinal homeostasis. Short-chain fatty acid producing genera increased in the treatment group, decreasing the level of pro-inflammatory cytokines and influencing fat and energy metabolism (Feng *et al*., 2022).

Similar results were seen in a study using Da Wu Golden Phoenix hens and a treatment of 500mg/kg FA. Again, the caecal digesta were analysed as in the previous study the same region was amplified. Of the 2 542 operational taxonomic units identified, 457 were unique to the treatment and 1 044 to the control while the alpha diversity was unaffected.

Firmicutes and *Megamonas* levels were elevated in the treatment group. *Firmicutes* are Gram-positive and prevent pathogen intrusion. Actinobacteria and proteobacteria were decreased, the former aid pathogenic bacteria and the latter are Gram-negative and include *Escherichia coli* and *Salmonella*. There was a decrease in *Enterobacter* which causes infections of the urinary tract, lower respiratory tract, bloodstream, and soft tissues (Xiao *et al*., 2022).

Conclusion

Fulvic acid has the potential to improve feed intake, FCR and immune function, support intestinal functions through increasing digestive enzymes, promoting tight junctions and gut barrier functions. It may increase beneficial bacteria and keep gut flora balanced, potentially decreasing ammonia production.

There is much debate due to variations in the results of studies on the use of FA, specifically in the poultry industry (Feng *et al*., 2022). Due to the wide variation of findings and inclusions used as well as a lack of research, the direct effects, benefits, and inclusion levels of FA need to be further investigated (Tang *et al*., 2023).

References available on request. For more information, send an email to Danielle van der Merwe at 22622144@sun.ac.za or phone 072 877 9322.


INNOVATING
FROM WHAT NATURE PROVIDES
METEX ANIMAL NUTRITION keeps on investing in R&D in order to maintain its technical leadership in animal nutrition. Backed up by 40 years of know-how, we are a trusted partner, providing innovative solutions for our direct customers which impact the whole chain of animal production.
Pioneers in the production of amino acids, we further explore the potential of fermentation processes in order to develop new functional solutions that answer the new livestock challenges: thinking performance and sustainability together.
METEX
ANIMAL NUTRITION
TAILORED FOR ANIMALS, INSPIRED BY NATURE
metexanimalnutrition.com
YARA
Knowledge grows
MAN_Innov_ADfp_ZA_202211

AFMA INTERVARSITY WRITER'S CUP 2024: WINNER ROUND 3 / OWN RESEARCH

The palatability of a green seaweed, *Ulva lactuca*, for sheep

By B Sutherland, JHC Van Zyl and CW Cruywagen, Stellenbosch University

Feed intake forms an integral part of animal productivity (Van, 2006). Low intake leads to a low production rate, more metabolisable energy used for maintenance, and poor feed conversion efficiency (Forbes, 1995).

Palatability can be assessed by measuring or estimating feed intake, or by witnessing how the animals perceive the feed and like to consume it (Horrocks and Vallentine, 1999). Palatability can be described as the ability of a feed to stimulate the animal to consume the feed, and it depends on organoleptic factors (Paga *et al*., 2022) and physical characteristics (Jarrige *et al*., 1995). Short-term intake and the maintenance of bodyweight (BW) can indicate whether a feed ingredient is palatable when included in ruminant diets (Baumont, 1996).

Seaweeds have been identified as a possible alternative ruminant feed source due to specific livestock feeding regulations, an increasing human population, increasing food security demands, food-feed competition, and continuous climate change and global warming (Meissner, 1997; UNPD, 2015; Halmemies-Beauchet-Filleau *et al*., 2018; Morais *et al*., 2020).

Ulva lactuca in livestock feed

Ulva lactuca is a green seaweed identified to have a favourable nutritional composition comparable to that of a medium-quality forage, including lucerne hay, with a high protein content (Arieli *et al*., 1993; Ventura and Castañón, 1998). This seaweed also has additional advantages including exceptionally high production rates, production in seawater regardless of the weather, and no arable land is needed for production (Nasseri *et al*., 2011; Van der Spiegel *et al*., 2013; Halmemies-Beauchet-Filleau *et al*., 2018).

Most studies on feeding *U. lactuca* to livestock have included small quantities of up to 10% in the diet at a time (Abdoun *et al*., 2014; El-Waziry *et al*., 2015; Soliman, 2022). Studies regarding the palatability of seaweed in ruminant diets are scarce. Therefore, this study aimed to assess the palatability of *U. lactuca*, to determine the potential it has of being included as a feed ingredient in ruminant maintenance diets to replace lucerne hay on a one-to-one basis.

Materials and methods

An *in vivo* study consisting of 40 Dohne Merino ewes with eight repetitions per treatment, was conducted at Stellenbosch University to determine the effect of *U. lactuca* in sheep maintenance diets. The standard maintenance diets contained increasing levels of 0% control diet (C), 5% *Ulva* meal (U5), 10% (U10), 20% (U20) and 40% (U40) of harvested *U. lactuca* from the coast of South Africa. The *U. lactuca* replaced lucerne hay in maintenance diets on a one-to-one basis.

The sheep were given two days to adapt to the pelleted diets as they were kept permanently on kikuyu pasture prior to the trial. After the adaptation period, they received the pelleted diets *ad libitum* for 14 days.

Raw data was captured in Microsoft Excel (Microsoft, Redmond, Washington, United States), with analysis of variance tests and relevant multiple comparisons tests performed using Statistica (TIBCO Statistica® 14.0.1) software. Differences were declared significant if $P < 0,05$ with 95% confidence intervals describing unknown parameter estimations.

Results and discussion

The inclusion of 0, 5 and 10% *U. lactuca* in maintenance diets for sheep did not differ between treatments with relation to the BW, BW change, feed conversion ratio (FCR), dry matter intake (DMI), water intake (WI) or faecal score of the sheep (*Table 1*).

Ewes from the U20 and U40 treatment groups were removed after two days due to severe decreasing BW losses of more than 0,5kg/d and very low DMI of less than 2% of BW DMI per day, since the first day of the adaptation phase at the start of the trial. Normal daily DMI of sheep is documented to

Table 1: Mean ± SEM production parameters over the entire trial period of ewes per treatment group (n=8) fed maintenance diets containing increasing levels of *U. lactuca*.

Item	Treatment			P-value
	C	U5	U10	
Average bodyweight (ABW) (kg)	38,64 ± 0,24	38,71 ± 0,25	38,42 ± 0,25	0,73
Average bodyweight change (kg/d)	0,14 ± 0,03	0,15 ± 0,03	0,15 ± 0,03	0,95
Total bodyweight change (kg)	1,95 ± 0,39	2,06 ± 0,40	2,14 ± 0,40	0,95
Average daily dry matter intake (DMId) (kg/d)	1,30 ± 0,06	1,34 ± 0,07	1,21 ± 0,07	0,38
Average daily dry matter intake (% BW/d)	3,36 ± 0,14	3,46 ± 0,14	3,16 ± 0,14	0,36
Total dry matter intake (kg)	18,21 ± 0,89	18,82 ± 0,92	16,93 ± 0,93	0,38
Average daily water intake (ℓ/d)	3,21 ± 0,28	3,38 ± 0,29	3,64 ± 0,30	0,59
Total water intake (ℓ)	44,98 ± 3,98	47,33 ± 4,08	50,97 ± 4,14	0,59
Average feed conversion ratio	11,80 ± 5,77	8 ± 5,92	9,89 ± 6	0,90
Average faecal score	1,71 ± 0,17	1,88 ± 0,18	1,93 ± 0,18	0,66

be approximately 2% of BW per day for maintenance (NRC, 2007), indicating that the DMI of less than 2% is not sufficient for maintenance. Thus, there wasn't enough raw data to perform a statistical analysis and the data was subsequently omitted from all analyses.

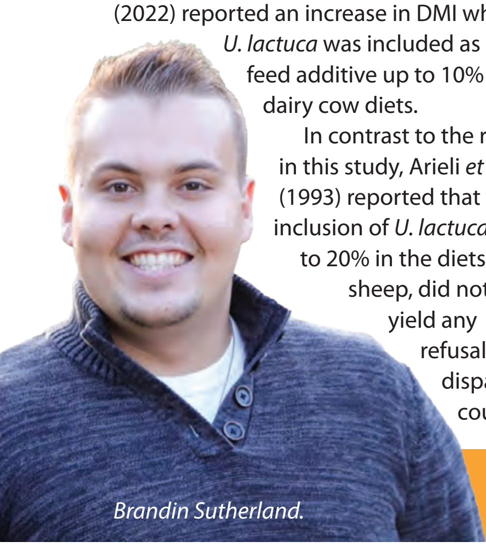
The removal of the animals could potentially be attributed to the higher mineral and salt content, giving the feed a bitter taste. This is supported by Applegate and Gray (1995), Forbes (2010) and López *et al*. (2021) who all concluded that an increased salty taste could lead to decreased DMI and lower palatability, with Van der Spiegel *et al*. (2013) and Paga *et al*. (2022) hypothesising that an increased mineral content decreases DMI.

High amounts of minerals found in *U. lactuca* can be attributed to their capability to absorb and accumulate inorganic substances including minerals, heavy metals and other mineral contaminants from the aquatic environment (MacArtain *et al*., 2007; Van der Spiegel *et al*., 2013; Makkar *et al*., 2016; Morais *et al*., 2020).

Water intake, DMI and BW are inter-correlated showing a positive relationship (Kraly, 1984; Arias and Mader, 2011; NASEM, 2016; Ahlberg *et al*., 2018). This can be seen from the results where no differences were reported between treatments in terms of the water, dry matter and BW measurements.

Abdoun *et al*. (2014) and El-Waziry *et al*. (2015) reported no differences in BW gain, feed intake or average daily gain when *U. lactuca* was included in up to 5% levels in growing lamb diets. In contrast, Soliman (2022) reported an increase in DMI when *U. lactuca* was included as a feed additive up to 10% in dairy cow diets.

In contrast to the results in this study, Arieli *et al*. (1993) reported that the inclusion of *U. lactuca* up to 20% in the diets of sheep, did not yield any refusals. This disparity could be explained by possible improper rinsing of the seaweed during harvesting, leading to elevated salt levels which would have affected DMI as *U. lactuca* inclusion increased (Applegate and Gray, 1995; Forbes, 2010; López *et al*., 2021; Paga *et al*., 2022). The salt content in the U20 and U40 diets (25 and 33, respectively) was more than double and triple that of the control diet (11g/kg DM). The ash content of the U20 and U40 diets (101 and 132g/kg DM, respectively) was also relatively higher than that of the control diet (77,1g/kg DM).



Brandin Sutherland.

The voluntary WI of sheep should be two to three times that of their DMI (DARD, 2016). The average daily WI of the three treatments was two to three times higher than their average DMI, suggesting that WI were all within normal parameters for sheep (*Table 1*).

The absence of differences in FCR of ewes between the C, U5 and U10 treatments was expected due to vectors, DMId and ABWΔ, determining FCR (Coetzee, 2020; Davison *et al*., 2023) not displaying any differences (*Table 1*). A lower FCR indicates that the feed is more efficiently digested (USAID, 2011; Coetzee, 2020). Since the diets were formulated to maintain BW, the relatively poor FCR values in the current trial were not unexpected.

Faecal scoring was adapted and applied from previous studies (Le Jambre *et al*., 2007; Armeni *et al*., 2023), with the most common faeces observed being individual pellets clumped together, which represents a score of two. Thus, the increased inclusion of *U. lactuca* in maintenance diets did not influence faecal consistency and the ewes digested the diets with ease.

A longer adaptation period is advised to enable the rumen microbes to adapt and efficiently utilise the seaweed component of the diet (Makkar *et al*., 2016). The ruminal micro-organism profile can be changed within seven to ten days when adapting to a seaweed-based diet (Orpin *et al*., 1985; Collier *et al*., 1998). This is supported by a study by Hansen *et al*. (2003) where the sheep ate only seaweed, proving the microbial adaptation to a seaweed-based diet.

Conclusion

Including *U. lactuca* up to 10% in sheep maintenance diets have been successful without affecting the production parameters and palatability of the diet. Higher *U. lactuca* inclusion levels proved to be too unpalatable. Future research should investigate the inclusion of this seaweed in diets containing artificial sweeteners which could possibly yield better results.

References available on request. For more information, send an email to Brandin Sutherland at brandin@gmail.com or phone 076 313 7441.


GLOBAL FOOTPRINT
LOCAL COMMITMENT
Your partner of choice in a globally integrated feed supply chain network.
SEABOARD
TRADING
A DIVISION OF SEABOARD OVERSEAS AND TRADING GROUP
SERVING SOUTHERN AFRICA'S ANIMAL FEED AND GRAIN INDUSTRIES SINCE 1996
DURBAN +27 31 581 4500 | CAPE TOWN +27 21 753 6600 | INFO.ZA@SEABOARDTRADE.COM | WWW.SEABOARDTRADE.COM