MILK SOUTH AFRICA

Promoting a healthy dairy community

MELK SUID-AFRIKA

Research & Development

SYNOPSIS OF SELECTED PROJECTS

2015 to 2024

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SA MILK PROCESSORS' ORGANISATION

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MELKPRODUSENTE-ORGANISASIE
MILK PRODUCERS' ORGANISATION

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CONTENTS

Milk South Africa
Melk Suid-Afrika

Summarized results of selected Research & Development Projects since 2015

The Research & Development mandate of Milk SA

Milk SA promotes Research and Development that is functional in respect of the strategic direction of the South African dairy industry, namely to improve the competitiveness of the dairy industry, broadening the market for dairy products and empowerment of previously disadvantaged individuals.

Environmental issues are of increasing importance and require research and interaction with international bodies and different South African government departments, as well as interaction between members of the South African dairy industry, in order to deal with environmental issues of collective importance.

The fields of knowledge relevant to the dairy industry are especially the fields of soil, plants, dairy animals, milk and other dairy products, environmental issues and production, processing and manufacturing technologies.

Milk SA takes actions that promote coordination between research institutions, research that is functional in respect of the strategic direction of the South African dairy industry and the practical application of local and international research results by the South African industry. These actions are taken in consultation with Government research institutions and other research institutions.

Statutory levies are employed towards this mandate, while supplementary funding is sought where possible.

Structures and functional arrangements

All R & D projects are evaluated, prioritized and commissioned within the ambit of the structures and functions described below:

- **Dairy R & D Committee (DRDC)** evaluate and prioritize project proposals, and make recommendations to the Board regarding funding and future requirements.
- **R & D Forum** is an informal body which advises on R & D challenges in the whole spectrum of farming and processing activities and may suggest R & D projects for consideration by the DRDC. The R & D Forum consists of key opinion leaders in their relevant Specialist R & D Reference Groups, plus members of the DRDC with such expertise and experience.
- **R & D Management Committee (ManCom)** ensures that requirements and proposals submitted are real, of importance at grassroots level, and in line with Milk SA's guidelines and policies. To that effect, ManCom advises the DRDC.
- **Animal Health & Welfare** and **Environmental Sustainability** are sub-programmes under the R&D Programme.
- Milk SA liaises with a variety of **national and international stakeholders** with either R & D capacity or particular expertise and may collaborate with them when appropriate.

A. Programme: Disease control

A.1. Fasciolosis (Liver fluke)

BACKGROUND

Fasciola species are liver flukes that infect cattle and reduce productivity by 15-30%. The infection can affect general health and feed intake which may result in morbidity, reduction in milk production and weight gains.

The high incidence of infestation, particularly in the Tsitsikamma area, prompted an investigation into the prevalence, seasonal occurrence and favourable/unfavourable environmental conditions of the intermediate snail host (Lymnaea truncatula) of the liver fluke parasite.

If these factors are understood, the intermediate host should be controlled more effectively, and as a result the development and infestation of the parasite which needs the snail to complete its life cycle can be inhibited.

This has become important as there is evidence that liver fluke is developing resistance to all available anthelmintics, including the preferred triclabendazole.

PROJECT TITLE

Fasciola hepatica: Impact on dairy production and sustainable management on selected farms in South Africa.

AIM:

To investigate the potential threat of the liver fluke parasite (*Fasciola* spp.) to production of dairy cattle, in order to develop effective, sustainable on-farm methods of control / management.

SUMMARY OF RESULTS:

The prevalence and distribution of the snail host over seasons are water and temperature dependent, but rain versus irrigation often have different outcomes as it affects the texture of muddy patches and therefore the suitability of the snail's habitat.

Open water is essential for the parasite to infect the snail, whereas the snail can survive well in wet mud, in the absence of open water. Another factor is the parasite depresses the health of the snail in unfavourable times such as in summer, particularly if it is also dry, since the snail is not well adapted to high temperatures. This implies that relatively high proportions of the infected snails will not survive long enough under such conditions for completion of the development of the parasite in them.

A crucial further factor is that the fluke's eggs need to be deposited in the vicinity of the snails long enough before the open water is present for the eggs to mature by the time they are required in the open water, and being ready to hatch soon after they have become submerged in the water. The mature eggs can then hatch and successfully infect the snails, in this way paving the way to infect the cattle.

These results suggest that pasture-based dairy farmers should manage the open water areas carefully, such as often cleaning the water troughs and fencing-off water and mud patches when their animals graze nearby. This should significantly assist to control liver fluke infestation.

A.2. Mastitis

BACKGROUND

Despite widespread global focus on udder infection and mastitis curing and prevention, mastitis control arguably remains the single most costly input in dairy herds, also in South Africa. Thus, as elsewhere, Milk SA has embarked on a comprehensive R & D programme in assistance to dairy farmers. Apart from effective testing, sample analysis and managerial measures to support control, an increasing problem is that of antimicrobial resistance and in isolated instances biofilm formation, which negatively affects effective treatment, and which accordingly received prominent attention. Several projects have been conducted.

PROJECT TITLE

Control of mastitis by somatic cell count (SCC) levels and other means.

AIM:

To establish managerial measures of farmers in the prevention and control of mastitis.

SUMMARY OF RESULTS:

In a survey among dairy farmers, it was found that in routine evaluation of cows, groups and/or herds, SCC was relatively low at 67% for cows and groups and 53% in whole herd testing. The majority of high SCC cows were however treated. Only 1% of dairy farmers reported clipping or flaming udders.

Only 50% of dairy farms pre-dip, and a third either do not strip or wipe and/or do not use gloves. Allowing for an ideal stimulation time of between 60 to 120 seconds, only 11.1% of

rotary and 18.2% of other parlours were in this range.

The average bulk milk SCC was 287 000 cells per ml and were similar across management types and regions. About two-thirds of farmers routinely identify cows with an elevated SCC, either by testing individual cows and/or groups. Of these, two-thirds consider cows with a SCC of more than 500 000 cells per ml eligible for treatment.

The mastitis incidence of 31.8% was higher than the median of 20% to 25% seen elsewhere in the world and ranged from 3.5% to 93%. Milk samples for culture and microbial identification were collected on 29.2% of farms and 20.2% requested antimicrobial sensitivity, which is lower than world standards.

One-fifth of dairy farmers reported using antibiotics in an off-label manner (including higher dosage, shorter treatment intervals and/or an extended duration of treatment), and not always doing so under the supervision of a veterinarian.

The results of management procedures to control mastitis are disturbing. In addition, dairy farmers' attitudes toward mastitis are alarming, with only a third testifying that they had enough knowledge of the condition. It was concluded that there is an urgent need for systematic extension and training as mastitis is costing the industry millions.

PROJECT TITLE

Mastitis costs in South African dairy herds.

AIM:

To evaluate the cost of mastitis, farmers need to report on a structured questionnaire, the results of which may not always be highly accurate. The results nevertheless of a representative sample of herds, distinguishing between *losses incurred subsequent to the disease (failure cost, FC) and those invested to prevent the disease (preventive cost, PC) were evaluated.*

SUMMARY OF RESULTS:

The incidence of mastitis was 32% and the cull rate due to mastitis was 6%. The average total cost (TC) of mastitis was at the time (2017) R1 982 per cow per year, with FC contributing R1 604 and PC R378.

Milk loss due to subclinical mastitis (SCM) was the most significant, accounting for 73% of FC and 59% of TC. Other FC estimated was mastitis-related culling (14%), milk discard (12%) and clinical mastitis (CM) treatment (1%).

Contributing to PC was post-milking teat disinfection (36%), blanket dry cow treatment (16%), liner replacement (15%), pre-milking teat disinfection (12%), routine whole herd milk testing programs (SCC portion alone, 12%), followed by vaccination for mastitis, veterinary consulting

limited to udder health, milker gloves and udder health consultants, all less than 2.5%.

The variation between farms was marked with FC ranging from R744 to R2 992, PC from R55 to R940 and TC from R1 002 to R3 728.

PROJECT TITLE

Effect of heat stress on udder health.

AIM:

No previous study has elucidated the reason/s why heat stress increases SCC in bulk tank milk. Thus the objective was to establish whether the summer rise in bulk tank SCC is largely due to an increase in the SCC of "chronic" mastitis cows, and to ascertain whether the increase in SCC is due to an increase in intra-udder infection during the warm season, a response of the "chronic" udder to heat stress, or some combination.

SUMMARY OF RESULTS:

In the study, two groups of cows, referred to as high heat (HH) and low heat (LH) sensitive were established. When tested, HH and LH cows were responsible for 78% and 50% of the increase in bulk tank SCC in summer respectively, and therefore it was concluded that season is a risk factor in the causal pathway towards the summer rise in SCC.

This was substantiated when the temperature-humidity index (THI) model was implemented, which showed that HH and LH cows produced 92% and 24% of the increase in SCC's when transitioning from THI conditions below 65 to those above 70. When compared to the Central region, EC, KZN and WC had an increasing risk of producing more somatic cells in this context, with some farms having a very high number of HH cows of more than 70%, and these farms are expected to experience a more drastic summer rise in SCC.

PROJECT TITLE

Bulk tank testing in support of mastitis control.

AIM:

The goal was to demonstrate the use of routine bulk tank (BT) milk testing for the monitoring of bacterial counts in bulk milk combined with differential cultures and counts to identify the source of high bacterial counts.

SUMMARY OF RESULTS:

The results showed that high psychrophile counts in bulk tank samples were common in all herds. In some herds, this was largely attributable to a high incidence of sub-clinical mastitis caused by environmental streptococci. In other herds, the problem seemed multi-factorial, influenced by inadequate pre-milking practices, problems with cleaning of equipment and inadequate cooling of milk. BT was therefore useful as a further method to detect inadequacies in dairy herd management and possible mastitis causing pathogens.

PROJECT TITLE

Antimicrobial resistance (AMR) of *Staphylococcus aureus* and coagulase negative staphylococci (CNS) in SA dairy herds.

AIM:

The genus *Staphylococcus* comprises various species and strains which are pathogenic. A characteristic of staphylococci is their ability to develop resistance to antibiotics. An understanding of *S. aureus* reservoirs and transmission is essential for effective control. The first and main objective was therefore to investigate the antibiotic surveillance data of the unique maltose-negative *S. aureus* ST 2992 in various provinces, seasons and SCC categories over time. The second objective was to compare the results of the maltose-negative *S. aureus,* previously identified as an emerging pathogen, to those of maltose-positive *S. aureus.*

SUMMARY OF RESULTS:

Staph. aureus isolates were 100% resistant to amoxicillin, ampicillin and penicillin G, 95% resistant to cephalexin, 82% to streptomycin, 75% to oxacillin, 64% to erythromycin, 50% to tobramycin, 46% to tetracycline and 39% to vancomycin. Although of concern, at least none of the *Staph. aureus* isolates showed complete resistance to all antibiotics tested.

With respect to provinces, AMR differed between maltose-negative (MN) and maltose-positive (MP) *Staph. aureus* types. Resistance of MN indicated little difference between provinces and

seasons for ampicillin, penicillin G and cephalexin. In general, there was more resistance by MN than MP *Staph. aureus* isolates to most of the antibiotics used.

As a further concern, the results showed resistance of the MN strain isolated from milk samples to antibiotics that are only used in human medicine, which implies a possible transfer from humans to animals, i.e. a zoonotic effect.

Overall, the difference in AMR profiles between MN and MP *Staph. aureus* is useful information to farmers and veterinarians, as it indicates to different management and treatment protocols for MN *Staph. aureus* which, although more resistant than the MP counterpart, does not seem to be a chronic intra-udder infector, therefore preventing unnecessary culling.

As a follow-up to the zoonotic evidence above, several CNS/NAS strains from mastitis cases and from humans that were in contact with infected dairy cows were isolated.

The β-lactam encoding resistance gene was the most common resistance determinant detected and was identified in 29% of the cattle and 75% of the human *Staph. aureus* isolates.

Human *Staph. aureus* isolates from three sampling sites clustered together with bovine isolates recovered from milk samples at these sites. The human isolates were indistinguishable from the cattle *Staph. aureus* isolates present at the respective sites. This is disturbing because it suggests bacterial transmission which should be closely monitored.

PROJECT TITLE

Mastitis: Coagulase Negative Staphylococci (CNS) threat to udder health.

AIM:

The objective was to understand and quantify the potential threat of Non-aureus Staphylococcus (NAS) to udder health.

SUMMARY OF RESULTS:

Three hundred NAS were identified, the most common NAS strains isolated being *S. chromogenes, S. epidermidis* and *S. haemolyticus*. The numbers increased as lactation progressed.

A probable correlation between the NAS strains and SCC levels was also investigated. Of the three most prominent isolates, *S. epidermidis* had the highest percentage of isolates with SCC in excess of 1 million cells per ml milk followed by *S. haemolyticus* and the most commonly isolated NAS overall was *S. chromogenes*.

Furthermore, not many differences were found between the isolation of the three prominent NAS strains in pasture and TMR herds, although there was a slightly higher percentage of

S. chromogenes and lower percentage of *S. haemolyticus* in pastured-based systems than in TMR herds.

It was concluded that NAS at this stage is not yet a major threat, but the strains should be monitored.

PROJECT TITLE

Effect of machine take-off time on the prevalence of mastitis.

AIM:

Milking machines differ in layout and settings and continue to be a challenge to the primary immune system of the udder namely the teat canal. Different levels of teat end vacuum during machine milking may influence milking performance and teat condition. The aim of the project was to establish to what extent variation in teat end vacuum is responsible for teat damage and infections in dairy herds.

SUMMARY OF RESULTS:

It was found that even in a milking system where the automated cluster removal settings have a short reactive time (short milk tube and a flow meter lower than the level of the udder) the take-off time overall could be improved.

The average time of over-milking in the herds tested was 1.25 minutes under more or less ideal conditions. The work was supported by a literature review which confirmed that all vacuum levels need to be monitored, and in addition, liners, milk yield and automated cluster removal switch-point settings need to be regarded as bearing a risk of teat damage.

They affect milking speed and vacuum levels at total, peak and over-milking. An equilibrium should be reached between optimal milking speed and risk of teat damage. An increased switch-point setting shortens milking time and decreases over-milking and claw vacuum to preserve teat-canal integrity.

PROJECT TITLE

AMR and biofilm formation by the mastitis pathogen *Streptococcus uberis*.

AIM:

To limit AMR, the antibiotics to which specific mastitis pathogens such as *Str. uberis* are resistant to, must be known. A complicating factor is biofilm formation which is a resistance strategy

followed by many pathogens, which is more difficult to control than non-biofilm forming species.

In this context, virulence factors of *Str. uberis* have been discovered that can transfer resistance genes among members of the biofilm micro-community. The objective here, therefore, was to determine the in vitro susceptibility of *Str. uberis* to several antibiotics, and in addition, to investigate the implications of biofilm formation.

SUMMARY OF RESULTS:

Most isolates from the group with clinical mastitis had moderate biofilm production. Those isolated from the high SCC group had mostly strong and those from the low SCC group mostly low biofilm production.

The percentage resistance to the antibiotics clindamycin, daptomycin, linezolid, rifampin and tetracycline were between 5 and 8%. Of these, clindamycin and tetracycline are used for treatment of dairy cattle.

Biofilm formation in the isolates tested for antibiotic susceptibility was 47.3% for weak positive, 34.9% for moderate positive and 17.8% for strong positive. Overall, little AMR was detected in the *Str. uberis* isolates.

The highest susceptibility was detected in the group with moderate biofilm production (97.6%) and the least (92.2%) in the isolates that had strong biofilm production. It was thus concluded that at this stage biofilm formation by *Str. uberis* is of concern, but not AMR.

A.3. Sporidesmin toxicity (Facial Eczema)

BACKGROUND:

Sporidesmin is a toxin released by the fungus Pseudopithomyces chartarum, which infests grass pastures, in particular ryegrass. It results in photosensitivity in sheep and cattle, commonly known as Facial Eczema.

Outbreaks in the Eastern Cape caused significant economic losses to farmers in the area and highlighted the importance of this condition to the dairy industry.

Typical signs are secondary photosensitivity (skin inflammation and icterus), very high serum concentrations of Gamma-Glutamyl Transferase (GGT) and large numbers of spores on grazed pastures.

Supplementation with high levels of zinc oxide (ZnO) in the feed are comparatively effective, but contributes significantly to the cost of milk loss and premature culling of cows which could be 60% higher than usual.

The condition is causing the industry millions, prompting a comprehensive research programme supported by Milk SA.

PROJECT TITLE

Prevalence and diagnostic investigation of sporidesmin toxicity on pastures in the Eastern Cape.

AIM:

Major objectives were to -

- Estimate the prevalence of sporidesmin toxicity in pasture-fed dairy cattle based on histological examination of liver tissue from slaughtered cattle;
- Determine whether liver biopsy samples are potentially useful as a diagnostic test for sporidesmin toxicity;
- Establish whether the liver enzyme GGT is an acceptable indicator of liver damage caused by sporidesmin toxicity; and
- Conduct spore counting of fungus as an early warning to farmers of sporidesmin build-up.

SUMMARY OF RESULTS:

Abattoir liver samples showed clear evidence of typical periductular fibrosis, a lesion which characterizes sporidesmin toxicity. Other non-specific changes both in liver parenchymal tissue and in bile duct tissue were noted. Even though these are non-specific, they are described by other authors as early changes associated with sporidesmin toxicity.

The liver biopsy samples showed lesions in both the left and right lobe, which indicate that liver biopsy samples are representative of changes in other parts of the liver and can therefore be used for diagnostic purposes.

The liver enzyme GGT was consistently increased in cattle with affected skin originating from farms with high spore counts. Of all the parameters used, GGT appeared to be most useful, but it is known that it does not detect early cases. None of the other blood chemistry parameters tested seemed to be useful to pick up early cases.

The spore counting service to farmers as an early warning tool proved useful.

PROJECT TITLE

Exploring the Facial Eczema problem in dairy cattle in the Eastern Cape of South Africa, with a focus on the fungus *Pseudopithomyces chartarum*.

AIM:

The objectives are to -

- Resolve the taxonomy of the genus *Pseudopithomyces* with focus on *Pseudopithomyces chartarum* and to determine which species are associated with Facial Eczema;
- Determine whether *Pseudopithomyces chartarum* is seed-borne;
- Determine which *Pseudopithomyces* species predominate the outbreak areas in the Eastern Cape; and
- Study the relationship between sporidesmin production and the population diversity of *Pseudopithomyces chartarum*.

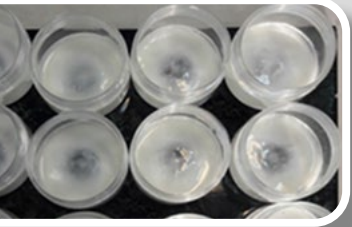
SUMMARY OF RESULTS:

A total of about 1000 gene regions from different isolates have been sequenced, including two from New Zealand through an existing MoA. At this stage it does appear that the sporidesmin-producing species predominates in the Eastern Cape.

Pseudopithomyces chartarum appears not to be seed-borne, but this should be confirmed by next generation sequencing (NGS), which offers several benefits for exploring the mycobiome of grass seed compared to traditional sequencing methods.

Furthermore, a method to detect sporidesmin has been successfully developed. Further studies aim to produce sporidesmin in quantities suitable for purification. These steps are crucial for routine analysis of sporidesmin detection from pasture and toxicokinetics experiments.

B. Programme: Milk flocculation / gelation



BACKGROUND:

Milk flocculation and gelation describe the phenomena relating to protein stability that could lead to the rejection of milk for processing or to defects in long-life milks such as UHT milk. Both phenomena create problems in the industry and therefore an extensive research programme has been initiated by Milk SA.

Flocculation applies to the protein precipitate that is formed in the test tube when ethanol unstable milk is subjected to the alizarol test. The ethanol concentration in the test varies from 68% to 76% and milk that flocculates is then declared unfit for further heat processing and rejected. Gelation is a major factor limiting the shelf life of UHT milk.

The defect manifests in the formation of a semi-solid to solid coagulum or gel in the milk over time. The gel which forms is a protein matrix initiated by interactions between the whey protein β-lactoglobulin and the κ-casein of the casein micelle during the high heat treatment. Several projects have been conducted.

PROJECT TITLE

The significance of proteolytic psychrotrophs as a cause of milk flocculation / protein instability.

AIM:

Flocculation of milk is one of many defects due to proteolytic enzymes of psychrotrophic bacteria which destabilizes the milk protein (casein). The psychrotrophic bacteria have the ability to proliferate at cold storage temperature of less than 7 °C.

While growing, these bacteria then produce the proteolytic enzymes which destabilizes the casein. The commonly used psychrotrophic count method employs incubation at 7 °C for 10 days which means that by the time the results are known, the raw milk will already be spoiled.

The aim here, therefore, was to evaluate methods to rapidly and reliably detect the psychrotrophic bacteria in unpasteurised milk.

SUMMARY OF RESULTS:

The results illustrated that the rapid qualitative Psychro-Fast test, which gives results within 48 hours, can be used to not only indicate the presence of psychrotrophic bacteria in unpasteurised milk, but the pink colour intensity can be used to indicate the degree of psychrotrophic bacterial contamination.

Other findings indicated that -

- The hygienic practices at farm production level may have a significant effect on the quality of the raw milk especially in terms of the presence of psychrotrophs;
- Colder environmental temperatures may have a significant effect on the level of proteolytic psychrotropic counts, which in turn emphasizes the importance of good cooling practices;
- Lower counts of the total bacteria, total coliforms and the Pseudomonas count of raw milk resulted in milk with a better quality which in turn resulted in delayed flocculation;
- Gram-negative psychrotrophic bacteria were the prevalent bacteria at the time that flocculation occurred, which indicated that these bacteria and their proteolytic enzymes were associated with milk flocculation; and
- Pseudomonas spp., particularly Ps. fluorescens, were the prevalent genus at the time of flocculation.

PROJECT TITLE

Studies to determine the effect of proteolytic enzymes in unpasteurized milk on flocculation and gelation.

AIM:

The main objectives were to:

- Obtain a trustworthy and repeatable assay for plasmin/protease;
- Use a quantitative version of the RP- HPLC to possibly identify unpasteurised milk with high proteolytic activity before UHT processing;
- Obtain more relevant information on the activation of plasminogen to plasmin; and
- Study the role fat and its components (FFA) play during plasminogen activation in milk.

SUMMARY OF RESULTS:

Software were created of chromatograms representative of peptide peaks liberated by each proteolytic enzyme and which could distinguish satisfactorily between the bacterial protease profiles and the plasmin profiles present in unpasteurized and UHT milk.

It was also established that the sensitivity of the method should make it possible to detect whether the origin of the flocculation is from psychrotrophic bacteria (contamination) which can survive low temperatures, or whether it results because of heat induced proteolysis in the milk manufactured for extended shelf life.

Milk fat was found to play an important role in the susceptibility of casein towards proteolytic attack. It was evident that UHT fat-free milk is more susceptible to bacterial protease than UHT low-fat and full-cream milk. In contrast, plasmin protease hydrolysed UHT fat-free, low-fat and full-cream milk equally.

During the treatment of milk fat with Pseudomonas lipase enzyme, it was evident that the FFA'S released during fat hydrolysis activate plasminogen to plasmin protease. It was found that most of the FFA's between C4-C18:1 had the ability to activate plasminogen to plasmin.

A further significant finding was that proteolysis by plasmin protease occurred to a greater extent at refrigeration temperature than at room temperature.

Thus, it is clear that not only psychro-tolerant bacterial protease results in proteolysis at lower temperatures, but native plasminogen can be activated during storage under refrigeration temperatures and as a result, plasmin can effectively contribute to gelation.

PROJECT TITLE

Evaluation and validation of methods for the detection of psychro-tolerant bacteria and proteolytic enzymes in milk.

AIM:

The general aim was to develop methods for early detection of bacteria and enzymes influencing milk flocculation/gelation to ensure the quality of raw milk and the products produced from the milk. The objectives were to -

- Validate the azo-casein method for determination of proteolytic activity in producers raw milk samples;
- Test producer milk samples using the validated azo-casein and commercial Pierce method;
- Evaluate producer milk samples with the accelerated psychro-tolerant count method and the Pseudomonas count method on selective media;
- Evaluate producer milk samples with the PsychroFast test and compare the results to the accelerated psychrotolerant counts and Pseudomonas counts, and
- Repeat the above-mentioned bacterial counts and enzyme activity tests at specific time increments during incubation of the milk samples at 7 °C until the samples flocculate with the alizarol test.

SUMMARY OF RESULTS:

Results and recommendation: The alizarol test, PsychroFast test, accelerated proteolytic psychrotolerant count and *Pseudomonas* count methods, may all be used by UHT processors to rapidly determine the psychrotolerant bacterial quality of the raw milk before UHT processing.

If the results of these methods indicate normal pH with the alizarol test, a white to light pink colour in the PsychroFast test and the accelerated proteolytic psychrotolerant counts, and *Pseudomonas* counts of less than 2 log cfu/mL (< 100 cfu/mL), the milk may be UHT processed. Otherwise, the milk should only be pasteurized.

PROJECT TITLE

The effect of potassium content of pastures on heat stability of milk.

AIM:

The objective was to determine if high levels of potassium (K) in the pasture contributes to heat instability of milk and therefore contributes to flocculation. The following questions were raised:

- Whether the high levels of K in pasture will reduce the heat stability of milk; and
- Whether the high levels of K in pasture will reduce the phosphorous (P) content of milk.

SUMMARY OF RESULTS:

Potassium levels were progressively increased in the concentrate mix. The pasture grazed was of high quality and contained a K content of 5.2%. With supplementation, this resulted in a K content in the total diet of the cows on the high K treatment of substantially more than 5%.

The higher K levels in the diet significantly reduced the alcohol stability of the milk and also reduced the protein, lactose and MUN content of the milk. The high K level also resulted in reduced milk Ca, K, P and Mg.

The lower milk P content may partly explain the reduced protein stability of the milk as P linkages are present in the structure of casein micelles and are crucial to maintain the stability of milk protein. Farmers were therefore advised to carefully monitor and control the K levels in the soil and pastures.

PROJECT TITLE

The effect of Ca/P ratio in Jersey cows grazing kikuyu pasture on milk flocculation and milk composition.

AIM:

The aim of the study was to determine if changing the Ca:P ratio in the diet of cows grazing kikuyu pasture in late summer from 2:1 to 1:1 will increase the phosphate content of milk and improve protein stability. The hypothesis was that Increased dietary phosphate level will increase the phosphate content of milk and thereby reduce flocculation.

SUMMARY OF RESULTS:

The average alizarol level passed was not improved by feeding more P and the mineral content of milk was not affected. Therefore, increasing the P in the diet did not improve the protein stability of the milk. Milk production and milk composition was also not affected.

It was concluded that the protein stability of milk is clearly complex as it is affected by both environmental and nutritional stress and maybe also the genetics of the cow, as some cows' milk flocculated irrespective of the Ca:P ratio they received in the diet.

C. Programme: Pastures

BACKGROUND:

Pastures are the backbone of pasture-based dairy production systems and should provide optimum quality and biomass to support the maximum possible energy, protein and micro-elements to lactating cows. The possible supply is however sometimes unclear as it depends on soil health, pasture type and seasonal influences, and therefore e.g. N application and mineral availability under specific conditions remain in question.

PROJECT TITLE

Impact of fertilizer application rates on pasture yield and greenhouse gas emissions.

AIM:

The first objective was to investigate the influence of fertilizer application on the yield of dairy-pasture mixes, with the aim of identifying fertilizer guidelines which would optimise pasture productivity and profitability.

Other objectives were to:

- Analyse greenhouse gas (GHG) samples taken from pastures treated with various rates of nitrogen fertilizer;
- Determine the global warming potential of dairy pastures through a Lifetime Cycle Assessment;
- And develop and implement a Lifetime Nitrogen Efficiency and an Lifetime Cycle Assessment in selected South African dairy systems.

SUMMARY OF RESULTS:

In dairy-pasture systems, N application is often thought to be directly proportional to pasture yield. The question is, whether it holds true.

This was evaluated using data from 153 pasture camps over five years. Fertilizer application rates were grouped into three treatments: <200, 200-350 and >350 kg N per hectare (ha) and the herbage yield response over the five years was recorded.

There were no differences between treatments in total annual herbage yield, suggesting that the about 200 kg N per ha per year was probably sufficient.

Nitrogen level had a weak but significant negative correlation with total annual yield and only accounted for 6% of the yield variation. Because of the lack of herbage yield response with the higher N applications, N use efficiency was the best at the low level of application.

From these results, a second question emerges: if there is little response at high N application levels, what are the environmental consequences of the N not used in herbage growth. This was tested over three years on rotationally grazed pastures.

Different levels of N fertilizer were applied, namely 0 (N0), 220 (N20), 440 (N40), 660 (N60) and 880 (N80) kg N per ha per year. The additional N-input from the manure of the grazing cows was estimated as 450 kg N per ha per year on all treatments.

The average pasture camp N-balance over the three years for the N0, N20, N40, N60 and N80 were -119, +86, +299, +501 and +706 kg N per ha per year respectively. The highest GHG (~2.7 CO_2eq per kg energy corrected milk [ECM]) was associated with the N80 treatment and the lowest with the N0 and N20 treatments (~1.4 kg CO_2 per kg ECM).

The farm N-balance as well as the N-footprint were positively correlated with increasing N-fertilizer rates. Methane from enteric fermentation (~44% ± 2.7) and nitrous oxide (N2O) (~15% ± 3.1) emissions were the main contributors to the CHG.

It was concluded that fertilizer N applied at a level of 220 kg N per ha per year (N20) in addition to the animal excreta N is sufficient to ensure adequate pasture yields of about 20 ton DM per ha per year to achieve a pasture milk yield of about 17 ton ECM per ha and a low carbon footprint. This is in line with the results in the first study.

PROJECT TITLE

Seasonal effect on dairy cow trace mineral status in the Tsitsikamma, Eastern Cape.

AIM:

The overall aim was to advance the practical application of trace mineral supplementation during periods of high environmental stress on pasture-based dairies to ensure optimum cow health and productivity. Objectives were to -

- Determine dairy cow trace mineral status seasonally, together with that of pasture and drinking water content; and
- Evaluate study results and apply this knowledge to practical pasture-based dairy cow trace mineral nutrition.

SUMMARY OF RESULTS:

Stress effects were anticipated to result in inflammation which is classified as a non-infectious stress, heat stress being an example, which could implicate higher requirements. However, there were very little indication of either inadequate tissue levels of the trace minerals, or stress.

Although season did effect the trace mineral status of the dairy cows, the opposite of what was maybe expected was observed. The expectation that chronic inflammation associated with seasonal stressors, e.g. heat stress, would increase trace mineral demand to the extent of reducing trace mineral status was expected.

This may have supported the need to improve trace mineral supplementation during such stressful times. However, there was no consistent trend with spring through to autumn, when high temperatures were observed.

This may suggest that the trace mineral status of the study cows was adequate and the requirement of the immune system for activation not so large as to the extent of depleting mineral reserves, or it could simply imply that heat stress occurrences were infrequent and of too short duration to have a significant effect.

D. Programme: Environment

BACKGROUND:

Environmental protection of the dairy farm and the ecosystem in which it operates is paramount to the sustainability of the enterprise and beyond its borders. Thus, the responsibility also implies protection of wetlands and river systems from polluting substances which may pass to neighbouring farms and communities. In addition, dairy farms, as all agricultural systems, have the obligation to limit their carbon footprint. If this can be done whilst benefitting economically, this is a win-win situation. Accordingly, Milk SA embarked on an extensive research programme to address this obligation.

PROJECT TITLE

Buffer zones for wetlands and rivers in the dairy sector – A case study to determine best practice guidelines for improved wetland and river management.

AIM:

To contribute to the sustainability of the dairy sector through the development of best practice guidelines for improved wetland and river management through the implementation of sector specific buffer zones.

SUMMARY OF RESULTS:

The results obtained culminated in a guideline for farmers which is on the Milk SA website, the title being: "Best practice guidelines for improved wetland and river management on dairy farms in South Africa".

Some conclusions and recommendations are:

- Strategies for a specific site should be implemented, e.g. stream fencing is most effective when implemented along headwater streams and as a result, is a more suitable option for farmers in the headwater catchments.
- Similarly, the benefits of riparian buffers are most tangible when continuous strips of unbroken buffers are implemented.
- Since sustainable dairy farming is an essential requirement to ensure that receiving freshwater systems are protected from harmful dairy farm wastewater, trade-offs have to be made between dairy production and the associated environmental impact. The challenge is that environmental and economic goals often conflict, and a middle ground must be found to achieve a level of balance on dairy farms.
- To conserve scarce water resources rehabilitation of degraded wetlands may be the best option.
- Also, the potential benefits of constructed wetlands should be investigated as the long-term benefits will outweigh the loss in grazing land and costs of construction.

PROJECT TITLE

A feasibility analysis of low cost biological waste water treatment options for dairy farms in South Africa.

AIM:

Dairy wastewater is characterised by high biological and chemical oxygen demand, suspended and dissolved solids, fats, sulphates, oils and nutrients such as ammonia phosphates, which are harmful to the receiving environment. Therefore, it is critical that produced wastewater is treated and stabilised appropriately before being discharged into any terrestrial and aquatic environments. There is also an urgent need to reduce the volume of water utilized and to identify efficient and cost-effective approaches to treat dairy waste water. These issues provided the motivation for the study.

SUMMARY OF RESULTS:

The farmers participating in the study indicated that wastewater quality is not monitored and they also did not indicate water quality issues associated with the dairy waste water. However,

the characterisation of the dairy waste water and a comparison of the water quality standards required for irrigation with wastewater with high organic content found that all participating farms sampled were not compliant with a number of water quality standards, highlighting the need to introduce measures to enhance dairy waste water quality, prior to disposal of waste water onto pastures.

The results from the phycoremediation laboratory experiments suggest that algal consortia have potential in diluted dairy waste water and that further enrichment and adaptation is required for the consortia to be effective in higher concentrations of dairy waste water. The micro-algal consortia used in phycoremediation out-competed native microbes.

However, this was based on a controlled environment laboratory experimental setting. Further additional adaptation experiments have to be carried out to enhance the removal efficiency of Chemical Oxygen Demand (COD).

PROJECT TITLE

A systems dynamic approach to incorporate environmental indicators into economic outcomes of dairy production systems in SA.

AIM:

To develop a tool based on a system dynamics model to assess greenhouse gas emissions (GHGE) and carbon capture and storage on farms, by analysing critical nutrient flows in order to determine whether farms are net emitters of carbon (i.e. sources) or sequestrators thereof (i.e. sinks).

SUMMARY OF RESULTS:

The model was developed which will assist dairy farmers and the industry in general with estimating carbon emissions, and carbon capture and storage capacity (including sequestration) of different production systems. In support, an online tool has been developed to estimate the carbon footprint (emissions only) of a dairy farm, which can be accessed from: https://assetresearch.org.za/environmental-indicators-dairy-production-systems-phase1/

Furthermore, a comprehensive web-based DESTiny tool (Dairy Environment Sustainability Tool) was developed which estimates the net on-farm nutrient and net carbon flows (inclusive of both sources and sinks) for dairy production, which can be accessed from: https://assetresearch.org.za/on-farm-carbon-capture-and-storage-capacity/

The latter tool includes a user-interface which allows remote users, researchers, practitioners, farmers, and technicians ease of access while integrating the system dynamics models with on-farm realities.

PROJECT TITLE

Application of the system dynamics model to estimate the relative environmental footprint of milk and imitation plant beverages.

AIM:

To estimate and compare the environmental footprints of milk and plant beverages in a South African context, with emphasis on:

- The water use (in litre) per nutrient index value, and per litre of product;
- The land (area per ha) use per nutrient index value, and per litre of product;
- The energy use (in MJ) required per nutrient index value, and per litre of product; d) the greenhouse gas (GHG) emissions (in kgCO_2e) per nutrient index value, and per litre of product; and e) the economics of production per nutrient index value, and per litre of product.

SUMMARY OF RESULTS:

A sustainability index providing a score to milk and plant beverages within South Africa was developed, based on the following:

- The Nutrient-Rich Food Index (NRFi), which incorporates factors like nutrient bioavailability, food matrix effects, age and sex-specific dietary needs, demographic strata, global nutrient contributions, and associations with diseases or protective effects;
- Additionally, protein quality was evaluated and included;
- The approach taken also leveraged life-cycle analysis principles with parameters within farming and production systems, including carbon emissions and storage, water usage, fertiliser application, land use, energy consumption, recyclable materials, and waste management; and
- To comprehensively gauge sustainability, the approach furthermore employed consumer and producer indicators and self-reported inquiries on aspects such as employee numbers, production efficiencies, profitability and others, which are considered along with product pricing in relation to country-specific poverty levels.

The results showed that milk was inferior to the plant beverages in LCA when compared per litre product, but superior when compared per nutrient index. Milk was also superior in relation to country-specific poverty levels and cost of production.

E. Programme: Sustainable production

BACKGROUND:

The enhancement of productivity on dairy farms should always be a major priority, which could be by genetic means, management improvements, employing new technologies, or following good agricultural practices. These measures should enhance sustainability in a continuous tight economic environment and therefore will always receive particular attention by Milk SA.

PROJECT TITLE

Genetic and performance monitoring from automatic milking systems (AMS) data.

AIM:

The objective of the study was to extract and analyse herd performance data from producers employing automatic milking systems (AMS) to monitor genetic and other performance progress in order to make recommendations.

SUMMARY OF RESULTS:

Historical herd performance data were analyzed from a TMR and pasture-based system, focusing on heifer fertility, sire rankings, and survivability. Key differences emerged between systems: 36% of heifers calved between 25–26 months in the TMR herd, while 53.5% of pasture-based heifers calved at 22–24 months.

Time-trend analysis revealed a declining Age at First Calving (AFC) across both systems, highlighting improvements in heifer rearing and fertility management. Progeny performance analyses strongly correlated sires and offspring survivability (TMR: $R^2 = 0.91$, Pasture: $R^2 = 0.97$), emphasizing AMS software's role in sire selection.

However, survivability declined with successive lactations, with the highest exits occurring between the heifer phase and third lactation (Pasture: 75%; TMR: 83%). These findings demonstrate the critical role of management software in recording historic fertility data, progeny performance, and survivability in South African dairy herds.

PROJECT TITLE

Introduction of efficiency measures in automatic milking system software.

AIM:

To develop efficiency measures for production parameters in dairy herds using automatic milk recording system data.

SUMMARY OF RESULTS:

The following is of significance: Different ways can be used to define efficiency. Generally, gross efficiency is defined as the ratio of one kg of fat-corrected (or energy-corrected) milk per unit of estimated net energy consumption.

Efficiency is related to feed intake, live weight (LW), body condition score, milk yield, milk composition and changes in LW. For the genetic improvement of efficiency in dairy herds the genetic merit of sires to be used in the artificial insemination (AI) industry should be established.

As efficiency changes over the lactation period, standard measurements should be used to determine the efficiency of cows. Residual feed intake (RFI) is one way of estimating the efficiency of production.

Reasons for considering RFI include -

- onsiderable variability in the trait;
- It being moderately heritable; and
- It appears largely independent of other production traits.

It can therefore be incorporated into a selection index aimed at simultaneously improving multiple traits. However, RFI is expensive to measure because it requires careful measurement of individual animal intake and LW gain, but it is possible to achieve acceptable accurate DMI or RFI prediction results based on standard milk component data, LW and stage of lactation. Such equations are obtainable from the literature.

Locally developed prediction equations for TMR systems for cows weighing more than 500kg include:

- DMI (kg/day) = 8.81 + 0.212 Milk (kg/day) + 0.010 LW(kg), and to calculate gross feed efficiency (GFE) [milk (kg)/DMI (kg)], the cow's own milk yield is divided by the calculated DMI
- Using milk composition data, GFE can be calculated as:
  GFE (kg/kg) =1.881 + 1.344BFY −0.003LW, where
  BFY = butterfat yield (butterfat % x milk yield)

PROJECT TITLE

Practical implementation of SANS 1694 with the DSA audit.

AIM:

The purpose of the project is to -

- Through the application of the DSA audit on a number of farms, all possible issues be identified and confirmed that would require amendment of the SANS 1694 standard as well as the DSA audit format and criteria;
- Compile a formal proposal/report and submit it as the authoritative document to the SABS Standards writer by the manager of the Regulations and Standards Project of Milk SA; and
- Based on the submission to SABS and subsequent outcome amend the DSA audit criteria accordingly.

SUMMARY OF RESULTS:

- The animal welfare audit programme was completed and ready for implementation. Audits are being conducted in electronic format on electronic devices such as tablets to support improvement of effectiveness and efficiencies, and professional services.
- Advertisements, an industry circular and a webinar in respect of animal welfare were done. In this context, the DSA presented at the RUVASA Congress in May 2022 to promote the DSA animal welfare audit programme amongst rural veterinarians in the interest of reaching sustainability development goals in South Africa.
- Regular communication has been with the SPCA (with specific reference to animal welfare issues and handling of bull calves) which resulted in a formal letter addressed to stakeholders in the value chain.

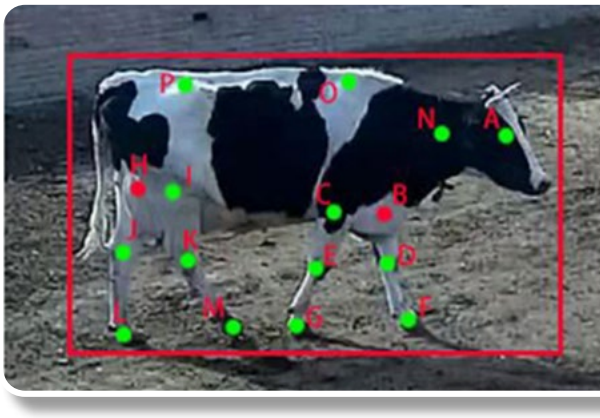
PROJECT TITLE

Investigation of alternative precision recording systems for collection of novel phemones in the dairy industry.

AIM:

The objectives were to develop a number of tools, which can be integrated or used separately, to detect, record and monitor dairy cattle health and welfare traits over time. The data recorded will be validated for management and application in genetic selection. Specifically, these tools will make use of 2D and 3D imaging and machine learning technologies to enable automated detection and tracking (monitoring) of body condition, claw health and lameness.

These automated methods will aim to mimic and improve upon traditional scoring methods.



SUMMARY OF RESULTS:

For body condition scoring (BCS):

- A novel approach was the use of three cameras instead of just one to perform automated body condition scoring. This approach was made possible by utilising a model aggregation technique called ensemble modelling.
- The motivation for using ensemble models was to reduce the generalization error of the prediction. As long as the base models are diverse and independent, the prediction error of the model decreases when the ensemble approach is used.
- The results showed that by evaluating all the data as a whole, several BCS predictions are produced per cow. However, by grouping these predictions together, a single prediction per cow was produced. The overall best-performing approach was when all three camera models were combined. Within 0.25 and 0.5 BCS this model yielded an accuracy of 83.67% and 95.92% respectively, which is highly satisfactory.

For lameness and claw health:

- The most useful features to evaluate lameness scoring is the back, the hooves and the head movement of the cow. The YOLO object detector which was used, showed a significant increase in performance due to the increase in the number of training samples. This resulted in better back extraction and hoof detection, which is important for automatic lameness prediction.
- The results showed that it was possible to successfully extract three smaller parts of the back of a cow and to stitch them together to show the back in total.
- It was also shown that it is possible to fit a circle to the back, and that the circle radius is positive to the lameness scoring accuracy when it was used as a feature.
- The hoof tracking algorithm was also shown to be quite accurate, when compared to the estimated ground truth paths of the hooves.

F. Programme: Market access

BACKGROUND:

Opportunities to increase market access of milk and dairy products, including export, should always be a main thrust. If successful, it would stimulate production, broaden the market, create opportunities for new entrees and may be beneficial to the whole economy of dairy production.

PROJECT TITLE

Investigation of Dairy Industries in selected African countries.

AIM:

The goal was to establish possibilities for trade in Sub-Saharan Africa and to understand benefits and possible constraints which may be limiting. Some of the objectives relevant to the countries investigated include:

- The customs duties on import;
- Measures by the government which aims to influence or determine the quantity and price;
- Measures by the state (including control boards) which aim to influence or control the production, prices and/or marketing;
- The international trade agreements and the rules of origin with regards to unprocessed milk and other dairy products contained herein, of the countries that influences the cusoms duties on imports;
- Initiatives by the public sector and other initiatives to promote the production and marketing of unprocessed milk and other dairy products; and
- Initiatives by the public sector to promote the use of dairy products.

SUMMARY OF RESULTS:

The objectives were addressed. Results from Botswana, Namibia, Eswatini, Lesotho, Mozambique, Angola, Zimbabwe, Zambia, Tanzania, DRC, Comoros, Madagascar, Seychelles, Malawi and Mauritius have been completed. The final report is available at Milk SA and SAMPRO.

G. Programme: Food safety and quality & Human health

BACKGROUND:

The safety and quality of milk and dairy products are not negotiable, and therefore Milk SA through the DSA created an impressive structure and human resources for testing and advising dairy-related and commercial institutions in the quest to ensure safety and quality. Supporting research focusses on testing for undesirable substances, supporting the regulatory environment and products that may enhance the health of consumers.

PROJECT TITLE

Probiotic yogurt with anticandida and antilisteriosis characteristics.

AIM:

The goal was to develop an acceptable synbiotic (probiotic + prebiotic) yoghurt product containing selected probiotic strains with the potential to prevent candidiasis, listeriosis and diarrhoea.

SUMMARY OF RESULTS:

Prebiotic inclusion did not have any apparent benefit on the probiotic viability, which means that the final yoghurt product will be made without any prebiotic addition. With respect to the probiotic strains, challenges before the stage of final selection were survival at adequate levels in cold and oxygen conditions, low pH, homogenization, and counteracting the biofilm-forming ability of *Listeria monocytogenes*.

Due to its intrinsic survival ability, *Bifidobacterium animalis*, in particular some of the subspecies, was the suitable organism for use in the probiotic yoghurt production, as they are stress adaptable, and as some of the lactobacilli such as *L. rhamnosus* inhibited *L. monocytogenes* in vitro, a combination of e.g. *B. animalis subsp. lactis BB 12* and L. *rhamnosus GG* which have proved to survive well in yoghurt, should be useful. These probiotic organisms may also assist in supporting obesity reduction, as a favourable gut microbiota is positively correlated to that effect.

PROJECT TITLE

The significance of *Enterobacteriaceae*, coliforms and *Escherichia coli* in milk in the South African market with the aim of updating microbial specifications in R1555 of 1997 (Act 54 of 1972).

AIM:

Regulation 1555 is under revision and there appears to be a notion to follow international trends to specify only *Enterobacteriaceae* and not coliforms and *E. coli*. DSA is not convinced that this is in the best interest of the industry and therefore the aim of the project was to measure the status of these organisms in milk.

Specific objectives were -

- Risk assessment to determine the significance of *E. coli* and coliforms as indicator organisms of hygiene and food safety;
- The isolation, characterisation and antibiotic susceptibility of *Enterobacteriaceae* species; and
- Source tracking of *E. coli* strains detected in pasteurised milk and how PRPs, HACCP and ISO 22000 can be implemented to overcome contamination after processing.

SUMMARY OF RESULTS:

The results showed that 54% of samples met the criteria set by R.1555 for coliform counts in pasteurised milk, 88% were compliant for *E. coli* and 68% had *Enterobacteriaceae* counts under 100 cfu/ml. With regard to packaging, milk in sachets had significantly higher coliform counts, indicating possible inferior hygiene compared to other packaging methods.

It was concluded that, although specifications for *Enterobacteriaceae* have not been incorporated into the South African milk regulations, this study underscores the importance of specifications and reaffirms the potential significance of *E. coli* and coliforms as indicator organisms within the SA dairy industry.

Therefore, it is recommended that all groups should be included in the revised regulations (R.1555), as the results do not support the international notion that the *Enterobacteriaceae* should suffice.

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