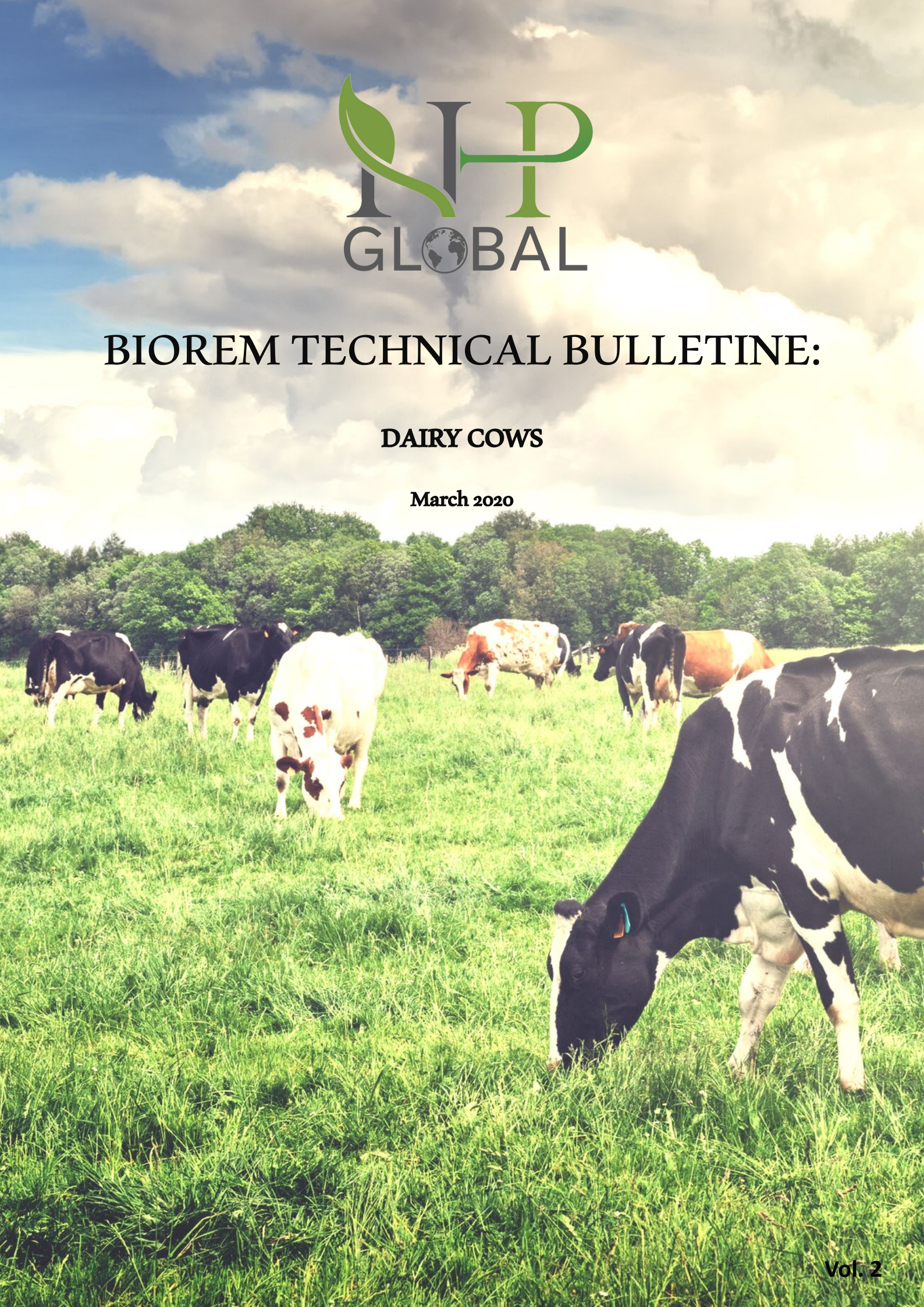

BIOREM TECHNICAL BULLETINE:

DAIRY COWS

March 2020

Vol. 2

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Trademarks and Registration

X™ indicates trademarked of product mentioned
X* indicates product is registered in terms of Act 36/1947

BIORED Reg no. V 20924
BIOSIN Reg no. G 1114
BIOREM Reg no. G 958

SIMILARITIES AND DEFINITIONS

BIORED: A natural bioflavonol antioxidant
BIOSIN: A probiotic suspension consisting of a broad spectrum of Lactic Acid producing organisms.
BIOREM: A probiotic consisting of a broad spectrum of Lactic Acid producing organisms grown on alfalfa.

3D illustration of *L. bulgaricus* one of the strains in the Biorem-culture

1. SCOPE

For dairy production units the three major causes for production loss are:

Stress (causing loss of favourable digestive micro-organisms)

Acidosis (clinical and subclinical)

Mastitis (clinical and subclinical, leading to high somatic counts in milk)

In general stress impacts negatively on the animal as a whole, but the contribution of stress on acidosis and mastitis have not been quantified.

2. STRESS

Although dairy cow production is in general more intensive than beef production their nutrition is still anaerobic fermentation which takes place in the rumen. Intensive feeding systems with a high grain content have been developed to cater for increased milk production. This influences the composition of the micro-organisms in the gut. Optimal functioning of the micro population is essential for maximum milk production, and a cost-effective feeding program.

The effect of stress on the digestive system of ruminants has been well documented both internationally and under Southern African conditions. These symptoms can be alleviated by amending the composition of the gut micro-organisms with the use of a probiotic inoculum.

3. PROBIOTICS

Veterinary companies have often offered probiotic products, claiming that they improve production. Very often these "newly found strains" have been advertised as to be the answer to the problem. However to our knowledge no publication has ever fully explained the reasons for the drastic loss or even chronic decrease of condition or production during these high production, stress related situations. Neither have we ever seen an acceptable solution for this condition.

Our research and observations over a period of more than 30 years has led us to believe that the manifestations of stress in dairy cows can be solved by our unique product range. Under normal high production conditions, a degree of stress is always present. We have therefore concentrated on ways of helping the animal cope better with this stress.

Effect of stress:

When an animal is placed under stress the following happens:

i. In the body we have a dual innervation system called the sympathetic and parasympathetic system. When stressed the sympathetic system is in ascendance and the reaction is known as the "fight-or-flight" reaction.

ii. During this reaction the hormones that help the body cope with this stress namely adrenaline and cortisol is secreted. These hormones increase heart rate and respiration and cause the veins and arteries supplying the gut to contract while the blood supply to the muscles increase.

iii. This happens both in the long term (chronic) and short term (acute).

iv. Acute stress is normally caused by factors such as the transport of the animal, adverse weather conditions and feeding insults can normally be alleviated by removing the stressor. This type of stress leads to feed related morbidities, loss of appetite and even mortalities. It can also cause significant changes in the microbial composition in the digestive tract especially the rumen.

v. Chronic stress has a negative effect on the microbial composition of the gut (in diverse species such as ruminants and ostriches) and together with decreased blood flow to the digestive tract leads to a decrease in condition and production, poor growth as well as a decline in food utilization, resulting in poor feed conversion ratios.

See **ANNEXURE A:** The quantitative ratio between pathogens and the total anaerobic bacteria in stressed versus healthy animals.

4. MANAGING ACUTE STRESS IN DAIRY COWS

Acute stress in dairy cows could be related to conditions such as new environments or transport to a new environment, change in feed, mating, the calving process, separation of the calf, harsh weather conditions.

Various trials indicate that a significant improvement occurs when ruminants are treated with BIOSIN* after acute stress in feedlots. It should be noted that the cause of the stress should also be removed.

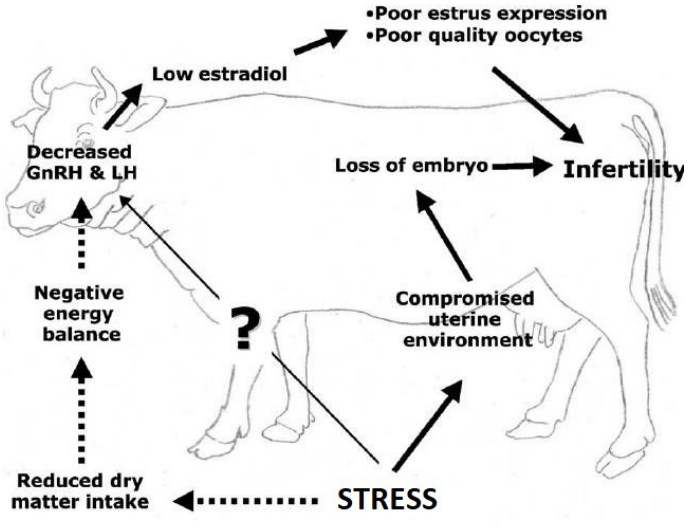
ANNEXURE B: Effect of Biosin® treatment on steers after a high stress incident (processing at the feedlot).

5. MANAGING CHRONIC STRESS IN DAIRY COWS.

Chronic stress is more difficult to observe in ruminants, as manifestation of symptoms are not always visible without sophisticated testing methods. This type of stress can lead to a decrease in milk production, increased incidence of (chronic) mastitis, high somatic counts in milk and poor milk quality. In an industry that is already under pressure this can determine the survival of a dairy operation. This situation is made worse by the fact that these losses are not always easy to detect and management is often not aware of the situation.

5.1. Identifying the problem

With an optimal microbial population in the digestive system of ruminants, feed is optimally digested. In stressed animal there are a decrease of total number of microbes and this have a negative effect on performance, resulting in decreased nutrient digestion, energy utilization, and microbial protein synthesis (King *et al*, 2011).



5.2. Probiotic: Biosin®

Under anaerobic conditions at 37°C as experienced in the rumen, microorganism flourish if nutrients are available. Under normal condition opportunistic pathogenic organism are also present in the rumen, but these organisms do not pose a threat as their numbers are kept low and they cannot dominate due to the competition from other organisms. This is also the case for most of the commercial probiotics offered in the market place and for this reason their effect is limited.

In diets with a high grain content non-fibrous carbohydrates (mainly starch) fermentation by amylolytic bacteria, e.g., Streptococcus bovis and Lactobacilli spp. produces organic acids (Mickdam et al., 2016). During this process, S.bovis, the most prominent amylolytic bacterium in the rumen, proliferates rapidly and produces lactate while limiting the production of volatile fatty acids (Chen *et al*., 2016). During the early period, an insignificant amount of lactate is quickly metabolized by lactate utilizers, especially Megasphaera elsdenii, and is converted into acetate, propionate, and butyrate. However, once asynchronous fermentation occurs between lactate-producing and lactate-utilizing bacteria, the lactate accumulates in the rumen and the pH of the ruminal fluid drops rapidly (Kenney et al., 2015). This induces rumen acidosis. In general, if the rumen's pH declines to below 6, it is recognized as subacute rumen acidosis (SARA) whereas a ruminal pH below 5.0 is defined as acute rumen acidosis (ARA).

When this happens the composition of the micro-organisms in the gut change as organisms such as the Megasphaera species that utilizes lactate, die, resulting in rumen stasis, as well as other pathological conditions, such as systemic acidosis.

With acidosis the following must be kept in mind:

- The mortality rate of important digestive micro-organisms increase as the pH of the rumen drops and at a pH of lower than 5 digestive fermentation is severely inhibited.
- With rumen acidosis the rumen must be alkalized;
- Megasphaera, a lactate and lactic acid consumer, can only re-establish when the pH has returned to normal and enough lactic acid producers are present.
- Biosin® is the ideal inoculant to re-establish a viable lactic acid bacteria population after the occurrence of rumen acidosis.
- Biored® a natural antioxidant positively influences the composition of rumen microbial populations and helps to protect the integrity of the gut wall and helps to limit damage caused by acidosis.
- Pathological manifestation of subacute/acute acidosis cannot be treated by probiotic administration if the probiotic organisms do not multiply in the digestive tract. An effective probiotic must act as inoculum and multiply in the digestive tract.
- To ensure survival and multiplication in the digestive tract the inoculum must be "stress buffered".

5.3. Biosin® Features

- A wide spectrum of lactic acid producing organisms;
- Strains that inhabit every segment of the digestive tract;
- Strains are "stress buffered", thus can survive harsh conditions. Trials indicated that in feedlot steers, even 60 days after a single treatment feedlot steers still exhibit a significant better average daily gain than a control group. **(ANNEXURE B)**;
- In the digestive tract the organisms in Biosin® start to multiply immediately and organism numbers reach levels where they exert a significant effect within 4 hours;
- The combination of strains has a synergistic effect against pathogens;
- BIOSIN® consists of a kit to which only water has to be added. After an incubation period of 2 at room temperature the product is ready for use.
- The fact that water is added on the farm during preparation makes it easy to use and reduces transport cost, making the product cost effective.

6. MASTITIS

6.1. Milk production

Nearly 70% of the energy requirements of ruminants is supplied by volatile fatty acids. Volatile fatty acids are the product of fermentation of starch and plant fibre (cellulose and hemi-cellulose) by micro-organisms in the rumen. Both starch and fibre consist mainly of glucose molecules bounded to each other in chains and branched chains. In the rumen the bindings between the different glucose molecules is broken down by microbial enzymes and the glucose molecules are further broken down to produce volatile fatty acids (propionic, acetic and butyric acid). The different volatile fatty acids are absorbed and transported to the liver via the portal vein system.

In the liver up to 70% of propionic acid are used for gluconeogenesis while the other volatile fatty acids do not contribute significantly. Of the glucose between 60 and 85% is used for milk production. Lactose is produced by the combination of glucose and galactose. Galactose is also derived from glucose as the precursor for galactose is uridine diphosphate-galactose which is also derived from glucose (Lin *et al*., 2016).

6.2. Causes of mastitis

Mastitis is caused by several different pathogens with the main mastitis-causing pathogens being Escherichia coli, Streptococcus uberisand and Staphylococcus aureus. It is the most common disease in dairy cattle and can have huge financial implications for dairy farmers. (Gu *et al.,* 2009) reported that subclinical mastitis could be found in 70% of milk producing dairy cows and that mastitis could induce increased formation of free radicals in milk, leading to oxidative stress.

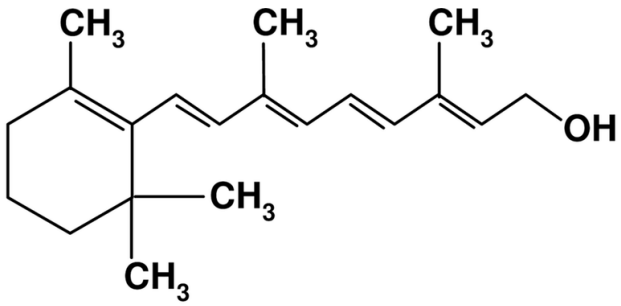
6.3. Vitamin supplementation

Natural anti-oxidants such as vit E and selenium play an important role in inhibiting and reducing sub-clinical mastitis. Supplementation with 1000 IU/day Vit E during the dry period and 4000 IU/day during lactation decreases the incidence

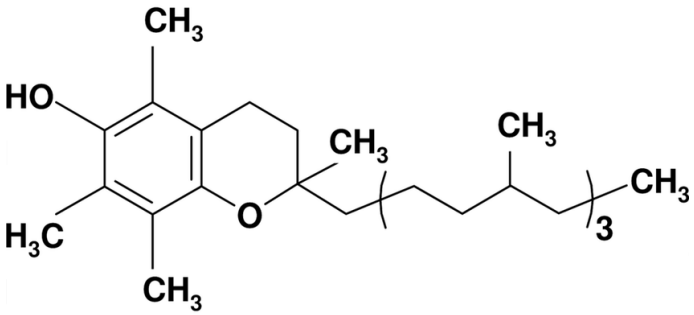
of sub-clinical mastitis between 57 and 88% (Chatterjee et al, 2005; Hogan et al, 1990).
Vitamin E also protects against lipid peroxidation by harmful lipid free radicals. It enhances phagocytic activity of neutrophils, macrophages and lymphocytes. (Chawaia et al 2004) reported that the supplementation of 1000 IU/day had the following effect on dairy cows:
45% reduction of the infection of mammal gland (IMI) at calving;
35% reduction of clinical mastitis;
48% reduction of the duration of IMI;
63% reduction of the duration of udder quarter infection;
70% reduction of somatic cell counts.

Vitamin E and Se deficiency lead to impaired phagocytosis by polymorphonuclear neutrophils (PMN). Dietary supplementation of cows with Se and Vitamin E result in a more rapid PMN influx into milk following intramammary bacterial challenge. It increases intracellular killing of ingested bacteria by PMN (Smith et al, 1997), as well as lowering the frequency and shortening the duration of clinical mastitis (Smith et al, 1984). Cows supplemented with 740 IU of vitamin E throughout the dry period had a 37% lower incidence of clinical mastitis during the next lactation compared to cows fed ad libitum haylage providing an estimated 320 IU vitamin E per cow per day.

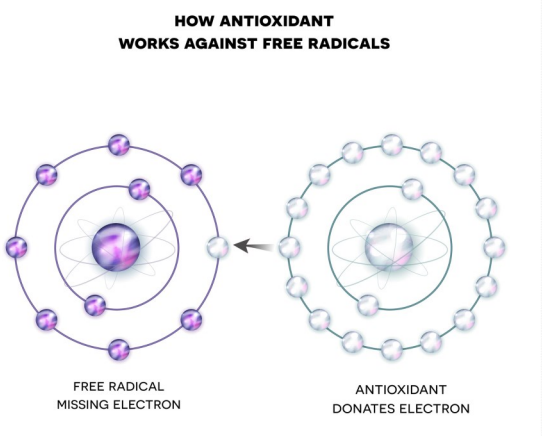
Low plasma concentrations of vitamin A (<80 µg/100 ml) and β-carotene (<200 µg/100 ml) were associated with increased severity of mastitis (Chew *et al.* 1982). In a study where diets of dairy cows were supplemented with 53,000 IU vitamin A per day or 173,000 IU per day or 53,000 IU per day plus 300 mg of β-carotene per day starting three weeks before drying-off and continuing through the dry period, the cows that were fed with the vitamin A plus β-carotene had fewer new infections during the early dry period (27% of previously uninfected quarters) than cows on the other treatments (49% for low vitamin A and 50% for the high vitamin A treatments) (Dahlquist and Chew, 1985)



Vitamin A as Retinol ($C_{20}H_{30}O$; M_W 286.45)



Vitamin E as DL-α-Tocopherol ($C_{29}H_{50}O_2$; M_W 430.7)



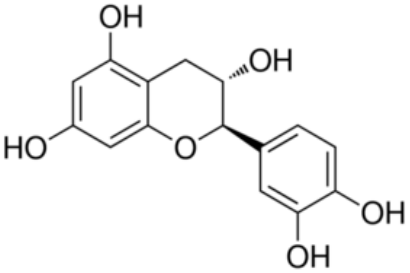
7. BIORED®

Biored® is composed of a large variety of natural occurring flavonoids and the antioxidant properties of most of these flavonoids are well documented:

- Robinetin (Panche *et al.,* 2016)
- Dihydrorobinetin (Panche *et al.,* 2016
- Robtin
- Rutin (Choi *et al.,* 2002)
- Catechin (Choi *et al*., 2002)
- Leucorobinetindin
- Leucofisitenidin
- Dihydrorobinetin
- Myricetin (Ross and Kasum, 2002)
- Quercetin (Choi *et al.*, 2002)
- Fisitin (Sahu *et al.,* 2014)

It has been demonstrated that in both steers and broilers Biored® can replace 50% of the industry standard supplementation of both vitamin A and E and in steers this significantly improved average daily gain (Kriel et al, 2019). The effect antioxidant and vitamin sparing effect of Biored® therefor supplements the effect of Vitamin E and A. This can lead to improved udder health and the ®prevention or treatment of both clinical and sub-clinical mastitis contributing towards improved milk production.

ANNEXURE C: In feedlot steers, supplementation of 200 gram Biored® per ton feeds as well as 50% the industry standard inclusion of vitamin A and E in the feed performed better in terms of weight gain than the group of steers fed a ration supplemented with 100% of industry standard inclusion of vitamin A and E without Biored®. Growth results corresponded with vitamin A and E levels in liver and subcutaneous fat samples.
In feedlot lambs no statistical difference was observed between treatments where lamb diet was supplemented with 150 grams per ton Biored® and 50% of industry standard vitamin A and E and 100% vitamin A and E without Biored® in terms of growth and feed conversion. An improvement in fiber digestibility as well as shelf life of meat was observed.



Catechin, a major compound in Biored's flavonoid composition


1203
1202

NOTES

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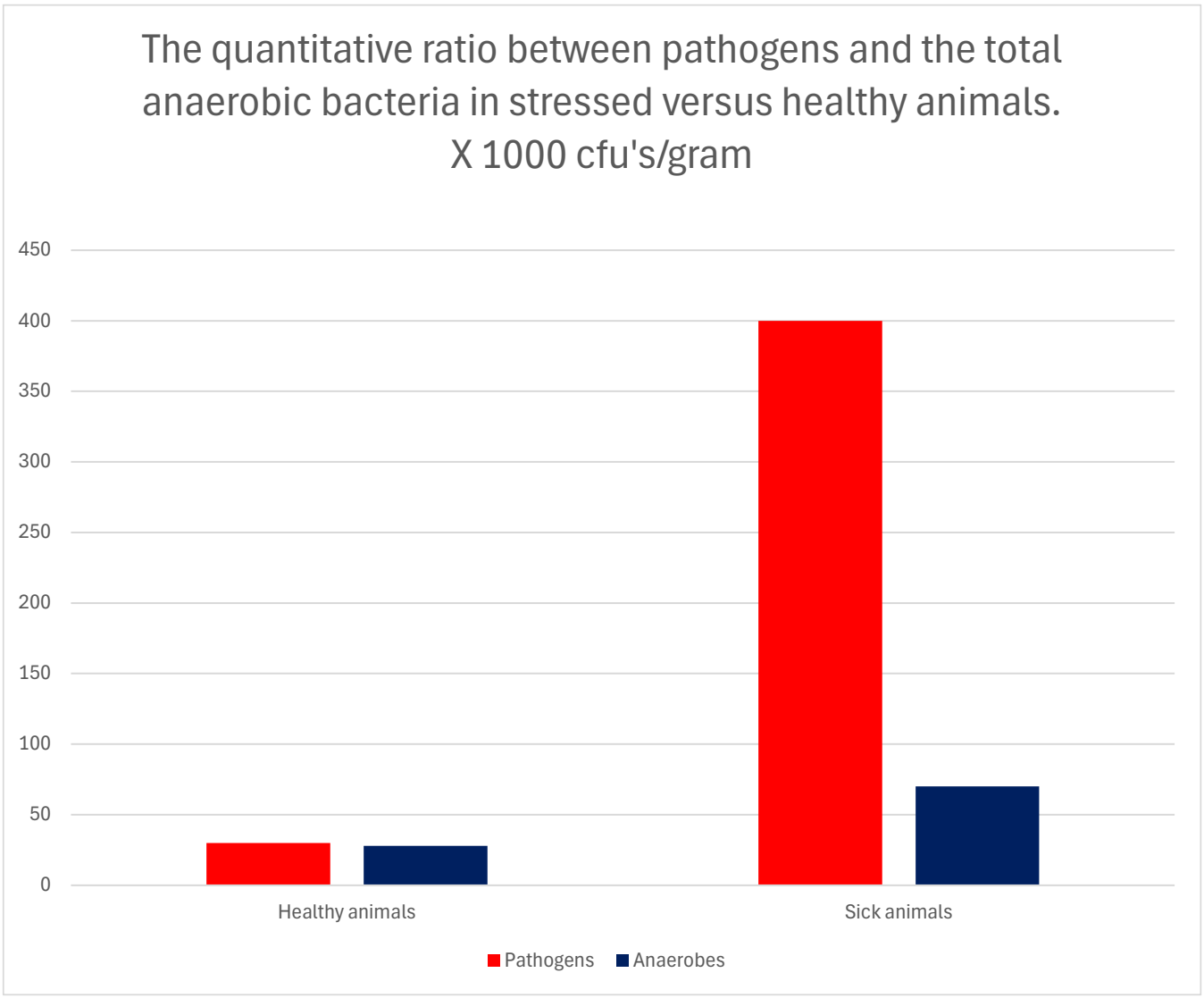
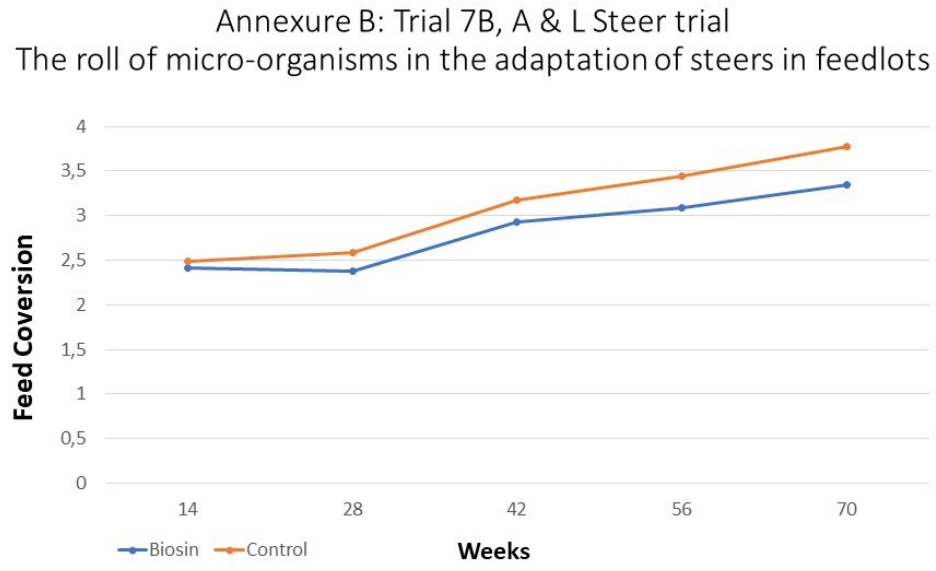
Annexure A

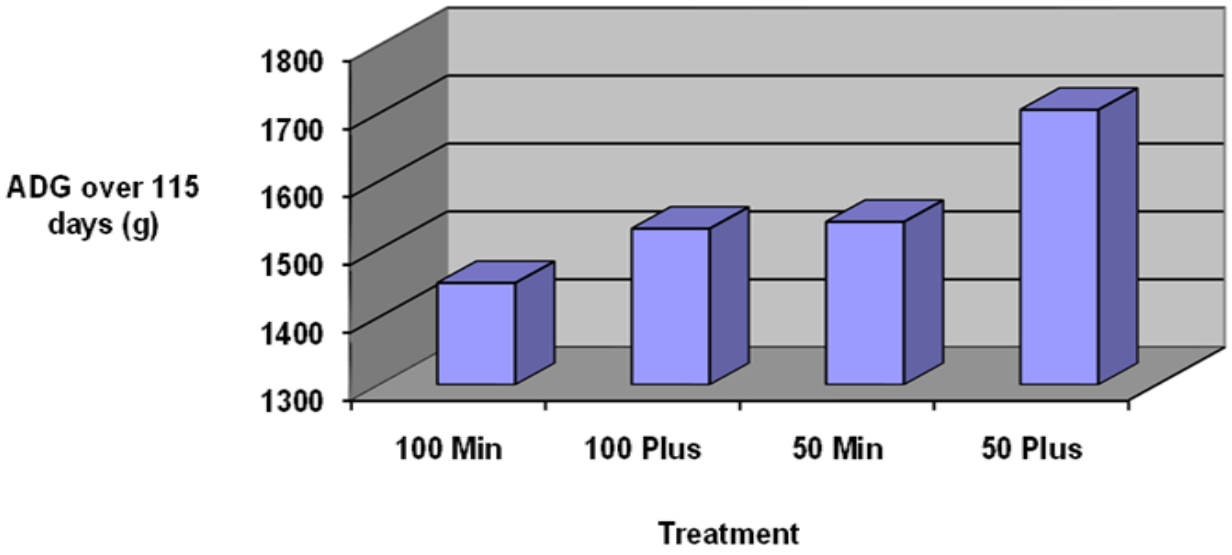


Annexure B



Biosin:	40 ml	n=17	Relative stress condition:	High
Control:	40 ml Plasebo	n=17	Objective:	Mass Gain, GDT, Feed Conversion

Annexure C


ADG over 115 days (g)
1800
1700
1600
1500
1400
1300
100 Min
100 Plus
50 Min
50 Plus
Treatment

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