# THE EFFECT OF NUTRITIONAL NUCLEOTIDES AND PARENTERAL GLUCOCORTICOIDS ON IMPROVING IMMUNOGLOBULIN ABSORPTION AND GROWTH BY NEONATE CALVES - REDUCING THE CARBON FOOTPRINT OF DAIRY CALVES.

by

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Submitted in accordance with the requirements
for the degree of

DOCTOR OF PHILOSOPHY

in the subject

ENVIRONMENTAL SCIENCE

at the

UNIVERSITY OF SOUTH AFRICA (UNISA)

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CO-SUPERVISOR: DR. AJ BARTKOWIAK-HIGGO

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The undersigned, appointed by the Dean of the Graduate School, have examined the thesis entitled:

**THE EFFECT OF NUTRITIONAL NUCLEOTIDES AND PARENTERAL GLUCOCORTICOIDS ON IMPROVING IMMUNOGLOBULIN ABSORPTION AND GROWTH BY NEONATE CALVES - REDUCING THE CARBON FOOTPRINT OF DAIRY CALVES.**

Presented by WILHELM STERNBERG SCHOOMBEE

A candidate for the degree of Doctor of Philosophy (PhD. Env. Sci)

and it is hereby certified that in their opinion it is worthy of acceptance.

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**ABSTRACT**

Antibiotics are routinely used in modern livestock production systems to treat and prevent disease as well as to enhance livestock production and/or outputs. This widespread use of antibiotics has led to a growing worldwide interest in antibiotic-free animal production. The addition of feed supplements such as nucleotides to improve intestinal health as well as the early development of the immune system needs to be investigated as an environmentally-friendly nutritional management activity. In this study four (4) groups of newborn Holstein calves (n=24) were evaluated after being treated as follows: Group 1 (Negative control), Group 2 (Investigational Veterinary Product (IVP) 1 – Oral supplement containing nucleotides, vitamins, essential fatty acids, essential amino acids, pre-biotics and trace-minerals), Group 3 (IVP 2 – Oral supplement containing nucleotides only at concentration and dosage equal to IVP1) and Group 4 (IVP 3 - Parenteral glucocorticoids).

Results of the study indicated that:

*Pre-colostral Serum IgG titres* – After titration of the serum the anti-BVDV results showed no difference between the study groups. All calves recorded a SP ratio of zero value (0.00 ± 0.2) indicating that they had not been nursed by their mothers prior to the start of the study. This was an entrance requirement for the calves to be enrolled into the study.

*Apparent Efficiency of Absorption % (AEA%)* – Literature shows that an AEA% result of between 20%-30% is good and 35% is excellent. The AEA% recorded for this study fell within this range although there was no statistically significant difference shown between the groups. In spite of a strictly controlled study protocol, 7 of the 24 calves (29%) still suffered from FPT in this study. The result falls within the range of 19% - 37% reported on United States of America (USA) farms by Doepel and Bartier in 2014.

*Serum cortisol* - When compared to the control group, there were no statistical significant difference evident for group 2 and group 3. However, the difference between the Glucocorticoid – Group 4 and the control and other groups was statistically significant (P = 0.0001; table 12) at the various time intervals. It was also

evident that the inclusion of parenteral glucocorticoids (group 4) may have assisted in prolonging the natural “gut closure”.

*Gut closure* – a positive 2 point linear regression forecast line indicated an increasing trend in IgG absorption post-24 hours for group number 4 whilst groups 1, 2 and 3 each had a negative 2 point linear regression forecast line (figure 23).

*Weight / ADG ($D_{42}$)* - Group 2 showed a statistically significant increase in mean $D_{42}$ weight ($P = 0.0042$) of 59.167 kg ± 3.545 kg when compared to the other study groups and compared to the control group ($P = 0.0227$). A comparison of the relative increase in mass of groups indicated that Group 2 efficiently achieved this result with a statistically significant ADG of 0.536 kg ($P = 0.014$) compared to the other study groups and compared to the control group ($P = 0.022$). In addition, *weight / FCR* – FCR was calculated for all groups as follows: G1 – 4.000 kg, G2 - 2.593 kg, G3 – 2.703 kg and G4 - 3.012 kg feed required for the production of 1 kg meat. The results indicated that the group 2 feed input was the most economical followed by groups 3, 4 and control respectively.

In conclusion, it is evident that a nutritional supplement containing nucleotides, vitamins, trace- minerals, essential fatty acids, essential amino acids and pre-biotics may contribute significantly to important economic indicators such as weight gain, ADG and FCR on a commercial dairy farm.

**PERSONAL DETAILS**


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**DEDICATION**

In 1990 I embarked on a journey to gather as much knowledge of my field of interest as possible. This was done with the purpose to empower myself. I now dedicate this work to all who shared this journey with me and everyone who impacted on it on the way.

A special thank you to my wife and two sons who contributed to this work with time spent at home whilst I was studying, and my parents who motivated me with continuous words of encouragements.

To my sons, always remember to aim for the moon because even if you miss you will still be amongst the stars.

*There is no knowledge that is not power*

Ralph Waldo Emerson

**DECLARATION**

Student number: 4117-773-8

I declare that "The effect of nutritional and parenteral glucocorticoids on improving immunoglobulin absorption and growth by neonate calves – reducing the carbon footprint of dairy calves" is my own work and that all the sources that I have used or quoted have been indicated and acknowledged by means of complete references.

_____ MARCH 2015

SIGNATURE DATE

(WS Schoombee)

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**ABBREVIATIONS**

| | | |
|---|---|---|
| A | - | Adenine |
| ACTH | - | Adrenocorticotropic hormone |
| ADG | - | Average daily gain |
| AEA | - | Apparent efficiency of absorption (%) |
| ATP | - | Adenosine triphosphate |
| BW | - | Body weight |
| BM | - | Birth mass |
| C | - | Cytosine |
| CBC | - | Complete blood cell count |
| CMM | - | Critical mating mass |
| CNS | - | Central Nervous System |
| $CO_2$-eq. | - | Carbon dioxide equivalent |
| CRH | - | Corticotropin-releasing Hormone |
| DNA | - | Deoxyribonucleic Acid |
| EAA | - | Essential Amino Acids |
| EDTA | - | Ethylenediaminetetraacetic acid |
| EFA | - | Essential Fatty Acids |
| EG | - | Esophageal groove |
| ELISA | - | Enzyme-linked Immunosorbent Assay |
| *E.coli* | - | *Escherichia coli* |
| FBC | - | Full blood count panel |
| FCR | - | Feed conversion ratio |
| FPCM | - | Fat and protein corrected milk |
| FMDV | - | Foot and Mouth Disease |
| G | - | Guanine |
| $G_1$ | - | Indicates the investigational calf group number |
| GCs | - | Glucocorticoids |
| GHG | - | Greenhouse Gas |
| GIT | - | Gastrointestinal Tract |
| GWP | - | Global warming potential |
| hrs | - | Hours |
| IFA | - | Indirect fluorescent antibody test |

| | | |
|---|---|---|
| Ig | - | Immunoglobulins |
| IM | - | Intra-muscular |
| IVP | - | Investigational Veterinary Product |
| Kg | - | Kilogram |
| L | - | Liter |
| MAAC | - | Metal Amino Acid Chelates |
| NPN | - | Non-protein-nitrogen |
| OVI | - | Onderstepoort Veterinary Institute |
| RBC | - | Red blood cells |
| RNA | - | Ribonucleic Acid |
| ROI | - | Return on investment |
| RPO | - | Red meat producers' organization |
| SC | - | Supply chain |
| SG | - | Specific Gravity |
| SOP | - | Standard operating procedure |
| SP | - | Sample to positive ratio |
| T | - | Thymine |
| $T_0$ | - | Time of birth (The sub-script number e.g. $T_1$ is an indication of the hours post birth i.e. 1 hour in this case) |
| Tg | - | Tg = 1 million metric tons |
| U | - | Uracil |
| UV | - | Ultra violet light |
| VFA | - | Volatile Fatty Acids |
| WBC | - | White blood cells |

**ACKNOWLEDGEMENTS**

I thank the following individuals and/or organizations for their contributions towards this thesis:

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*Education is the most powerful weapon that you can use to change the world*

Nelson Mandela

**TITLE**

**THE EFFECT OF NUTRITIONAL NUCLEOTIDES AND PARENTERAL GLUCOCORTICOIDS ON IMPROVING IMMUNOGLOBULIN ABSORPTION AND GROWTH BY NEONATE CALVES - REDUCING THE CARBON FOOTPRINT OF DAIRY CALVES.**

ABBREVIATED TITLE

THE EFFECT OF NUTRITIONAL NUCLEOTIDES AND PARENTERAL GLUCOCORTICOIDS ON IMPROVING IMMUNOGLOBULIN ABSORPTION AND GROWTH IN CALVES.

# 1. CHAPTER 1 – BACKGROUND

Intensive animal management requires effective disease control and so antibiotics are routinely used in modern livestock production systems to treat and prevent disease as well as to increase and enhance livestock production and outputs. However, pharmaceutical interventions are only successful when the animal's immune system is simultaneously enhanced. Unfortunately, when antimicrobials are used as livestock growth promoters they are commonly included in feed and water at sub-optimal concentrations (Cromwell, 2001) and this practice ultimately leads to the selection of antibiotic-resistant bacteria in the gastrointestinal tract (GIT), providing a potential reservoir of antibiotic-resistant bacteria ready for distribution into humans and the environment (Andremont, 2003). The transfer of antibiotic-resistance genes from, and through, faeces into indigenous soils and water may occur (Daane et al., 1996; Nielson *et al.*, 2000) and may persist due to the organisms' adaptability for survival in aquatic and terrestrial ecosystems. Bacteria have shown that they can, in nature, readily exchange genetic material and, thus, allow the transfer of various antibiotic-resistance mechanisms that that may already be present in the microbial environment (Salyers and Amabile-Cuevas, 1997).

The development of antibiotic resistance in humans has led to a growing worldwide interest in antibiotic-free animal production (Bager *et al*., 2000). Following the fortification of human infant milk formulas, research has shown that the addition of nutritional nucleotides improved intestinal health as well as the development of the immune system (Kulkarni *et al*., 1994). The role of nucleotides in the maintenance of intestinal gut health may well result in dietary nucleotides substituting as an alternative to conventionally used antibiotics in the feeding of young animals. If this is true, it may provide the livestock industry with alternatives to using antibiotics in animal feed. However there is only limited information about the role of nucleotides in the development of the immune system and the physiological requirement for nucleotides in young animals (Kulkarni *et al*., 1994).

Neonatal animal disease, morbidity and early mortality in the first days and/or weeks post-partum have been linked to a number of factors, including the lack of colostrum feeding, poor quality colostrum, insufficient quantity colostrum fed (McGuire *et al*.

1976), poor timing of colostrum feeding (Stott *et al.*, 1979a) and failure to transfer passive immunity from colostrum (FPT) within the first 24 hours post-partum. In particular, FPT has been associated with significant losses of suckling neonatal calves and several studies have reported a prevalence of between 10% and 68% of FPT worldwide (McGuire *et al*., 1982; NAHMS, 2002; Trots-Williams, 2008; Doepel and Bartier 2014). The net result of FTP is antibiotic intervention to cure disease and/or stimulate growth.

Maternal and fetal blood supply are separate so as maternal antibodies are not transferred across the placenta to the fetus during pregnancy, the bovine placenta is a "closed system" and, thus, calves are born devoid of detectable levels of immunoglobulins (Ig) (Stott *et al*., 1979; Doepel and Bartier 2014). Furthermore, newborn calves are unable to produce their own antibodies within the first weeks after birth and so it is essential that neonates acquire immunoglobulins from their dam's colostrum (passive immunity). Additionally, the replication of vital leucocytes is reduced by the animals increasing vulnerability to stress which has a negative feedback on the neonate immune system. It is, thus, necessary to investigate ways to improve and stimulate the development of the bovine active immune system in the early life of the neonate calf. As antibiotics and/or their residues may be detrimental when introduced to the environment, there is a need to provide an alternative intervention that more rapidly stimulates active immunity in the newborn calf and by so doing reduces the use of antibiotics in the production system that, in turn, ultimately ensures a more positive impact on the environment (Cromwell, 2001).

The long-term, overall and indirect concept aims of the current study are twofold: to assist the gut health of neonate calves by attempting to improve their digestion processes within a healthier GIT environment and, secondly, to aid in reducing the "carbon footprint" of the study area (dairy) by reducing the use of antibiotics, and/or the effect of its by-products associated with the rearing of dairy calves and ultimately aiding in improving production on the specific study area.

Success in these objectives will be determined by noting a quantitative improvement in calf growth and feed conversion ratio compared to a negative control group.

A management intervention such as the early post-partum addition of a nutritional supplement to the colostrum feedings might result in the activation of neonate T cells (Van Buren *et al.*, 1985) but also in increased absorption of colostral immunoglobulins (Ig) and increased blood serum levels of Ig post-partum. The occurrence of FPT might be reduced whilst the early development of the active immune system might be stimulated, hence reducing the need for antibiotics to prevent or cure disease. By so doing the growth and feed conversion ratio might be improved without the use of expensive and environmentally unfriendly growth promoters and in the process reduce the carbon footprint of dairy operations in South Africa.

Key words: Nucleotides, absorption of IgG, cortisol, FPT.

## 1.1 PROBLEM STATEMENT

Failure of passive transfer (FPT) of immunity has been associated with significant losses of lactating neonatal calves' post-partum and several studies have reported prevalence between 10%-68% of FPT worldwide (McGuire *et al.*, 1982; NAHMS, 2002; Trots-Williams, 2008). This results in antibiotic intervention to cure disease and/or stimulate growth. As antibiotics and/or their residues may be detrimental when introduced to the environment, there is a need to provide an alternative intervention that more rapidly stimulates active immunity in the newborn calf and by so doing reduces the use of antibiotics in the production system that, in turn, ultimately ensures a more positive impact on the environment (Cromwell, 2001).

## 1.2 HYPOTHESES

H1: A management intervention such as the early post-partum addition of a nutritional supplement consisting of nucleotides essential vitamins, minerals, fatty acids, amino-acids and a pre-biotic to colostrum fed to calves improve feed conversion rate and calf growth and general gut health.

H0: A management intervention such as the early post-partum addition of a nutritional supplement consisting of nucleotides essential vitamins, minerals, fatty acids, amino-

acids and a pre-biotic to colostrum fed to calves will have no effect on feed conversion rate or on calf growth and general gut health (presence of diarrhea).

H2: A management intervention such as the early post-partum addition of a nutritional supplement consisting of nucleotides essential vitamins, minerals, fatty acids, amino-acids and a pre-biotic to colostrum fed to calves will improve feed conversion rate, calf growth and general gut health (absence of diarrhea).

## 1.3 STUDY AIMS AND OBJECTIVES

The long-term, overall and indirect concept aims of the current study are twofold: to assist the gut health of neonate calves by attempting to improve their digestion processes within a healthier gastro-intestinal tract (GIT) environment and, secondly, to aid in reducing the "carbon footprint" of the study area (dairy) by reducing the use of antibiotics, and/or the effect of its by-products associated with the rearing of dairy calves and ultimately aiding in improving production on the specific study area.

Specific objectives of the study are:

1) To evaluate the effect of the addition of nutritional nucleotides to colostrum on immunoglobulin (Ig) absorption of the newborn calf immediately post-partum by noting the presence of maternal antibodies to immunologically naïve calves.
2) To evaluate the influence of serum cortisol concentrations on the absorption of Ig in neonatal calves.
3) To evaluate the effects of parenteral cortisol on the rate of maturation of intestinal epithelial (natural gut closure at 18-24 hours post-partum).
4) To determine/note any improvement in gut health as indicated by a reduction in neonate calf mortality and use of antibiotics to treat one of the major calf syndromes i.e. diarrhea, Feed Conversion Ratio (FCR), Average Daily Gain (ADG) and growth rates (kg) at the study site at the study site.

Success in these objectives will be determined by noting a quantitative improvement in (1) IgG serum levels (group 2 – Investigational Veterinary Product 1) compared to

the control group, (2) establish a negative correlation between serum cortisol and serum IgG (group 2 – IVP 1), (3) detect an increasing IgG absorption trend post – 24 hours (group 4 – IVP 3) compared to the other study groups, (4) calf growth (Greater kg weight gain during the study period compared to the control group 1) and in the feed conversion ratio (FCR – ratio of the amount of dry feed ration (kg) consumed to gain 1kg bodyweight).

# 2. CHAPTER 2 - LITERATURE REVIEW

## 2.1. INTRODUCTION

All phases of dairy production leave an environmental (carbon) footprint. The magnitude of this footprint impacts on and is measurable in three main areas: (1) *air quality and the atmosphere* – affected by green-house gas emissions, (2) *water quality and aquatic ecosystems* – affected by run-off containing animal drugs and pathogens and, (3) *soil and terrestrial ecosystems* – altered because of water run-off, pesticides and fertilizer use (Bertrand and Barnett, 2010; FAO, 2010).

The importance of early colostral Ig absorption by the neonatal calf for adequate transfer of passive immunity is well documented (Butler, 1983; Kruse, 1970). In spite of this knowledge, between 30% - 40% of dairy calves in the United States of America suffer from FPT (NAHMS, 2002). Past breeding strategies for dairy cattle have been aimed at achieving speedy genetic improvement, industry targets and raising profitability. However, whilst these strategies have been very successful they may very well impact negatively on the immunity of the animal (Lazarus *et al*., 2002; Zenger *et al*., 2007). The immune system is the body's natural defense mechanism designed to protect the body against invasion by micro-organisms including bacteria, viruses and fungi (Zenger *et al*., 2007).

A responsive immune system improves animal health and welfare and it is, hence, important for the producer to understand how to "build" a healthy immune system. Stress is known to influence and/or suppress the animal's immune system which may lead to increased episodes of disease (Olson *et al*., 1980; Olson *et al*., 1981; Quigley, 2007). Environmental conditions such as periods of extreme heat, cold and ambient temperatures outside the thermoneutral range for calves may affect and internal absorption and transport of Ig from colostrum whose composition may show a reduced Ig content. These conditions are predisposing factors towards a compromised animal immune system (Olson *et al*., 1980; Olson *et al*., 1981; Quigley, 2007). During conditions of stress, glucocorticoids are released by the adrenal cortex. Cortisol is the primary glucocorticoid in cattle and has been shown to affect the immune system (Minton, 1994; Fulford and Harbuz, 2005). An impaired immune system may predispose a calf to developing diarrhea which is the foremost cause of mortality and

morbidity in calves of less than 30 days old (Constable, 2004). The most common enteropathogens associated with calf diarrhea are *Escherichia Coli*, Rotavirus and Corona Virus, *Clostridium perfringens*, *Salmonella* spp. and *Cryptosporidium* spp. (Constable, 2004).

## 2.2. ANIMAL WASTE AND THE ENVIRONMENT

The size of intensive livestock production systems in South Africa is ever-increasing and as most of these systems are associated with confined-housing livestock production systems, these systems increasingly serve as a source of surface-, groundwater- and soil-contamination.

***Methane gas*** – is a potent, short-lived (9-12 years in the atmosphere) greenhouse gas with a global warming potential of 25 times that of carbon dioxide (100+ years in the atmosphere). When ruminants produce protein from plants methane is produced. Atmospheric concentrations of methane are ever increasing and this has made scientist investigate and examine the sources. Cattle have been identified as source contributing to global warming. The multi-factorial influence of cattle includes feed intake, composition of the feed as well as the status of ruminal microflora (Johnson and Johnson, 1995). Livestock produce approximately 80 million metric tons (Tg) of methane gas worldwide per annum, about 73% of this can be attributed to the global population of 1.3 billion cattle (Gibbs and Johnson, 1994). A typical dairy cow is estimated to produce in the region of 400 g/day or 109 kg to 126 kg of methane gas per year. Methane production starts as early as 4 weeks of age when the first feed is retained in the reticulo-rumen for fermentation and digestion and gas production rises rapidly during the reticulo-rumen development stage. It causes a significant loss of feed energy subsequently increasing feed costs. Much can be done to reduce methane production if a farmer can manage to improve feed conversion efficiency (FCR) during this period (EPA, 1993), by improving diets to include more non-structural carbohydrates or more starchy feeds, increase dietary fat content and/or crude fat.

***Manure management*** **-** At intensive enterprises, manure management is dependent on herd size, type of livestock and the stage of production at the farm. Historically, dairy operations have a large component of agronomic activity i.e. pasture-based production

for which high levels of nitrogen (N) and phosphorus (P) are required. For this purpose and that of waste management, waste and effluent are recycled over fields and pastures for fertilizer use. Although surface application of N is considered to be "environmentally unfriendly" due to the loss of N (up to 50%) it is considered to have some positive spin-off in that it reduces the pathogen load due to ultra violet (UV) exposure and environmental conditions i.e. exposure to summer and winter conditions (Hutchinson, 2004). Liquid effluent and manure is expensive to transport and, therefore, is applied as close to the source as possible. It is clear that the amounts of manure and production wastage produced in a dairy operation are vast and so there is ample opportunity for exposure, contamination and re-contamination of the environment.

***Antibiotics*** **-** Elevated levels of antibiotic resistance in humans and animals have been linked to the practice of indiscriminate use of antibiotics as growth promoters in intensified livestock production systems (Boxall et al., 2004; Gilchrist et al., 2007). Environmental contamination may result if good antimicrobial application practices are not maintained (Boxall *et al*., 2004; Gilchrist *et al*., 2007). Antibiotics are mostly water soluble with an affinity for a solid, soil phase which consequently affects its mobility in the environment (Tolls, 2001; Boxall *et al*., 2004). Most antibiotics sorb readily and to various degrees to the different clays and soils of the environment. Thus, the stronger the sorption of the antibiotic the better will be its mobility in the environment and the more this process will be facilitated by distribution of manure as a fertilizer (Boxall *et al*., 2004).

## 2.3. ENTRY OF ANTIBIOTICS INTO THE ENVIRONMENT

Many antibiotics may not be fully absorbed by the GIT and this may result in the parent compound and certain metabolites being excreted in the waste. It was estimated by Elmund et al. (1971) that as much as 75% of antibiotics administered to feedlot cattle might be lost to the environment following their excretion from treated animals. Oxytetracycline and tylosin are drugs that are routinely used in dairies and it was suggested that 50% - 60% and 67%, respectively, may be excreted unmetabolized in the urine and feces (Feinman and Matheson, 1978). Various routes exist whereby antibiotics enter the environment, including the disposal of unused drugs and their

containers and the use of waste material that contains remnants of drugs and or residues thereof (Buchberger, 2007). Other corridors of entry include waste products from grazing stock, feed and manure dust containing antibiotics and spills (Daughton, 2004).

## 2.4. PERSISTENCE OF ANTIBIOTICS IN THE ENVIRONMENT

Sorption dynamics dictate that most excreted antibiotics are concentrated in the solid phase (Boxall *et al*., 2004; Kolz *et al*., 2005). The application of both wet and dry manure to fields undoubtedly introduces breakdown products to the environment but persistence data are scarce and can be attributed to analytical difficulties (O'Conner and Aga, 2007). The danger of these metabolites pertains to the biological activities of the chemicals along with the parent compounds. Very few studies have determined the occurrence of veterinary antibiotics in groundwater (Boxall *et al*., 2004).

As there are few reports, thus, little is known about the mechanisms of degradation of antibiotics in the environment. However, these mechanisms may include: (1) hydrolysis as effluent as the most common way for antibiotics to be introduced into the environment (Huang *et al*., 2001); (2) photolysis from a soil interface providing a different photo-degeneration environment compared to an aqueous environments (Balmer *et al*., 2000) and (3) bio-degeneration of antibiotics, where little is known about this process suffice as to say that it can vary from limited to non-bio-degeneration (Kummerer *et al*., 2000).

## 2.5. OCCURANCE OF BACTERIA AND DEVELOPMENT OF RESISTANCE

Antibiotic resistance in bacterial pathogens begins, initially, with resistance among commensal gut bacteria since the intestinal bacterial ecosystem is extensive and often comprises hundreds of diverse species (Salyers *et al*., 2004). Secondly there is external selection for antibiotic resistance associated with every antibiotic treatment intervention, irrespective of the health status of the animals. The microbial population is then excreted in the waste from where the dominant resident bacterial populations undergo changes in terms of numbers and proportions (Andremont, 2003).

## 2.6. BACTERIAL SURVIVAL AND TRANSPORT IN THE ENVIRONMENT

The most significant route of bacterial introduction to the environment remains land application of effluent and manure, mainly because of its large biomass and the ability of the organisms to easily survive the transition from effluent to soil (Boes *et al*., 2005). The persistence and movement of such organisms in the environment continually poses a threat to food quality, human and animal health alike. Gavalchin and Katz (1994) concluded that native soil bacteria stand a greater chance of being adapted by the effect of antibiotics the longer the antibiotic waste persists in the environment leading to an increased selection for resistant traits by the bacterial communities in the soil (Schmitt *et al*., 2005). Survival and distribution of bacteria in the soil depends on soil moisture, porosity, pH, temperature and the resident bacterial community (McMurray, 1998).

## 2.7. IMMUNE SYSTEM

The collection of cells, tissues, molecules that mediate resistance to infectious disease and a humoral system (soluble biomolecules) function together and are called the immune system. When a calf is born its immune system is naïve, although fully developed. Thus, the calf is susceptible to environmental diseases which contribute to increased morbidity and mortality. Ultimately, the best defence that will ensure calf survival is the rapid ingestion of the correct quantity and quality colostrum that contains high volumes of IgG and leukocytes (Tyler *et al*., 1988).

The immune system consists of specialized cells and proteins, designed to protect the body against foreign material that enters the body, including bacteria, viruses and various other pathogens. An antigen is a molecule that interacts with an antibody, once recognized, or which alternatively induces a host to produce antibodies against that antigen. The immune system may be sensitized to act immediately upon a second entry by foreign material or by dangerous organisms (Alberts *et al*., 2002). Nutrition, especially vitamins and minerals, remains the key to a healthy and effective immune system as it can reduce the incidence and/or effects of disease. The building blocks of a healthy bovine immune system are a quality diet, efficient colostrum management,

antioxidants to promote lung and gut health and a disease-ready immune system (Abbas and Lichtman, 2007).

Essentially, the bovine immune system comprises of several complex lines of defence that defends the host against mainly environmental pathogens of bacterial and viral origin. These defence lines may overlap and are of varying specificity but are based on two pillars that have different functions at different times to provide a complete defense against disease: the innate defense system and the adaptive, or life-long, immunity (Abbas and Lichtman, 2007). The first line of innate defence is mechanical i.e. barriers like the skin and the mucous membrane on the surface of the GI-tract that, if intact, directly defends against pathogenic invasion. The initial immune response, or co-ordinated reaction against infectious microbes and/or antigens, is responsible for rapid but non-specific detection and elimination of invading pathogens by producing clones of antibody-producing cells or Ig. It does not require prior exposure to a specific pathogen and its defense does not result in life-long immunity (Abbas and Lichtman, 2007). An immediate innate immune response is inflammation involving cells such as macrophages and neutrophils. Both these cell types migrate rapidly to the site of invasion with neutrophils arriving first and macrophages at a later stage. These cell types are tasked with, through the process of phagocytosis, killing invader organisms (Alberts *et al.*, 2002).

Innate immunity significantly contributes to the body's defence, however, it is the mechanisms of the "adaptive immunity" that ensures long lasting protection against severe disease (Alberts *et al*., 2002; Abbas and Lichtman, 2007). This second line of defence known as the adaptive or "acquired immunity" develops relatively slowly and is dependent on learning and memorizing from every contact with a specific pathogen and then responding by developing a targeted response. Acquired immunity is possible through either receiving antibodies from an already immunised animal i.e. colostrum or by the stimulation or frequent challenge from pathogens i.e. bacteria, viruses or vaccinations. One can distinguish between adaptive cell-mediated immunity and humoral immunity (Alberts *et al*., 2002; Cortese, 2009). The hallmark of the adaptive immune system is its ability to recognize current, and to remember past, encounters with specific pathogens/invaders. This ability enables the immune system to recognize and respond to invaders much faster when encountered again.

Lymphocytes are the cells of the adaptive immune system. When lymphocytes encounter invaders they control the production and release of specialized proteins called antibodies that mark invaders for destruction by other immune cells (Cortese, 2009). This process might take seven to ten days during which time lymphocytes multiply to produce large numbers of antibody-producing cells. On completion of the action of the antibodies and cells of the immune system, the immune system remembers the specific invader, often for a lifetime, so that it may act quickly upon a next encounter to destroy invaders before any signs of disease may be seen. When regularly challenged vaccines induces this kind of life-long immunity (Cortese, 2009).

Cell mediated immunity is reliant on functional T-cells derived from bone marrow and the fetal liver that mature in the thymus gland. When in circulation T-cells account for between 40% - 80% of lymphocytes. Humoral immunity, in contrast, depends on B-cells. B-cells divide to become either plasma cells, which again produce antigen-specific antibodies, or memory cells which remember and respond to prior antigen exposure. This enables a much faster subsequent immune response associated with the release of large numbers of antibodies (Janeway, 2001).

### 2.7.1. IMMUNITY

Effective disease control of intensively managed animals is important and prevention must be the highest priority. The increase of immunosuppressive diseases is also making vaccination necessary; however, no intervention can be successful if the animal's immune system is not simultaneously enhanced. In addition, stress negatively impacts on the body's immune system.

Immunity can be defined as resistance to infectious disease. In normal healthy animals, innate protective barriers such as the skin, mucous membranes of i.e. the eyes, the respiratory tract and the digestive tract restrict and/or reduce the entry of bacteria, viruses or other disease-causing pathogens into the body. As described above, immunity may either be passive (from dam colostrum) or active when derived from the calf's own body after stimulation of the immune system from either natural infection or a vaccination response. Thus, active immunity involves cells that mediate an antibody response or passive immunity that involves uptake of external, pre-formed antibodies. During the first 24 hours of life the gastro-intestinal tract (GIT) is

able to absorb intact antibodies directly into the bloodstream, i.e. from colostrum from the cow, without digesting it (Quigley *et al*., 1998). Invading bacteria or viruses that are coated with antibodies are easier to ingest and be destroyed by the white blood cells circulating in the body.

### 2.7.2. IMMUNOGLOBULINS

Immunoglobulins (Ig) are a family of large glycoproteins that make up the major components of the host immune system; it is transferred from the cow's blood to her colostrum (colostrogenesis) at a rate of 500 g/week in the udder during the late dry period (4-6 weeks pre-partum) (Barrington *et al*., 2001). Immunoglobulin has a dual protective role. It is firstly absorbed and protects the animal against immune challenges and secondly, unabsorbed Ig has a local protective role in the GIT. A changing oestrogen and progesterone serum profiles initiate colostrum formation in the dam. The bovine mammary gland actively assists in regulating the immunoglobulin concentration in colostrum. Maximum IgG entry rates are between days 3 to day 1 pre-partum. Mammary epithelium does not synthesize immunoglobulins. The greatest IgG production and turnover is at parturition accounting for the large IgG concentration in colostrum (Sasaki *et al*., 1976). Most immunoglobulins enter colostrum through a selective receptor-mediated intracellular route (Lacy-Hulbert *et al*., 1999; Stelwagen *et al.,* 2009). Maternal serum Ig concentrations can reach~ 5.7 mg/ml at 28 days pre-partum and can decline to~ 1.4mg/ml at 4 days pre-partum. It is speculated that the decline in progesterone levels a week pre-partum is an initiating sign for the onset of colostrogenesis and the increase in circulating oestrogen, progesterone and prolactin at parturition induces the onset of lactation and it coincides with the ending of colostrogenesis (Guy *et al.*, 1994).

Antibodies generated against antigens are very specific i.e. antibodies made in response to a *Pasteurella* infection will only attach to the *Pasteurella* bacteria when these bacteria are encountered.

The synepitheliochorial structure of the ruminant placenta prohibits the direct transfer of Ig from the dam to the fetus. This means that eight different layers of membrane

separate the maternal and fetal blood supply resulting in the restriction of heavy molecular weight nutrient transport such as Ig (Fowden *et al*., 2006), hence newborn calves are deprived of Ig, and i.e. they are hypogammaglobulinemic. The ingestion and subsequent "successful" absorption of Ig essentially determines calf health, survival rate and future productivity of the calf. Whilst adequate transfer of Ig depends on the Ig concentration (mg/ml) and volume (L) of the colostrum fed, the efficiency with which the intestine absorbs the Ig from the passing colostrum depends on the timing of the colostral meal. (Besser and Gay, 1994). For this, at least 100 g of colostral Ig should be absorbed within the initial 12 hours of the neonate's life (Weaver *et al*., 2008).

Bovine colostrum contains three types of immunoglobulins: IgG, IgM and IgA accounting for 75%-90%, up to 5% and up to 7%, respectively, of the total Ig in colostrum. There are two additional subclasses of antibodies known as IgE and IgD as seen in Figure 1a. IgE is associated with allergic reactions when the host immune system overreacts to environmental antigens i.e. pollen, fungi and spores and is found in the lungs, skin and mucous membranes. When measured in high concentrations is also a good indicator of parasitic diseases. The functions of IgD are poorly understood. It appears in tiny amounts in the blood but primarily on the surface of B cells where it acts as a receptor for antigens (Junqueira, 2003).

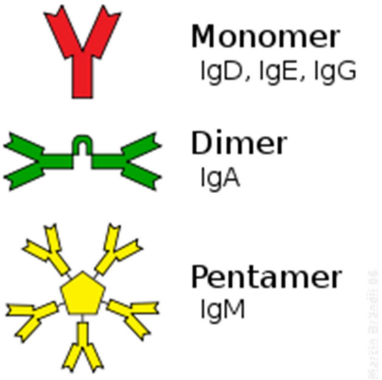


Figure 1a: Diagram of classes and the structure of immunoglobulin (Ig) (Woof and Burton, 2004).

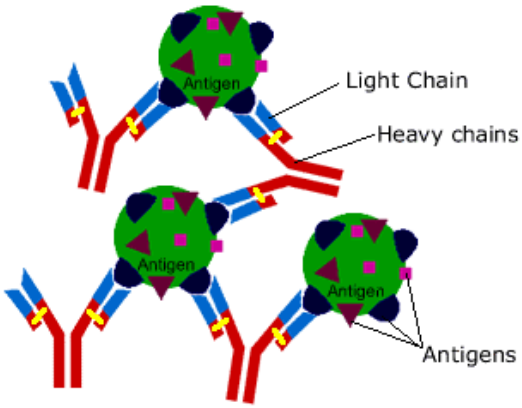


Figure 1b: Antibodies binding to antigens (Junqueira, 2003).

*Immunoglobulin A (IgA)* - is the second most common antibody and plays a critical role in mucosal immunity. Two isotypes of IgA are known: $IgA_1$ (predominant in serum) and $IgA_2$ (predominant in secretions). These are produced by the resident intramammary plasma cells of the *lamina propria* (thin layer of loose connective tissue under the epithelium together with which it forms the mucosa). After production, they are translocated, via the blood, across the mammary epithelial cells (MEC). Approximately 3 g - 5 g of IgA is secreted into the lumen daily and, around parturition, this transport is regulated by changes in the endocrine system (Barrington *et al*., 2001; Rincheval-Arnold *et al*., 2002; Wilson and Butcher, 2004; Stelwagen et al., 2009). The secretory form of IgA (sIgA) is the main Ig found in high concentrations within mucous secretions including tears, saliva, colostrum and secretions from the urinary tract, gastrointestinal and respiratory epithelium. It is also found in small amounts in the blood. Such sIgA is produced by B-lymphocytes adjacent to the mucosa. The secretory component of sIgA protects the immunoglobulin from being degraded by proteolytic enzymes, thus sIgA can survive in the harsh environment of the gastrointestinal tract and respiratory passages where it provides protection against microbes that multiply in body secretions.

*Immunoglobulin M (IgM)* - is the largest and third most common antibody in the circulatory system and is mainly found in the blood and lymph fluids. It is the first Ig to appear in response to initial exposure to antigens. It is a surface immunoglobulin produced by the $B_1$-cells (lymphocytes) which are derived from bone marrow. Because of its size, it is primarily found in the serum and it does not pass across the placenta. These two biological properties of IgM make it useful in the diagnosis of infectious diseases because the demonstration of serum IgM antibodies is an indicator of recent infection or, in a neonate's serum; this indicates intrauterine infection (Houghton Mifflin Company, 2004).

*Immunoglobulin G (IgG)* - is a Y-shaped protein complex and each IgG molecule has two antigen binding sites. It consists of two identical heavy chain and two identical light chain peptides (short chain amino acid monomers). It is the most abundant immunoglobulin isotype, found in all body fluids, and is almost equally distributed between blood and tissue fluids, constituting 75% of serum-immunoglobulins

(Junqueira et al., 2003). These molecules are synthesised and secreted by plasma B cells (lymphocytes) that are produced in the host bone marrow. These differ from other lymphocytes in that it has a protein B cell receptor that binds to antigens. The IgG antibodies are predominantly involved in the secondary immune response (the main antibody involved in the primary response is IgM). The IgG antibodies bind to various pathogens i.e. viruses, fungi and bacteria and by so doing protects the host from infection (Junqueira et al., 2003; Meulenbroek and Zeijlemaker, 1996).

The IgG antibody has several immune mechanisms with the two main mechanisms including binding to pathogens as seen in Figure 1b and by so doing rendering them immobilised or by coating the pathogens' surface, thus marking the pathogen for phagocytosis by immune cells. The presence of specific IgG generally corresponds to maturation of the antibody response (Alberts *et al*., 2002; Abbas and Lichtman, 2007).

This is the only antibody subtype that can pass through the placenta, thereby providing protection to the fetus. Along with IgA secreted in the colostrum, residual maternal IgG introduced through the placenta contributes to the protection of the neonate. Bovine colostrum, in particular, contains a high percentage of IgG (Meulenbroek, 1996). The two isotypes of IgG: $IgG_1$ and $IgG_2$, as can be seen in Table 1., (Larson *et al*. 1980) account for 80% of IgG and are predominantly absorbed from the calf's intestine (Butler, 1983). These IgG isotypes together provide passive immunity to the neonate. The transport of IgG begins approximately 5 weeks pre-partum and peaks at 1-3 days pre-partum (Sasaki *et al*., 1976). According to Doepel and Bartier (2014) milk from the second milking post-partum contains only on average 55% of the IgG levels found in milk from the first milking and, thus, when referring to the capacity to build passive immunity in the new born calf, only milk from the very first milking can be referred to as true colostrum. All milk from subsequent milking's should be referred to as transitional milk.

There is a negative relationship between the weight of colostrum in the first colostrum milking and the $IgG_1$ content thereof (Pritchett *et al*., 1991). Researchers suggest that the decrease in colostral $IgG_1$ concentration in large volumes of colostrum might be due to a dilution effect of the $IgG_1$ i.e. the more colostrum a cow milks the less likely chances are that it will contain a sufficient concentration of $IgG_1$. The first milking's'

weighing <8.50kg is reported to have a higher Ig concentration (Pritchett *et al.*, 1991). The difference between the immunoglobulin isotypes content of milk and colostrum is shown in table 1 below.

Table 1: The principal compositional difference between colostrum and mature bovine milk. Adapted from Larson *et al.* 1980; Pritchett *et al.*, 1991.

| | **Source of Immunoglobulin and titre (mg/ml)** | |
|---|---|---|
| **Immunoglobulin and isotype** | **Colostrum** | **Milk** |
| Total IgG | 32-212 | 0.72 |
| $IgG_1$ | 20-200 | 0.6 |
| $IgG_2$ | 12 | 0.12 |
| IgA | 3.5 | 0.13 |
| IgM | 8.7 | 0.04 |

#### 2.7.2.1. ONTOGENY OF THE BOVINE IMMUNE SYSTEM

Ontogeny commonly refers to the origin and subsequent development of an organism's lifespan. It is, in essence, the study of the structural change within a unit which may be a cell, organism or society of organisms etc. (Oxford English Dictionary - second edition, 1989).

The development of a calf's immune system starts already during early foetal development i.e. a foetal thymus is recognisable as early as forty days (40) of gestation and blood lymphocytes are circulating at day forty five (45) of gestation. Bone marrow and the spleen are evident at day fifty five (55) and lymph nodes appear at day sixty (60). Bacterial activity can be measured by day seventy five (75) of gestation and haemolytic activity by day ninety (90) (Osburn *et al.*, 1982). Neutrophils and macrophages which will ultimately be responsible for phagocytosis and the innate immune response are released into the blood around one hundred and thirty days (130) and it will be capable of phagocytosis late in gestation (Banks and

Mcguire, 1989). The B-cells responsible for the humoral immunity will be released between day fifty nine (59), as evidenced by IgM, and at day one hundred and thirty five (135) as evidenced by IgG. Peyers Patches are part of the secondary lymphoid tissues found in the lower ileum and only develop after day one hundred and seventy five (175) (Tizard, 2004). This differentiates the ileum from the duodenum and the jejunum where the duodenum has Brunner's glands while the jejunum has neither. Peyers patches sample antigens from their location in the gut lumen to then stimulate memory cells after the encounter. This triggers an immune response (Tizard, 2004).

### 2.7.3. COLOSTRUM

Colostrum is the fluid secretion from the mammary gland provided before and directly after birth. It contains proteins, lipids, carbohydrates, water, fat soluble vitamins, growth factors and nucleotides (Kehoe and Heinrichs, 2007) as well as cytokines (Hagiwara *et al*., 2000) and immunoreactive cells (Concha *et al*., 1980) needed by the neonate to sustain life. It is nature's first oral supplement designed to activate and support the developing immune system. The most significant proteins in colostrum include immunoglobulins (Ig), lactoferrin, transferrin, α-lactalbumin, β-lactoglobulin and albumin (Kehoe and Heinrichs, 2007). The IgG concentration may vary due to various factors including animal's disease history, lactation number, volume colostrum, season, breed, health and nutritional status. The survival of the neonate is ultimately dependent on the maternal, the passive/humoral and later the active/cellular immunity and colostrum is the only source of sufficient circulatory Ig for newborn ruminant farm animals where it provides the neonates with passive immunity for the first 30-90 days of life and direct protection of the intestinal tract against infection (Guy *et al*., 1994). Various attempts have been made to provide a source of Ig other than colostrum i.e. supplemental Ig products, but all these attempts showed moderate to poor results (Fratric *et al*., 2005).

Bovine colostrum contains between $1 \times 10^6$ and $3 \times 10^6$ cells/ml of immunologically active leukocytes. These consist of macrophages (40% - 50%), lymphocytes (22% - 25%) and neutrophils (25% - 37%). Compared to neonates that are fed on colostrum lacking leukocytes, neonates that are fed colostrum containing leukocytes have the advantage of developing antigen-presenting cells faster (Reber *et al*., 2005).

Leukocyte containing colostrum is essential as it is necessary for the development of the acquired immune response to pathogens and vaccines (Chase *et al*., 2008). Colostral concentrations of fat soluble vitamins often vary from cow to cow and may be dependent on individual cow reserves and largely on the season of calving. The fat-soluble vitamins are particularly important in building efficient immunity in the neonate and inadequate absorption from the gut may predispose the newborn calf to enteric infections (Kehoe and Heinrichs, 2007).

Effective management and feeding of good quality colostrum should reduce calf mortality and morbidity, strengthen immunity and increase longevity of animals. A newborn calf attains immunity by early ingestion of sufficient quantities of good quality colostrum. Thus, there is a synergism between nutrition and immunity as there is an increased need for nutrition from the cells responsible for the immune response; hence a poorer immune response is shown by animals deprived of good quality nutrition (Doepel and Bartier, 2014).

Colostrum from second, and later, lactation healthy cows generally has a greater Ig concentration as these cows have been exposed to more disease, antigens and/or vaccines in their lifetime. As they have had the opportunity to develop antibodies against such disease organisms it is preferred to feed newborn calves colostrum from such cows (Kruse, 1970; Devery-Pocus and Larson, 1983). Colostrum containing >50 g Ig/L is of good quality and a calf should be fed at least 2 L – 4 L good quality colostrum as a first meal. Newborn calves should ideally be fed colostrum as soon as possible after birth, preferably within an hour, and then again at 6 hours and 12 hours later, totalling 4 L – 8 L within 24 hours, as this will improve the chances of ingesting sufficient Ig where a minimum of 200 g of Ig is recommended to ensure sufficient transfer of immunity (Kruse, 1970; Arthington, 2001). Colostrum of good quality may be stored as fermented colostrum for up to 1 month or good quality colostrum from the first milking may be frozen for up to 12 months without a significant loss in Ig content (Arthington, 2001).

#### 2.7.3.1. COLOSTROMETER

Colostrum quality is directly related to its Ig concentration (gamma-globulins content) and is a function of specific gravity (SG: R-square value 0.3-0.4) or its density i.e. the denser the colostrum the greater the quality thereof. Farmers can, therefore, use a KRUUSE colostrometer or hydrometer as a reliable, economic, quick on-farm tool for determining individual cow colostral quality (Fleenor and Stott, 1980). The colostrometer reading can be influenced by the temperature of the measured sample. When the sample temperature is below room temperature (< 20˚C) the quality will be overestimated (sample will be too thick) and when the sample temperature is above room temperature (> 20˚C) its quality will be underestimated (sample will be too thin) (Morin *et al*., 2001 and Mechor *et al*., 1992). The ideal is thus to measure at exactly 20˚C (72˚F).

Colostrometers (Figure 2a) are calibrated in intervals of 5 mg/ml and divided into three colored regions i.e. green, yellow and red (Figure 2b). Along the length of the colostrum holding beaker is a 0ml-200ml scale. Fill it with colostrum up to 200ml. The three colored regions on the colostrometer measuring between 0-180/200 mg/ml is based on Ig content/ml. Place the measuring beaker on a level flat surface. Then float the glass colostrometer in the test colostrum. Poor colostrum is indicated by red and contains < 22 mg/ml Ig, moderate is yellow and contains 22 mg – 50mg/ml Ig, excellent is green and contains > 50 mg/ml Ig. Read the colostrum SG value where the fluid touches the free floating colostrometer. If the reading is red, do not feed as a first colostrum feeding at all (insufficient Ig), however if the reading is green feed immediately. Yellow reading colostrum may be saved for second and third colostrum feedings (Morin *et al*., 2001 and Mechor *et al*., 1992).

Figures 2a and 2b were sourced from Fleenor and Stott, 1980.

Figure 2a: Colostrometer.

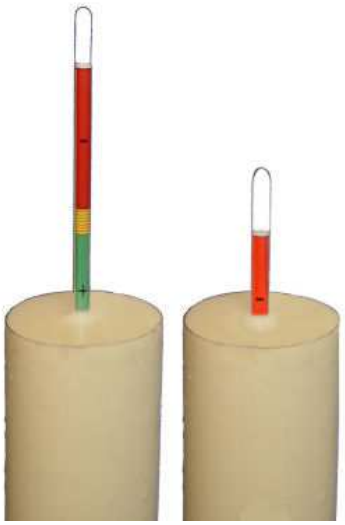

Figure 2b: Colostrometer reading.

### 2.7.4. FAILURE OF PASSIVE TRANSFER (FPT) AND THE TIMING OF THE INITIAL COLOSTRUM FEEDING

Newborn calves absorb Ig through the intestinal wall of the small intestine to acquire passive immunity. The timing of the initial colostrum feeding of a neonate is critical for a number of reasons:

1. The ability of the calf to absorb the large Ig molecules is short-lived as the cells that line the intestine mature shortly after birth due to rapid postnatal growth where fetal enterocytes are replaced by more mature enterocytes. This rapid cellular differentiation results in a spontaneous reduction in the uptake of Ig that is then digested as another, normal protein within the gut. This process is referred to as “gut closure” or impermeability and occurs around 24 hour’s post-partum. With gut closure there is an associated loss of passive immunization as no more Ig (responsible for passive immunity) is absorbed from that point in time (Schingoethe, 2001).

2. There is the possibility of pathogenic bacterial colonization of the intestine.
3. A delay in the first meal could be detrimental to calf health, vitality and future production of the calf (Quigley, 2002).
4. Ingestion of the first colostrum meal impacts in GIT development, function and hormones and its absorptive capacity (Blum and Hammon, 2000).

The process of Ig absorption, or pinocytosis, must occur before the calf's intestines become impermeable to the large Ig proteins. In the event of this process being unsuccessful it is referred to as failure to transmit passive immunity (FPT). This phenomenon may occur due to various reasons including none or insufficient quantities of colostrum being ingested, poor quality colostrum being ingested or allowing an extended period of time from birth to elapse prior to the initial colostrum feed. A neonate is considered to have FPT when the serum Ig titre is <10 mg/ml at 24 hours of life (Weaver et al., 2000).

The secretion of digestive, proteolytic enzymes is limited for a short period after birth. This allows maximum absorption of undigested Ig. However, from 12 hours after birth the enzyme secretion increases leading to a reduction in the ability of the Ig to reach the bloodstream. It is estimated that only between 25%-30% of Ig that enters the calf gut reach the bloodstream (Heinrichs and Jones, 2003).

At six hours post-partum, the gut wall's ability to absorb Ig is already reduced by 33% and at 24 hours post-partum this absorption is decreased by around 90%. However, colostrum remains necessary for calf vitality and to combat infectious organism's following the 24 hours post-partum. Unabsorbed antibodies function by lining the intestinal tract and preventing micro-organisms from attaching to the gut wall. If bacteria such as *E.coli* enter the digestive tract first, this defense mechanism will be ineffective as antibody absorption and attachment to the gut wall will be reduced and the neonate has increased risk of neonatal disease and early death (Heinrichs and Jones, 2003).

Thus, a strong correlation exists between FPT and calf illness and early mortality (Arthington, 2001). Good colostrum management practices and protocols including

the addition of nutritional supplements such as nucleotides to the colostrum feed aids in the defence of a calf against the effects of FPT (Mashiko *et al*., 2009).

## 2.8. NUCLEOTIDES

Most animals are required to produce millions of new cells every second for maintenance, considering that it is clear that trillions of nucleotides are required (essential) for cell proliferation during periods of rapid growth, reproduction, environmental challenges, and periods of limited nutritional intake, stress and recovery from disease. The internal supply mechanisms of the body i.e. endogenous *de novo* synthesis and the salvage pathway cannot always supply in the sudden increased demand of nucleotides, hence the suggestion by Carver and Walker in 1995 that the addition of nutritional nucleotides may employ advantageous effects on gastro - intestinal growth and development, reduced incidence of diarrhea, elevated IgG, various metabolisms i.e. lipid and liver functions (Uauy et al., 1994; Carver and Walker, 1995; Yau *et al*., 2003). Milk and colostrum contains high concentrations of nucleotides and it is estimated that up to 20% of the non-protein fraction of nucleotide supply, by the animal's diet, is contributed in this way (Uauy, 1989). A "shortage" of nucleotides may results in either reduced production or a slowdown in development, both of which leaves its mark in the producers' wallet. Under such conditions of short supply (joint capability of *de novo* synthesis and the salvage pathway) the addition of nutritional nucleotides might well assist the metabolic demand of tissues, requiring high levels of nucleotides to function i.e. intestinal mucosa, to perform optimally (Yu, 1998; Carver, 1999). For this reason nutritional nucleotides are referred to as a semi-essential nutrient in the diet of newborn, adult or sick mammalian animals (Yu, 1998; Carver, 1999).

In infants the addition of nutritional nucleotides have shown a positive influence on the immune response, metabolism of long chain fatty acids as well as an improvement in the gastro-intestinal tract restoration after injury when compared to non-supplemented nourishments (Gil *et al*., 1986; Carver and Walker, 1995; Pickering *et al*., 1998).

### 2.8.1. NUCLEOTIDE COMPOSITION

Nucleotides are low-molecular-weight bioactive groups of intracellular molecules with significant structural variation which are involved in many biochemical processes and of critical significance to cellular activity (Calver and Walker, 1995; Sauer *et al*., 2009). As the forerunner to nucleic acids and a vital part of coenzymes nucleotide functions include mediating chemical energy transfer and the metabolism of fats, carbohydrates and proteins (Carver and Walker, 1995; Cosgrove, 1998). Deoxyribonucleic acid (DNA) is a large molecule that constitutes the genetic material of a body. Nucleotides are the basic units or building blocks that are essential in the manufacture of new double helix DNA and ribonucleic acid (RNA) which is then used by new cells of all kinds. Nucleotides, nucleosides and nucleotide bases belong to the non-protein-nitrogen (NPN) fraction of milk and concentrations decrease during the lactation and/or nursing period (Schlimme *et al*., 2000). It is said that nucleotides may enhance growth and maturation as well as assist with the humoral and cellular levels of immunity (Schlimme *et al*., 2000).

A nucleotide consists of three basic components i.e. the nitrogen-containing ring or base, a five-carbon or pentose sugar and at least one (up to three) phosphate group/s (Figure 3a). The combination of a base (e.g. adenine) and a pentose sugar (e.g. deoxyribose) is referred to as a nucleoside (e.g. deoxy-adenosine). The further addition of a phosphate group (mono-, di- or triphosphate) results in a nucleotide (Rudolph, 1994; Voet and Voet, 1995). Molecules of DNA (Figure 3b) contain the sugar deoxyribose whilst RNA contains the sugar ribose. Nucleotide bases include two purines, adenine (A) and guanine (G), and also three pyrimidines, cytosine (C), thymine (T) and uracil (U). DNA contains the four bases A, G, C and T whilst RNA contains the four bases A, G, C and U. Complementary bases pair up so that C and G and, similarly, A and T pair up to allow the formation of the double-helix DNA. The order of the bases in DNA determines the genetic code. A sequence of nucleotides that is transcribed into RNA makes up a gene and triplets of nucleotides provide the code for the selection of amino acids that are linked to form proteins (Koppel, 2003).

Figures 3a and 3b were sourced from Koppel, 2003.

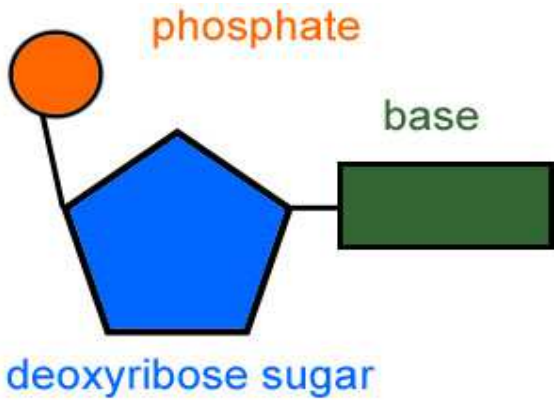


Figure 3a: Nucleotide structure

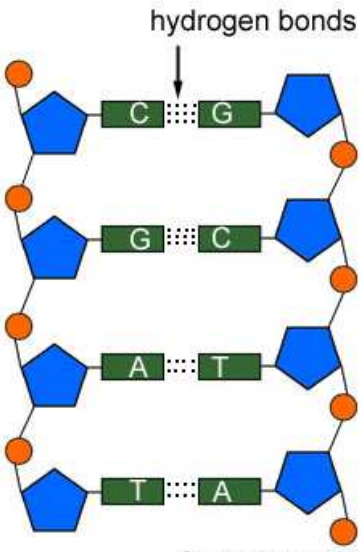


Figure 3b: DNA structure

### 2.8.2 ROLE AND IMPORTANCE OF NUCLEOTIDES

Until now antibiotics have been utilized to protect animals during periods of immunity gaps i.e. periods of stress. Antibiotic-free production systems are becoming sought after to avoid antibiotic resistance and the contamination of the environment. Nucleotides are normal components of a diet from animal or vegetable source (Sanchez-Pozo and Gil, 2002; Mateo *et al*., 2004), however, there is an increased demand for nucleotides during periods of cell division and growth (Tsujinaka *et al*., 1999). The nucleotide concentration of lactating mammals is species-specific and varies during the lactation period. Such variation may lead to a varied nucleotide demand between species and a reduction in availability may limit the performance of fast-growing and/or high-producing animals (Van Buren and Rudolph, 1997). Introduction of nucleotides is associated with accelerated intestinal maturation in humans and pigs; however, currently there are limited available data for dairy animals or their requirements for nucleotides (Gil and Sanchez-Medina, 1981; Gil and Sanchez-Medina, 1982; Sanchez-Pozo and Gil, 2002; Mateo *et al*., 2004).

Growth and development of the animal requires the continuous generation of new cells to replace dying cells in the body. All these cells metabolize nucleotides and are

reliant on DNA and RNA for mitosis (Schlimme *et al*., 2000). However, many cells including red blood cells, white blood cells, bone marrow cells and certain brain cells are unable to endogenously produce sufficient amounts of these nucleotides to cover their requirements. In addition, increased levels of stress in the body can increase nucleotide requirements as nucleotides are required to overcome the negative effects of the hormones secreted as a result of the stress condition (Carver and Walker, 1995).

In the event of an animal being unable to produce sufficient quantities of nucleotides it can only reverse/replenish these shortages by obtaining nucleotides as part of their feed intake. However, different feeds contain variable amounts of nucleotides and most are low in nucleotide content when compared to the needs of the animals (Koppel, 2003). The degree of nutrient absorption and animal growth is directly affected by the development and maturation of the intestine. Nucleotides affect this as well as the micro-flora of the intestinal tract and the integrity of the small intestine. Studies by Gil (1986) and Roselli (2006) have revealed that the addition of nutritional nucleotides to diets may alter the structure of the intestinal microflora by stimulating beneficial bacteria including bifidobacteria, and protects cells against enterotoxigenic bacteria which is known to increased membrane permeability. As they are immune-compromised, both pre-weaned and post-weaned dairy calves experience intense stress while they are undergoing rapid growth. Thus, these animals have major requirements for additional nucleotides during this time and there is a real need for nucleotides to maintain the structure and growth of the GIT immediately post-weaning (Barness, 1994; Tsujinaka *et al*., 1999). Dietary nucleotides contribute heavily towards the available circulating pool of nucleotides, essential at times when the production of leukocytes is stimulated (Kulkarni *et al*., 1994; Carver and Walker 1995)

### 2.8.3. EFFECT OF NUCLEOTIDES ON THE IMMUNE SYSTEM

The dietary supplementation of semi-essential nutrients (Uauy *et al*., 1989) such as nucleotides has been associated with both humoral and cellular immunity in humans and ruminants (Schlimme *et al*., 2000) but the exact mechanism is not clear (Kulkarni *et al*., 1994). Results from research studies with human infants, piglets and mice suggested that nutritional nucleotides play a role in the development, maintenance and enhancement of the immune system (Fanslow *et al*., 1988; Jyonouchi *et al*., 1993;

Jyonouchi, 1994; Pickering *et al.*, 1998; Zomborsky-Kovacs *et al.*, 1998). These results supporting the positive feedback on the immune system after nucleotide supplementation were confirmed in a study by Mashiko and co-workers in 2009, however, it was then suggested that calves needed to be supplemented for 10 days at least for the results to become evident (Mashiko *et al.*, 2009). Dissimilarly Kehoe and co-workers showed neither increased growth nor an effect on immunity in a 2008 study. Both studies by Kehoe in 2008 as well as Kentler in 2012 displayed no effect on either the severity or the incidence of diarrhea. Nucleotides appear to contribute to the circulating pool of nucleosides which in turn is available to stimulate leukocyte production and, hence, the increased need for nucleotides during periods of immunological challenge (Kulkarni *et al.*, 1994; Carver and Walker, 1995). These contrasting results supported the need for further field studies.

### 2.8.4. DIGESTION, ABSORPTION AND METABOLISM OF NUCLEOTIDES

Only nucleosides, bases and relatively small quantities of nucleotides can be absorbed by the small intestine and the duodenum has the greatest absorptive capacity (Bronk and Hastewell, 1987). Hence these compounds i.e. nucleoproteins, nucleic acids, free nucleotides and nucleotide adduct (Uauy, 1989; Uauy *et al.*, 1994) are referred to as the acting components of nucleotides (Schlimme *et al.*, 2000). More than 90% of all dietary nucleosides and bases are absorbed into the gastro-intestinal tract by enterocytes (Salati *et al.*, 1984; Uauy, 1989). This involves diffusion and specific $Na^+$-dependent carrier-mediated mechanisms (Bronk and Hastewell, 1987). The limited ability of nucleotides to pass through cell membranes is thought to be due to the phosphate group being negatively charged as well as the absence of a nucleotide transport system (Sanderson and Youping, 1994). Prior to absorption into epithelial cells, nucleotides need to be hydrolyzed by enzymes such as nucleoside phosphorylase, endonucleases and phosphodiesterases and, thus, the main absorptive compound is the nucleoside and it is this compound that is available for synthesis of purines and pyrimidines. This process is very efficient as it is said that more than 90% of the dietary nucleosides and bases are absorbed into the enterocytes in this way. The partially metabolized product enters the hepatic portal vein from the enterocytes from where it is transported to the hepatocytes for further metabolism. From the liver, the

product is released into circulation where after it enters the muscle tissue (Salati *et al*., 1984; Uauy, 1989).

### 2.8.5. STORAGE OF NUCLEOTIDES

In mature animals, nucleotides are continuously generated by *de novo* synthesis, salvaged and recycled from cells or tissues with a rapid turnover i.e. intestinal mucosa, skin, white and red blood cells (Uauy, 1989), the so-called "salvage pathway", or is absorbed from the animal's daily diet. Unabsorbed nucleotides are used elsewhere in the body in metabolism. However, young animals have an inadequate ability for *de novo* synthesis and need a source of nucleotides, particularly for cells with a high rate of replication i.e. cells of the reproductive tissues, immune and digestive systems (Uauy, 1989). Under these circumstances, nucleotides become an essential nutrient. In addition, nucleotide pools are greater in differentiated cells compared to undifferentiated cells suggesting that undifferentiated cells are much more dependent on dietary supplementation of nucleotides (Sanderson and Youping, 1994).

## 2.9. ANATOMY AND FUNCTION OF THE BOVINE DIGESTIVE TRACT

Two events in the neonate calf's life contribute to stress and ultimately lead to physiological changes in the GIT and immune system, viz. birth and weaning. Luckily, alterations in feeding and management practices may impact on both events to improve the future productive potential of the neonate (Pacha, 2000). The ontogenetic progress of the GIT comprises five developmental stages viz. (1) morphogenesis, (2) cytodifferentiation and fetal growth, including epithelium preparation for colostrum absorption, (3) birth and early suckling, (4) suckling period, (5) weaning to solid diet (Pacha, 2000). The digestive system of ruminants is unique as it allows to the most efficient utilization of energy from fiber compared to other herbivores. The bovine gastrointestinal tract as seen in Figure 4 comprises the mouth, grinding teeth (32), tongue, salivary glands, and esophagus, a complex stomach made up of four compartments, pancreas, gallbladder, the small and the large intestine. The stomach includes the rumen, vat or "pouch", reticulum or "honeycomb", omasum or "many plies" (because of its book-like resemblance) and the abomasum or "true stomach" (Heinrichs *et al*., 2003). This is shown in figure 4 below.

The digestive process begins in the mouth with the tongue harvesting the cud, then in the mouth chewing the cud, using 40,000 to 60,000 jaw movements per day. Saliva contains enzymes to aid in the breakdown of fat and starch. The animal then swallows and the cud passed through an approximately 1 meter esophagus into the rumen (Umphrey and Staples, 1992).

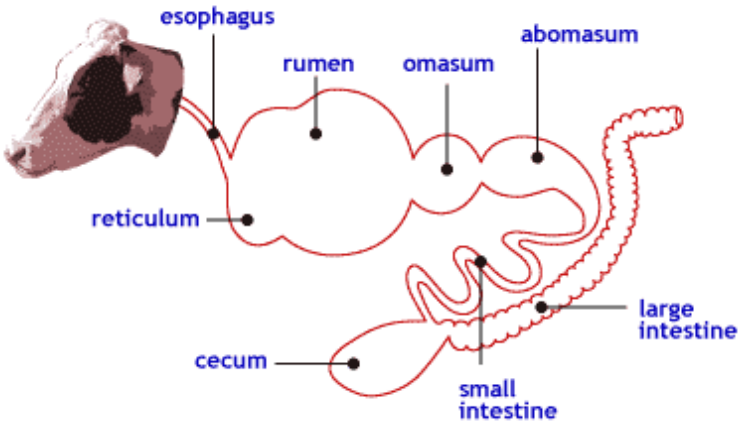


Figure 4: Ruminant digestive system. Sourced from Heinrichs and Jones, 2003.

*Rumen* (Figure 6) – The rumen is the largest (volume of up to 100 L) of the four compartments and is situated on the left side of the animal. The rumen or "fermentation unit" contains a variety of anaerobic microbes ($10^9$ to $10^{10}$ microorganisms per ml) that digest and ferment all feed consumed by the animal. These rumen microbes are highly dependent on vitamins and minerals for metabolic activity. It also acts as a storage vat. Carbohydrates are fermented in the rumen by the microbes to release adenosine triphosphate (ATP), the energy the microbes require for maintenance. Volatile fatty acids (VFA) produced by the fermentation processes are absorbed by the rumen and account for 70% - 80% of the energy source of the host. This absorption is enhanced by the good blood supply associated with the walls of the rumen. Tiny projections on the walls of the rumen, known as papillae, increase the surface area of the rumen and are responsible for continuous exchanges of nutrients, rumination and recycling of urea (Heinrichs *et al.*, 2003). The rumen is rich in water (85% - 90%), operates at a pH of between 6.4 and 7.0 and at a temperature of between 39˚C and 40˚C.

*Reticulum* (Figure 7) – The reticulum is a pouch-like structure, the tissues of which resemble a "honeycomb". It is separated from the rumen by only a small fold of tissues and collectively these two compartments are known as the rumino-reticulum. Dense feed is digested in this compartment. This compartment serves as a catchment area for heavy metal object i.e. wire that might be swallowed with the cut and aids in bringing boluses of feed back into the mouth for re-chewing (Heinrichs and Jones 2003).

*Omasum* (Figure 8) – The omasum, a globe-shaped structure that contains leaf-like tissue is the third compartment. It receives ingesta after it has been mechanically sized by the mouth and teeth that is digested by bacteria and protozoa. Water and electrolytes i.e. potassium and sodium, are absorbed by papillae from the digestive contents in this compartment. Ingesta between the leaves (up to 100 leaves) are subsequently drier than that that found in other compartments. Ingesta now pass to the next compartment (Heinrichs and Jones 2003).

*Abomasum* (Figure 9) – At birth the abomasum is approximately three to four times the size of the rumen and is the main digestive organ in early life. It is the fourth and last compartment of the ruminant stomach. The abomasum is similar to the stomach of mono-gastric animals and is the only compartment that has a glandular lining with many folds to increase the digestive area. Hydrochloric acid and digestive enzymes (pepsin) are secreted into the abomasum for breakdown of proteins to peptides and the rapid lowering of the pH of the arriving ingesta, pH 6, to an operating pH of 2.5 (Heinrichs and Jones 2003).

*Small intestine* (Figure 10) – This is composed of the duodenum, jejunum and the ileum and is approximately twenty times the length of the animal (approximately 40 meter). As most of the digestive processes are completed in the small intestine secretions of the pancreas and gallbladder are deposited here to aid in digestion. The pH is again elevated from pH 2.5 to around pH 7.5. Enzymes degrade proteins to amino-acids, starch to glucose and fat into fatty acids. Small finger-like projections known as villi (Figure 11) absorb these nutrients into the bloodstream and the lymphatic system. The duodenal glands produce and secrete nucleotidase to hydrolyze nucleotides to nucleosides and phosphoric acid, as well as nucleosidase to

hydrolyze nucleosides. Bile, a greenish yellow liquid, passes into the duodenum once secreted by the liver where it emulsifies fat and aids in pH regulation (Heinrichs and Jones 2003).

In newborn calves the microvilli are well developed and the small intestine is permeable to proteins. Ingesting high amounts of good quality colostrum ensures the survival of the mature mucosal epithelial cells (Figure. 11), whereas a lack of colostrum at this stage might reduce epithelial growth, reduce villi size in the jejunum and reduce crypt cell production in the duodenum and ileum. In the first week of life, villus size increases in the jejunum, crypt cell production increases in both the jejunum and duodenum and crypt depth will increase in the small intestine and the colon (Blättler et al., 2001). During this time immunoreactive Ig is localized within the enterocytes, suggesting that gut closure is a mechanism of orderly epithelial cell renewal (Trahair and Robinson, 1989).

*Cecum* (Figure 4) – The cecum, colon and rectum/anus is collectively known as the large intestine. The cecum is the area that joins the small and large intestine. Previously undigested fiber gets broken down here, however the exact significance of the cecum is not known.

*Large intestine* (Figure 4) – This is the final segment for passage of undigested feed. Although some bacterial digestion takes place in the large intestine the primary function is to absorb water.

At birth and for the first weeks of the calf's life the rumen, the reticulum and omasum are undeveloped and non-functional. During this period of the calf's life the abomasum is the largest compartment of the stomach. Fluid feeds such as colostrum and milk pass directly into the abomasum via the esophageal groove (EG). A reflex action, controlled by neural-stimulation, closes muscular folds from the reticulo-rumen at the bottom of the esophagus to form a tube-like structure (the EG) which prevents colostrum or milk from entering the rumen (figure 5). The process of formation of the EG is stimulated by suckling and milk protein (Heinrichs and Jones 2003). Water, however, enters the rumen and is not bypassed (unless consumed directly after milk feed). The rumen remains undeveloped for the entire period of milk

feeding and it is only once calves begin to feed on forage and grains that a microbial population is established in the rumen and reticulum. It is the end products of rumen fermentation that is responsible for the onset of the development of the rumen. The rumen becomes heavier with papillae development which increases the rumen surface and absorption of nutrients (Heinrichs and Jones 2003).

### 2.9.1. ESOPHAGEAL GROOVE (EG)

As soon as a calf swallows, any solids such as starter ration moves down the esophagus and passes through the EG (figure 5) just before it enters the main compartment called the rumen. Prior to weaning, any fluid feed take a different route. Stimuli such as suckling, anticipation to be fed and a variety of additional sensual and neural stimuli cause muscles around the esophageal groove to contract. These muscular contractions close the groove, allowing the fluid to bypass the rumen and flow directly into the abomasum for digestion (Blowey, 1994).

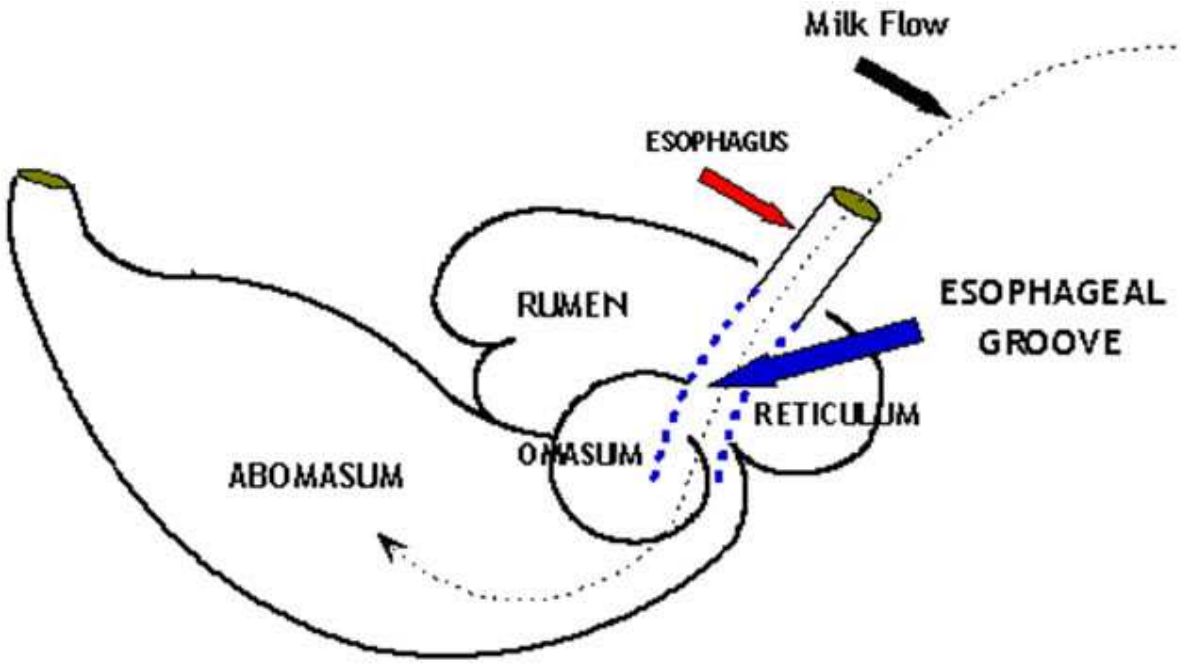


Figure 5: Esophageal groove that forms for a fluid feeding pre-weaning. Source: Merrick Animal Nutrition, Inc.

Figures 6-10 were sourced from Bowen, (2006).

Figure 6: Rumen papillae responsible for nutritional absorption.

Figure 7: Reticular epithelium “honeycomb” with numerous papillae studs.

Figure 8: Omasal leaves with ground ingesta lying between the folds.

Figure 9: Abomasum, the forth stomach, where digestion takes place.

Figure 10: Ruminant small intestine.

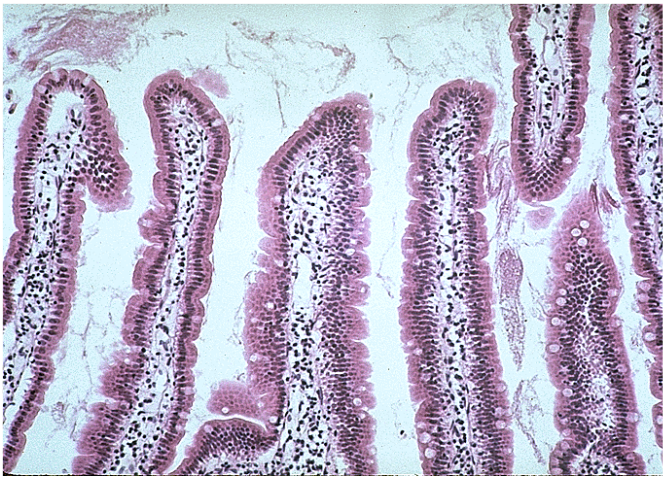

Figure 11: Ruminant duodenal villi. Sourced from: http://www.udel.edu/biology/Wags/histopage/colorpage/csi/csidv1.GIF. Accessed 21/07/2013.

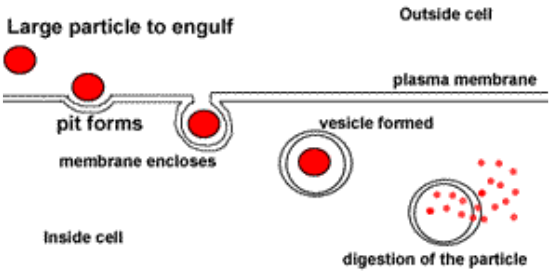


Figure 12: Process of pinocytosis. Sourced from: http://pixshark.com/dimg.php?i=http://www.patana.ac.th/Secondary/science/IBtopics/IBCell%20(01)/Pic1.4/endo2.gif. Accessed 21/07/2013.

### 2.9.2. IMPORTANCE OF MINERALS TO RUMINANTS

There are two general sources of the 17 minerals required daily by cattle i.e. natural feeds (forage and grains) intake and nutritional mineral supplementation. Further to that, macro minerals (Calcium (Ca), Phosphorus (P), Magnesium (Mg), Potasium (K), Sodium (Na), Chlorine (Cl), Sulphar (S), trace minerals (Iodene (I), Ferris (Fe), Cobalt (Co), Copper (Cu), Manganese (Mn), Zinc (Zn), Selinium (Se), Chromium (Cr) and vitamins need to be balanced to achieve optimal reproduction, production, health, immunity and growth (bone development, muscle movement and development of the nervous system). Strategic mineral supplementation is essential during critical periods of agricultural production i.e. 4-6 weeks before joining, 4-6 weeks pre-calving, during periods of stress e.g. post-partum, weaning, disease recovery and periods of convalescence. During these critical periods elevated blood levels of trace minerals as chelates are essential to support production, health and immunity (Grant, 1992).

### 2.9.3. METAL AMINO ACID CHELATED MINERALS

Essential trace minerals are individually and collectively involved in metabolism, reproduction, the immune system, growth, development and repair of tissues. They are needed in minute amounts but their uptake from the GIT might be impaired by antagonists. This has been shown where copper absorption is influenced heavily by the presence of molybdenum, sulphur and iron (Predieri *et al*., 2003)

Chelation is a natural or chemical process, meaning “bringing together’, whereby essential trace minerals are bonded with a chelating agent such as an organic acid i.e. amino acids to form complexes (chelates) and to enable minerals to move freely without the interaction between nutrients and the forming precipitation. Chelates are especially useful in animal nutrition where they reach the plasma intact and separate at the site of action. The significance of chelation includes increased availability of nutrients, prevention of mineral nutrients forming unabsorbable precipitates, reduced toxicity of minerals and the increased mobility of nutrients. Chelated minerals are preferred because their complex structures survive passage through the stomach and small intestines better where it is absorbed into the blood. Non-chelates are easily liberated in the same digestive system. Less chelated trace minerals are excreted in

animal faeces compared to inorganic minerals, thus resulting in less environmental contamination (Wright *et al.*, 2008).

Table 2. Essential trace-minerals, their functions and effects on health of their deficiency (Adapted from Suttle, 2010).

| **Essential trace-minerals** | **Functions** | **Deficiency** |
|---|---|---|
| Selenium *(Se)*<br>*Partner to Vitamin E* | 1. Essential to WBC development and the immune system | 1. Poor growth, infertility, mastitis and retained placentas<br>2. Muscle degeneration and dystrophy |
| Cobalt *(Co)*<br>*Synthesized to Vitamin B12 in the rumen by the rumen bugs* | 1. Required for general metabolism | 1. Anemia, poor health<br>2. Poor appetite, weak calves and poor growth |
| Copper *(Cu)* | 1. Essential for collagen synthesis of joints<br>2. Essential for ovulation | 1. Infertility<br>2. Impaired immunity<br>3. Anemia, impaired central nervous system |
| Zinc *(Zn)*<br>*Partner to Vitamin A* | 1. Essential to metabolism of EFA's and Vitamin A<br>2. Plays a role in cell division functions i.e. wound healing, hair growth and immunity | 1. Poor growth<br>2. Low sperm count, fertility<br>3. Scours<br>4. Immune deficiency |
| Iodine *(I)* | 1. Controls metabolism via the thyroid gland | 1. Fetal death, re-absorption, still birth<br>2. Birth of hairless offspring |
| Manganese *(Mn*) | 1. Essential to metabolism of fat and carbohydrates<br>2. Required for growth, reproduction and skeletal development | 1. Impaired growth and bone development<br>2. Gait abnormalities in neonates |
| Chromium *(Cr)*<br>*Impacts on stress in animals* | 1. Plays an important role in glucose and insulin metabolism | 1. Impaired immune function, glucose intolerance<br>2. Weight loss |

### 2.9.4. IMPORTANCE OF VITAMINS TO RUMINANTS

Vitamins are essential to the metabolism of ruminants and serves as antioxidants. These are organic compounds divided into two groups i.e. fat soluble (A, D, E, K) and water soluble ($B_1$, $B_2$, $B_6$, $B_{12}$, Biotin, Niacin, Folic acid, Vitamin C, Pantothenic acid, Choline and thiamin). Vitamins are normally adequately supplemented by the feed but it is beneficial to supplement vitamins (especially B-vitamins and C-vitamins) during periods of stress as colostrum is the neonate's primary source of vitamins and minerals (Quigley and Drewery, 1998). Vitamins K and the B vitamins are synthesized in the rumen by microorganisms. Fat soluble vitamins may occur in plant tissues as a pro-vitamin (precursor) e.g. carotene (1 mg) that is converted to Vitamin A (400 IU) by ruminants. There are no known pro-vitamins for the B-vitamins.

Table 3a. Essential fat soluble vitamins, their functions and deficiencies (Adapted from Suttle, 2010).

| Vitamins – fat soluble | Function | Deficiency |
|---|---|---|
| Vitamin A | 1. Essential for eyesight and the stability of all membranes<br>2. Necessary for healthy skin and teeth | 1. Poor growth and development<br>2. Impaired resistance to infections<br>3. Weak calves |
| Vitamin $D_3$<br>*Synthesized in the skin after exposure to sunlight* | 1. Essential to the absorption of calcium and phosphorus from the GIT for bone calcification | 1. Poor growth<br>2. Impaired immunity and fertility |
| Vitamin E<br>*Selenium assists in the absorption of Vitamin E* | 1. Vital for muscle development<br>2. Important for fertility and fetal growth | 1. Muscular dystrophy<br>2. Metritis<br>3. Retained placenta and ovarian cysts |
| Vitamin K (MSB)<br>*Produced by the gut bacteria* | 1. Essential for the blood clotting mechanism | 1. Retarded wound healing<br>2. Retarded growth |

Table 3b. Essential water soluble vitamins, their functions and deficiencies (Adapted from Suttle, 2010).

| Vitamins – water soluble | Function | Deficiency |
|---|---|---|
| Vitamin $B_1$ - *Thiamine* | 1. Essential for energy production | 1. Deterioration of heart, muscle, brain and nerves |
| Vitamin $B_2$ - *Riboflavin* | 1. Crucial in the liver to formation of enzymes<br>2. Essential in oxidation<br>3. Essential in the metabolism of proteins, fat and carbohydrates | 1. Lack of growth and milk production |
| Vitamin $B_3$<br>*Necessary for general health* | 1. Crucial in the liver to formation of enzymes<br>2. Essential in the metabolism of glucose and carbohydrates | 1. Changes in the GIT resulting in scours |
| Vitamin $B_5$ – *Calcium Pantothenate*<br>*Necessary for general health* | 1. Essential in the metabolism of a, fat and carbohydrates | |
| Vitamin $B_6$ - *Pyridoxine* | 1. Metabolism of Mg, Zn, sugar and EFA (Omega 3, 6) | 1. Inflammatory and skin disease<br>2. Disorders of heart, immune system |
| Vitamin $B_9$ – *Folic acid* | 1. Vital for the working of the central nervous system and development of the embryo | 1. Cerebrocortical Necrosis<br>2. Undeveloped embryo |
| Vitamin $B_{12}$<br>*Synthesized in rumen from cobalt by rumen bugs. Supplement calves as rumen in non-functional* | 1. Vital for the working of the central nervous system and post-partum development | 1. Small unthrifty calves<br>2. Nervous disorders |
| Vitamin H – Biotin | 1. Necessary for health of skin and hoof | 1. Dermatitis, cracking of skin and hooves |
| Vitamin C<br>*Good for stressed animals* | 1. Assists with detoxification and excretion of toxins and heavy metals | 1. Excess reduces availability of trace-minerals and amino acids |

*Prediction* - Vitamins are an essential part of the formulation of the Veterinary Investigational product 2 (IVP 2). Antioxidant vitamins such as vitamin A, E have been shown in previous research to enhance the immune function and reduce free radical harm (Politis *et al.*, 1995). This suggests a synergistic effect between vitamins and nucleotides. The synergistic effect of the vitamins as well as the other nutrients

discussed and contained in Investigational Veterinary Product 2 could make IVP group 2 perform better than group 3 in this study whilst group 3 should outperform the other study groups.

#### 2.9.4.1 IMPORTANCE OF AMINO ACIDS TO RUMINANTS

Amino acids are organic compounds that aid cells in keeping their structure as it provides cells with the necessary building materials. These essential amino acids (EAA) cannot be synthesized by the body and need supplementation. In the event of a lack of amino acids the body will scavenge muscle (proteins) to get amino acids. These EAA include lysine and methionine. In young calves they are essential for metabolism of essential fatty acid (EFA) i.e. omega 3 and omega 6 as well as neonatal growth. A deficiency will impair the immune system as amino acids play an important role in the activation of T lymphocytes, B lymphocytes, natural killer cells, macrophages and the production of antibodies. (Quigley and Drewry, 1998; Li *et al.*, 2007). There is researched evidence to confirm that the supplementation of essential amino acids to animals and humans with malnutrition and infectious disease increases the immune status hence reducing morbidity and mortality (Li *et al.*, 2007).

### 2.9.5. IMPORTANCE OF PREBIOTICS TO RUMINANTS

Prebiotics are non-digestible fermented food ingredients such as *galacto-oligosaccharides, fructo-oligosaccharides* and *inulin*, that stimulate the activity and/or growth of the internal gut *Bifidobacteria* and *Lactic acid bacteria (lactobacilli)* so as to benefit the host (Gibson and Roberfroid, 1995). As a result, they increase healthy digestion and increase resistance to GIT pathogens. Prebiotics are derived from indigestible carbohydrate fibers called oligosaccharides. The main sources of oligosaccharides include fruits, legumes and whole grains. When functioning optimally the host body can extract, retain and transport nutrients such as vitamins and minerals much more efficiently due to the prebiotic effect where prebiotics supply the energy necessary for bacteria to function properly (Macfarlane, Steed and Macfarlane, 2008).

## 2.10. GLUCOCORTICOIDS (i.e. CORTISOL)

*Stress* – According to fact sheet no. 6 of Farm Animal Welfare Education Centre (2013) stress is *"A set of physiological and behavioral changes elicited by aversive stimuli"*. Every animal, especially producing farm animals, will experience some level of stress during its lifetime. is perceived to be a reflex reaction to adverse or stressful conditions that generally decreases an animal's ability to produce to performance standards, might render it diseased or cause morbidity and/or mortality at individual and/or herd level (Rostagno, 2009). Stress can be acute or chronic and the latter is more likely to be experienced by animals reared under intensive conditions (Dantzer and Mormede, 1983). Stress can reduce the immune function as well as performance of the animal i.e. decreased feed intake, inhibition of oxytocin release reduced fertility and growth etc., dramatically. Chronic stress is very specific in its effect (diseases) of the immune response. The affected diseases include *Salmonella spp.* and *Pasteurella spp.*, both of which causes some of the major calf syndromes found at the study site (Broom and Johnson, 1993).

An objective measure of stress is related to plasma glucocorticoid (Cortisol) levels as well as behavioral changes of the animal (Koolhaas *et al*., 2011). Glucocorticoids (GCs) are essentially a class of naturally produced steroid hormones. They are produced by the *zona fasciculata*, the second of three layers comprising the outer portion of the adrenal cortex. The Oxford dictionary (second edition, 1989) defines GCs /glu·co·cor·ti·coid/ (-korʹt-koid), as "any of a group of corticosteroids (e.g. hydrocortisone or cortisol) which are involved in the metabolism of carbohydrates, proteins, fats and have anti-inflammatory and immunosuppressive activity".

The response by an animal to stress is regulated by two elements i.e. Hypothalamic-Pituitary-Adrenal axis (HPA) and the Sympatho-Adreno-Medullary system and commonly occurs in response to a stress reaction and/or a low level of circulating blood GCs. Hence the serum cortisol (hydrocortisone) level represents a measure of the animal's inherent stress level. Cortisol secretion is controlled by the central nervous system. Once the animal is stressed the *hypothalamus* will release hypothalamic peptide corticotropin-releasing hormone (CRH) which in turn will stimulate the release of adrenocorticotropic hormone (ACTH) from the *anterior pituitary*. Cortisol is then released from the adrenal cortex in response to the ACTH. The release of cortisol during periods of stress may have a significant effect on a

number of physiological pathways related to disease response, however, GCs contribute significantly to the maturation of organs i.e. lungs, in the prenatal and neonates of various species of animals Hence, GCs are commonly recommended for their potent anti-inflammatory and immunosuppressive properties but excessive GC levels may lead to the inhibition of bone formation, suppression of calcium absorption and it may retard wound healing (Schmidt *et al*., 2004).

In 1979, Johnston and Oxender investigated the effect of injecting ACTH into post-partum calves on immunoglobulin absorption. They found that injecting glucocorticoids elevated the serum Ig concentrations of calves compared to calves that did not receive ACTH. The study further suggested that the ACTH enhanced Ig serum concentrations additionally prevented premature intestinal closure. Studies demonstrated that calves which did not receive injections of ACTH were born with higher cortisol serum concentrations showed improved Ig absorption compared with calves born with low serum cortisol concentrations (Chen *et al*., 1999).

The reason for measuring the plasma cortisol levels of all the calves is, thus, to determine the amount of stress each calf experienced from birth until the end of the study to explain poor growth spike/s which might occur as a result of a high stress experience of an individual calf and to determine at what rate the cortisol increased or decreased through the course of the study. The main reason for the group 4 – parenteral glucocorticoid study group of this study was to determine, when compared to the control group, whether or not the parenteral glucocorticoids could delay the onset of natural gut closure post-24 hours, hence potentially increasing the absorption of the total IgG-mass over the prolonged absorption opportunity. This may also allow the absorption of more resident gut *E.coli* spp. and/or *Salmonella* spp. pathogens. However, the greater volumes of colostrum, containing as it does various maternal leukocytes, oligosaccharides and peptides with antimicrobial properties, that is ingested by the calf should kill these pathogens (Quigley, 2002; Progressive Dairyman, 2013).

## 2.11. APPARENT EFFICIENCY OF ABSORPTION (AEA)

The apparent efficiency of absorption (AEA) indicates how much of the IgG fed to a neonate actually enters the calf's bloodstream and rarely exceeds 35% (Arthington *et*

*al.*, 2000). This can be measured on-farm to determine whether or not the different factors involved in colostrum management are effective i.e. whether passive transfer of Ig was successful.

The determination of AEA is a means of balancing the variability in actual blood Ig concentrations by accounting for differences in plasma volume as related to body weight (BW). By estimating the mass of Ig ingested from the quantity and quality of colostrum and from determining the plasma Ig concentration following Ig absorption as well as the plasma volume, one can calculate the efficiency of IgG absorption. The AEA reduces rapidly post-natally as shown in Figure 13 below.

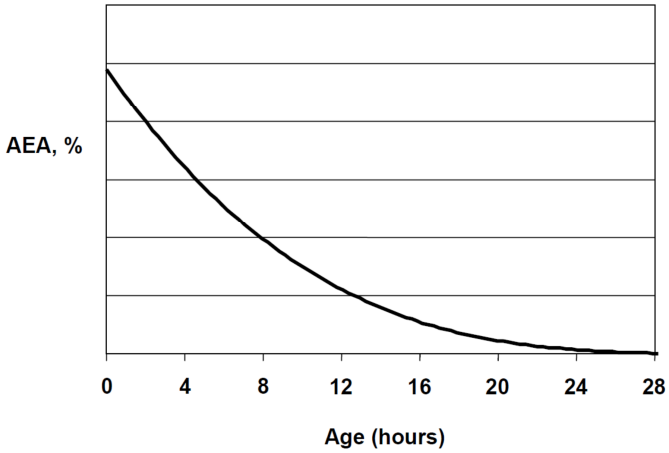


Figure 13: Changes in AEA of IgG with increasing age of calves. (Sourced from Quigley, 2007; Gvozdic et al., 2008).

The percentage AEA can be calculated by using the following formula:

$$\% \text{ AEA} = [(\text{plasma IgG (g/L)} \times \text{PV (L)} \times 100) / \text{IgG intake (g)}]$$

Where,

- IgG (Plasma IgG in g/L or mg/ml is measured by blood sample from the calf), this value is a measure of the degree of passive immunity; it however does not indicate efficiency of the IgG absorbed prior to gut closure.
- PV (plasma volume in g/L or mg/ml is estimated from Table 4)
- IgG intake = g/L (Colostrum quality) x volume colostrum ingested (L)

Table 4, as compiled by Quigley and Drewry in 1998, illustrates estimates of the PV as approximately 9.1% of body weight; (Quigley 2002) values for Holstein and Jersey calves at various body weights, and ages (hours) post-colostrum feed. This is a standard reference table (Quigley and Drewry, 1998) for AEA% calculation.

Table 4: Estimated plasma volume (PV) values of calves at various body weights and ages post colostrum feed (Adopted from Quigley *et al*. 1998).

PLASMA VOLUME IN CALVES

Estimates of plasma volume (PV) in Holstein and Jersey calves

| BW | Age of Holsteins in hours | | | | | | Age of Jerseys in hours | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| (Kg) | 22h | 23h | 24h | 25h | 26h | | 22h | 23h | 24h | 25h | 26h |
| | | | | | | ml | | | | | |
| 20 | … | … | … | … | … | | 1604 | 1719 | 1835 | 1950 | 2065 |
| 25 | 2279 | 2394 | 2509 | 2625 | 2740 | | 1912 | 2028 | 2143 | 2258 | 2374 |
| 30 | 2587 | 2702 | 1818 | 2933 | 3048 | | 2221 | 2338 | 2452 | 2567 | 2682 |
| 35 | 2895 | 3011 | 3126 | 3242 | 3357 | | 2529 | 2645 | 2780 | 2875 | 2991 |
| 40 | 3204 | 3319 | 3435 | 3550 | 3665 | | … | … | … | … | … |
| 45 | 3512 | 3628 | 3743 | 3859 | 3974 | | … | … | … | … | … |
| | | | | | | % BW | | | | | |
| 20 | … | … | … | … | … | | 8.03 | 8.94 | 9.84 | 10.75 | 11.65 |
| 25 | 9.11 | 9.58 | 10.04 | 10.50 | 10.98 | | 7.58 | 8.31 | 9.03 | 9.75 | 10.48 |
| 30 | 8.62 | 9.01 | 9.39 | 9.78 | 10.16 | | 7.28 | 7.88 | 8.49 | 9.09 | 9.69 |
| 35 | 8.27 | 8.60 | 8.93 | 9.26 | 9.59 | | 7.07 | 7.58 | 8.1 | 8.62 | 9.13 |
| 40 | 8.01 | 8.30 | 8.59 | 8.88 | 9.16 | | … | … | … | … | … |
| 45 | 7.81 | 8.06 | 8.32 | 8.57 | 8.83 | | … | … | … | … | … |

Correlated for 10-min sampling by multiplying estimated PV by 0.906

Example of calculation:

A 45 kg Holstein calf with an IgG titre of 8 g/L (measured in lab) has an estimated PV of 3.743 L at 24 hrs after consuming colostrum of quality 100g IgG/L (table 4, page 61):

AEA% = [(8 g/L x 3.743 L) / 100 g IgG] x 100

= 29.944 %

~ 30%

The calf used in this example has a good AEA% as research suggests an AEA% of approximately 35% being excellent for maternal colostrum (Kehoe *et al.*, 2007).

The following formula, adapted from Kehoe *et al*, (2007) can be used to calculate the plasma Ig concentration of the neonate on-farm:

$$\text{Plasma IgG (g/L)} = \frac{\text{Colostrum quality (g/L) x colostrum intake (L) x (AEA)}}{\text{plasma volume (PV)}}$$

Because our atmosphere is essential to sustained life on earth, the carbon footprint of dairy production is under debate at regional, national and international levels. Many of these debates revolve around livestock's contribution to climate change and its alleviation as virtually every step of livestock production contributes directly and/or indirectly to climate change (Mitloehner, 2012). These anthropogenic changes in the climate are likely to have a serious impact on the environment as we know it.
The following four definitions are adapted from Flysjo, (2011).

*Carbon footprint* – is the total amount of greenhouse gas (GHG) emissions associated with a specific product along its supply chain (SC). It is expressed in kilograms (kg) carbon dioxide equivalent ($CO_2$-eq.) of fat and protein corrected milk (FPCM). (table 5).

*$CO_2$-equivalent emission* – is the amount of $CO_2$ emitted that would cause similar time integrated radiative forcing over a given time as an emitted amount of GHG or mixture of GHG. $CO_2$-eq. = GHG x Global warming potential (GWP).

*Global Warming Potential (GWP)* – is an indicator that reflects the relative effect of a GHG in terms of climate change over a specific time i.e. 100 years compared to the same mass of $CO_2$.

*Fat and protein corrected milk (FPCM)* – is milk of which the fat has been corrected to a standard of 4% and protein corrected to a standard of 3.3%. The purpose of this is to be able to compare milk from any animal and/or breed on a common basis.

The total contribution of global dairy activities i.e. calf/replacement stock rearing, milk production, processing and transport to the total anthropogenic emissions is estimated to be 2.4 $CO_2$-eq. per kg of FPCM at farm gate level (FAO, 2010). This represents approximately 2.7% of the total. Of this, methane ($CH_4$) is the largest contributor at <50%, nitrous oxide ($N_2O$) between 30% - 40% and $CO_2$ makes up the rest of the total emission. Methane is mainly derived from enteric fermentation in the rumen, $N_2O$ from the use of fertilizers and manure in the soil and $CO_2$ is related to respiratory processes and the manufacture and use of fertilizer to improve crop production. A production system that includes a combined meat and milk production strategy, like the trial site, is more efficient at achieving a low GHG emission per unit (Mitloehner, 2012).

Although $CH_4$ production is a characteristic of all ruminants, it can be reduced by altering the animals' type of diet (more starch than forage) to increase FCR, to achieve critical mating mass earlier and to calve well grown heifers earlier than the norm at 22-24 months of age hence reducing the number of followers on the farm to

achieve the same net results (growth and milk production targets) with fewer resources. Increased efficiency also results in a reduced waste output. Reduced herd numbers due to increased productivity ultimately implies less land required for forage and less fertilizer used which will result in a saving of $N_2O$ and $CO_2$ as well. Besides a reduced carbon footprint, these biological efficiencies can also correlate with economic performance (Dolle and Baptiste, 2011) see figure 14.

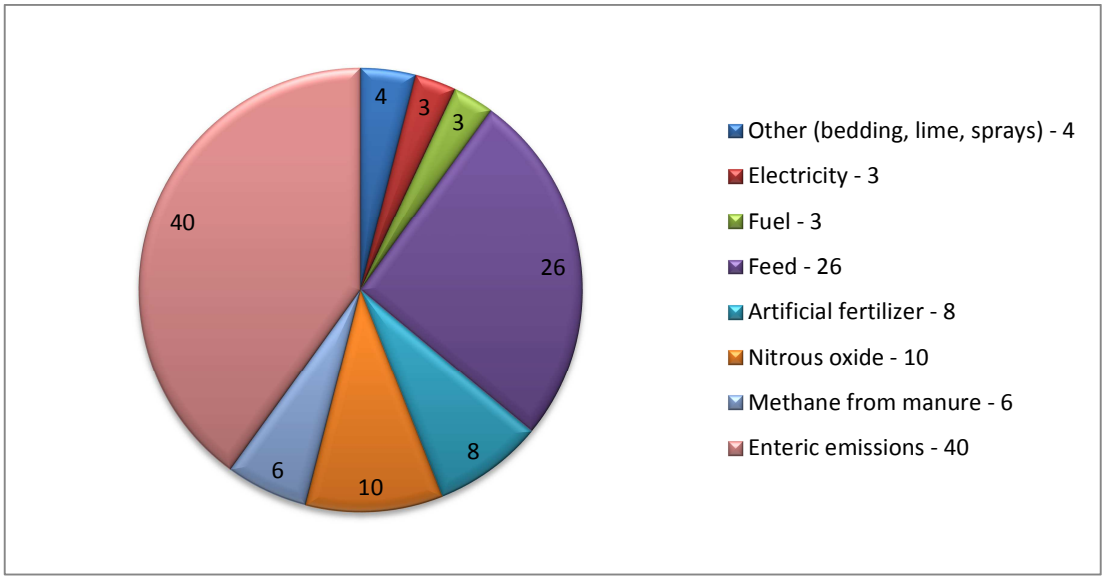


Figure 14: Breakdown of the average farm emissions adapted by source (Dolle and Baptiste, 2011).

Table 5: Past and current concentrations of important greenhouse gases.

| Gas | Pre-industrial concentration | Current concentration | GWP |
|---|---|---|---|
| Carbon Dioxide ($CO_2$) | 277 ppm | 382 ppm | 1 |
| Methane ($CH_4$) | 600 ppb | 1,728 ppb | 23 |
| Nitrous Oxide ($N_2O$) | 270-290 ppb | 318 ppb | 296 |
| ppm = parts per million, ppb = parts per billion, GWP = Global warming potential (years as a factor of CO2) | | | |

Source: WRI (2005).

*Full Blood Count (FBC)* - A full blood count (FBC), also known as a complete blood cell count (CBC), full blood exam (FBE) or a blood panel by laboratory evaluation of

bovine serum may be helpful in determining disease, infection and/or prognosis. Several components and features of blood are routinely measured i.e. red blood cells (RBC) which carry oxygen, white blood cells (WBC) which fight infection, platelets which assist in blood clotting, haemoglobin which is the oxygen-carrying protein of RBCs and haematocrit which is the proportion RBCs to plasma in the blood. Abnormal increases or decreases of these cell counts may indicate an underlying condition.

To avoid *in vitro* cellular changes it is important to properly collect, handle and store all blood samples. Peripheral blood is most commonly collected from the ruminant jugular or coccygeal (tail) veins and it is advised to avoid the use of the subcutaneous abdominal (milk) vein for blood collection as hematomas and abscesses may easily form post-collection (Jones and Allison, 2007). Ideally, animals should be calm and well restrained at the time of the sample collection to avoid abnormalities in the FBC as well as to reduce any haemolysis due to traumatic collection procedures. Whole blood is best collected by a rapidly and accurately placed 18 to 20 gauge, 1 to 2 inch needle. The blood is collected directly into an evacuated tube where ethylenediaminetetraacetic acid (EDTA) is used as an anticoagulant before gently inverting the sample four to five times to ensure proper mixing of the blood sample and the EDTA. In the event that the laboratory analysis is delayed, the samples should be cold-stored at approximately 4˚C for up to 24 hours. Platelet evaluation should best be done between 4-6 hours post-collection (Jones and Allison, 2007).

Table 6: Normal complete blood cell counts for cattle. Sourced from Jones and Allison, 2007.

| FBC | Cattle |
|---|---|
| RBC (x $10^6$ cells / µL) | 5.0 - 10.0 |
| WBC (µ/L) | 4,000 - 12,000 |
| Platelets (x $10^3$) | 100 - 800 |

## 2.13. CONCLUSION

Performance in the modern livestock industry is crucial as is shown where different parameters such as environmental factors, external stressors, genetics as well as various nutrient feed additives trigger and/or influence growth and development of dairy cows. Imbalances in specific feed nutrients can result in poor performance of an animal but this is a variable that can be monitored and controlled. Farmers must realize that optimized feed management not only improves farming yields but in addition this should be seen as an opportunity to impact positively on the environment.

# 3. CHAPTER 3 - RESEARCH METHODOLOGY

## 3. INTRODUCTION

The study protocol for this study was approved by the University of South Africa's College of Agriculture and Environmental Sciences (CAES) Ethical / Research Committee under application number 2013/CAES/038.

Research has indicated the possibility of increasing dairy production parameters by the addition of nutritional supplements to the neonate calf diet as well as delaying natural gut maturation in order to increase IgG absorption (Hough et al., 1990; Sangild, 2003).

The focus of this study was on neonate dairy calf immunity (IgG absorption, AEA% and gut closure), their management and the potential to increase production parameters (weight gain in kg, ADG, FCR) (Hough et al., 1990; Sangild, 2003).

A single large scale operator which uses dairying methods representative of large scale dairy farms in South Africa was chosen for this trial (Arthington, 2000; Fourie *et al.,* 2006; Murphy, 2014). This farm has comprehensive records, adequate facilities and the owner is interested and willing to participate.

A quantitative Complete Randomized Sampling Design (CRD) was chosen for this study (Yates et al., 2008). The outcome will generate awareness that will directly influence the rearing of dairy calves for optimal production.

## 3.1. STUDY AREA

*Geographical area* - The on-farm research study was conducted at "Witkop Dairy 2", one of three neighboring commercial dairy farms operated by dairy manager Mr. Simon Lavery as one enterprise and owned by Dr. Dawie Barnard (BVSc). The farm is situated in the district of Meyerton in the province of Mpumalanga, South Africa. All replacement heifers, approximately 1900♀+1800♂/annum, for the entire enterprise are reared at this single site. The farm GPS co-ordinates are: S 26˚30'09.2" E 28˚05'17.1". All necessary permission pertaining to this study was granted by the owner and farm manager before initiation of the study.

## 3.2. STUDY ANIMALS

### 3.2.1. ANIMAL SPECIES AND SAMPLE SIZE

*Study animals and sample size* - twenty eight (n=28) study animals (Holstein Friesland X Jersey dairy calves) of mixed gender were assigned to four treatment groups of seven calves each. All calves originated from a single commercial dairy enterprise situated in the Meyerton district of Mpumalanga, South Africa. To minimize temperature and seasonal variations, the research subjects were selected that were all born within 18 hours of each other. The type of farming operation and sample size of the various control and experimental groups used in this study is similar to published studies (Arthington, 2000; Keller *et al*., 2001; Jaster, 2005; Fourie *et al.,* 2006; De Paula Vieira *et al.*, 2010; Mokhber-Dezfooli *et al*., 2012; Gaillard *et al*., 2014; Murphy *et al*., 2014).

Although the sample size is small it was deemed appropriate as at least 7 international and 1 South African references could be cited where sample sizes between n=18 and n=30 were used for dairy calf studies. The sample size of n=28 has a confidence level of 95%, confidence interval of 18, power of 75%, margin of error of 16.04% and a base population of 250 000 dairy heifer calves (Agricultural, Forestry and Fisheries – National Department SA) (Raosoft). Various researchers have used similar sample sizes (Arthington, 2000; Keller *et al*., 2001; Jaster, 2005; Fourie *et al.,* 2006; De Paula Vieira *et al.*, 2010; Mokhber-Dezfooli *et al*., 2012; Gaillard *et al*., 2014; Murphy *et al*., 2014).

*Identification* - Each calf received an Aussie ear tag as soon as it was removed from the dam post-partum. $G_1$ received green ear tags, $G_2$ received yellow ear tags, $G_3$ received orange ear tags and G4 received white ear tags.

*Group allocation* - A Complete Randomized Sampling Design (CRD) was used with a random sequential allocation strategy used to group the calves i.e. 1 calf was allocated to each of the 4 investigational groups as they were born i.e. ($G_1$ (9) indicates the treatment group as $G_1$ and (9) indicates that the $9^{th}$ calf born on the day of evaluation was allocated to that specific group. This allocation strategy is demonstrated in Table 7 below.

Table 7. Allocation of test animals to the study groups.

| Group 1 | Group 2 | Group 3 | Group 4 |
|---|---|---|---|
| $G_1$(1) | $G_2$(2), | $G_3$(3) | $G_4$(4) |
| $G_1$(5) | $G_2$(6) | | $G_4$(8) |
| $G_1$(9) | $G_2$(10) | $G_3$(11) | $G_4$(12) |
| $G_1$(13) | | $G_3$(15) | $G_4$(16) |
| $G_1$(17) | $G_2$(18) | $G_3$(19) | $G_4$(20) |
| | $G_2$(22) | $G_3$(23) | $G_4$(24) |
| $G_1$(25) | $G_2$(26) | $G_3$(27) | |

*Inclusion criteria* – Only healthy calves from unassisted birth's that were witnessed at birthing were enrolled into the study. Cows were not allowed to nurse the calves and calves were not allowed to have circulating immunoglobulin hence the blood test at $T_0$.

### 3.2.2. GENERAL MANAGEMENT

*General management* - This commercial "share milking" dairy runs a Holstein Friesland X Jersey dairy cow herd as their main milking herd. The milking herd consists of +/- 4000 lactating animals of various lactation numbers, and is managed within a seasonal calving pattern.

The cows are bred by one-time artificial insemination only. All oestrus and breeding activities are accurately recorded. The peak calving season is between 1 March and 30 April annually, with 55% of the cow herd calving down in this period. The secondary calving season is between mid-July and August annually, with 45% of the cow herd calving down in this period.

All calves that were selected for enrollment into the study were born between the 5$^{th}$ and 6$^{th}$ August 2013. The births were uncomplicated and unassisted so as to minimize the effect of stress (cortisol) on the results of this study. All cows of which the calves were selected were certified healthy by a veterinarian. Both heifer and bull calves are reared on this farm. Bull calves are feed-lotted after weaning.

### 3.2.3. DISEASES AND TREATMENTS

*Diseases and treatments* - Foot and mouth disease virus (FMDV) is not endemic to this area and hence the herd has naïve status with regards to this disease. The herd receives annual vaccinations as well as booster vaccinations against Bovine Viral Diarrhea Virus (BVDV) with a multivalent vaccine from MSD whose trade name is Respiravax®. This vaccine protects against BVDV, Infectious Bovine Rhinotracheitis, Para influenza type 3, Bovine Respiratory Syncytial Virus and the 2014 calving season batch number was BNRM01. Other vaccines such as Scourguard 4KC® from Zoeitis provided protection against scours while Supavax from MSD provided protection against multiple Clostridial diseases. Calf scours are the most prevalent of the calf syndromes on this farm with an incidence of 13% in 2012 and 15% in 2013 (Witkop Dairy records). The cumulative mortality rate at this rearing station is approximately 7.5% / annum. *Salmonella* spp., *E.coli* and *Eimeria* spp. are the most prevalent diagnosed causative pathogens. In 2012 and during the same calving season, 70 heifer calves (from 4000 cows mated) were reported to have died because of these pathogens. These were diagnosed by a qualified veterinarian and confirmed by laboratory analysis of dung samples analyzed by Onderstepoort Veterinary Institute (OVI) in Pretoria. Because of the prevalence of multiple pathogens and a high calf mortality rate it is practice on this farm to single treat all neonates with Resflor (MSD), an antimicrobial combination of Florfenicol 300 mg/ml and Flunixin Meglumine 16.5 mg/ml as methaphylactic treatment to pneumonia as well as Potencil (Virbac), an antimicrobial combination of Amoxicillin and Colistin as methaphylactic treatment against GIT-related pathogens. All study calves were and remained untreated.

### 3.2.4. FEEDING OF THE STUDY ANIMALS

Each calf was removed from its dam immediately post-partum after it was cleaned and dried by the dam. It was then sexed, weighed, marked by insertion of an Aussie ear tag and penned in its study group on bedding of wood shavings. The cows were not allowed to nurse their calves. Two hundred liters of colostrum was collected from 27 freshly calved cows (multi- lactation), of the same herd, after the afternoon milking on the day (12 hours) before the study commenced. Colostrum quality (SG) was pre-determined by KRUUSE colostrometer (Cat.no: 290855) reading after

collection by an automated milking machine. Each colostrum sample (250 ml) was collected in a graduated measuring cylinder and allowed to cool down to room temperature (20˚C) before measurement. A reading in mg/ml was recorded at the point of buoyancy. It was then pooled once the quality was confirmed as good colostrum. The bulk drum containing the pooled colostrum was then stored in a walk-in fridge. The aim was to feed pooled colostrum of quality ≥50 g/L of IgG per feeding.

A pooled colostrum meal was then fed to each individual calf to ensure an equal colostrum feed/calf, using a polyethylene plastic esophageal tube feeder. At each time point each calf received the same pooled colostrum. Each calf in each study group received three pooled colostrum feedings of 1.50 L/feeding at before $T_6$, $T_{12}$, $T_{18\text{-}24}$ as well as its IVP treatment. Group 1 received in addition to the first three pooled colostrum treatments, one more pooled colostrum treatments at $T_{30}$ to confirm gut closure. Group 4 received in addition to the first three pooled colostrum treatments, three more pooled colostrum treatments at before $T_{30,}$ $T_{36}$ and $T_{42}$ to determine the possible extension of "gut closure" by the addition of a parental glucocorticoid.

The four groups received the following individual investigational veterinary product (IVP) treatments:

$G_1$: Negative control group received pooled colostrum only (4 feedings)
$G_2$: Investigational veterinary product 1 (IVP1) - the calves received colostrum (3 feedings) supplemented with oral Byboost Calf (IVP1) at 15 ml/feeding x 2 feedings mixed into the colostrum feeding.
$G_3$: Investigational veterinary product 2 (IVP2) – the calves received colostrum (3 feedings) supplemented with oral nutritional nucleotides at 15 g/feeding x 2 mixed into the colostrum
$G_4$: Investigational veterinary product 3 (IVP3) – the calves received colostrum (6 feedings) supplemented with Dexamethasone (trade name - Kortico) at 0.2mg/kg or 4ml/calf as a once-off intramuscular injection into the gluteus medius at $T_1$.

## 3.3. EXPERIMENTS

### 3.3.1. INVESTIGATIONAL VETERINARY PRODUCTS (IVP)

#### 3.3.1.1. IVP1 – ORAL SUPPLEMENTATION

Table 8: Composition of IVP 1 (Source: Ashkan Animal Health / Product Label).

| Composition (per litre) | | | |
|---|---|---|---|
| Vitamin A | 2000,000iu | Vitamin K (MSB) | 400mg |
| Vitamin $D_3$ | 100,000iu | Iodine | 1,000mg |
| Vitamin E ( as α-tocopherol acetate) | 14,000mg | Selenium | 350mg |
| Vitamin $B_1$ | 2,700mg | Cobalt | 1,600mg |
| Vitamin $B_2$ | 1,400mg | Copper (MAAC) | 5,000mg |
| Vitamin $B_3$ | 1,600mg | Manganese (MAAC) | 2,500mg |
| Vitamin $B_5$ (Cal. Pantothenate) | 2,600mg | Zinc (MAAC) | 9,000mg |
| Vitamin $B_6$ | 2,000mg | Lysine Hydrochloride | 3,600mg |
| Folic acid | 1,200mg | Methionine | 4,200mg |
| Vitamin $B_{12}$ | 6,200mg | Amino acid range | 2,0000mg |
| Vitamin C | 1,500mg | Essential Fatty acids | 1,000mg |
| Vitamin H (Biotin) | 3,000mg | Nucleotides | 5,000mg |
| **On a high energy base** | | Prebiotics | 2,000mg |
| *MAAC - Metal Amino Acid Chelate*<br>*MSB - Menadione Sodium Bisulfite* | | | |

The IVP1 is a ready-to-use liquid feed supplement for calves. IVP1 was administered orally via the colostrum/milk feeding at a rate of 15 ml/calf/colostrum meal for two consecutive colostrum meals. The composition is tabled in Table 7.

#### 3.3.1.2. IVP2 - NUTRITIONAL NUCLEOTIDES

The IVP2 contains pure nutritional nucleotides only. It was mixed into the colostrum meal and administered orally at a rate of 15g/calf/colostrum meal (Rx 2). The nucleotides used in both IVP1 and IVP 2 are of similar type/composition, origin and inclusion rate.

#### 3.3.1.3. IVP3 - GLUCOCORTICOSTEROIDS

The IVP3 used during the study contains dexamethasone at 2 mg/ml and was administered at a rate of 0.2 mg/kg or 4 ml/calf via the intramuscular route as a once-off treatment directly (+/- 30min) post-partum. Details of this procedure relate to

regulations No: 83/107 (Act 101/1965), Schedule (S4) and the details of the batch used in this study was: number 81728, Manufacturing date: 11/2012, Expiry date: 11/2013.

#### 3.3.1.4. BLOOD COLLECTION

Blood serum samples were collected into 10 ml yellow-topped tubes for cortisol and Ig titration evaluation at times $T_0$, $T_{12}$, $T_{24}$, $T_{27}$, $T_{30}$, $T_{33}$, $T_{36}$, $T_{42}$ ±10min and vial numbers were blinded. The first blood samples (5 ml/sample) were collected by veni-puncture of the calf jugular vein (left and right side) at $T_1$ but before the initial colostrum feeding. Invasive procedures and sample collection was supervised by a qualified veterinarian. The evacuated yellow top blood samples tubes were centrifuged for 10 minutes at 4000 rpm and then stored (upright) at 5˚C until being transported to IDEXX laboratory, around 24 hrs post-collection, to be processed for serum harvesting and immunoglobulin determination. This was done to confirm the IgG serum titre pre-colostrum and to measure the serum cortisol level per calf.

Further blood sampling (5 ml/sample) was done at specific intervals ($T_{12}$, $T_{24}$, $T_{27}$, $T_{30}$, $T_{33}$, $T_{36}$ and $T_{42}$ ±10 min) thereafter, to determine the following parameters:

(1) **The changes in blood serum IgG titre as a function of time –** ELISA test

(2**) The changes in serum cortisol levels as a function of time** – Cortisol test

(3) **Determination of gut closure**. Gut closure was determined by observing (on a linear regression scatter graph with a 2-point forecast trend line) when an absorption plateau and or incline in absorption had been reached. As normal gut closure is reported approximately 12 hrs - 24 hrs post-partum (Stott *et al*., 1979; Arthington *et al*., 2000; Weaver *et al*., 2008), the supply of pooled, good-quality Ig was continued until $T_{42}$ (group 4 only) to detect any extended gut closure pattern due to possible parental glucocorticoid activity.

(4) **FBC** – Full Blood Count.

**Determination of serum IgG titre** was performed using the quantitative ELISA - test method according to the manufacturer's (IDEXX labs) instructions. The AEA% was calculated to determine the percentage of the consumed mass of IgG absorbed by the calf (Schanbacher *et al*., 1993).

Holstein-Fresian colostrum was collected as described (Mokhber-Dezfooli *et al.*, 2012). A minimum of 200 L of unpasteurized colostrum from 27 freshly calved multiparous Holstein X Jersey cows was sampled and tested by means of a commercially available colostrometer after which the colostrum was collected by milking machine, pooled and fed to the calves that were enrolled into the study. This was done to normalize the colostrum i.e. to avoid erroneously feeding calves superior or inferior colostrum.

*Colostrum quality / serum evaluation (IgG)* - Colostrum of the first milking of 27 cows was measured for Ig quality by KRUUSE colostrometer and then pooled. The best quality colostrum that could be collected and pooled was measured at 1.044 (SG), colostrometer value, or 44.720 g/L IgG. This colostrum was regarded as being of MODERATE - GOOD quality. All newborn calves (n=24) were fed 1.5 L colostrum/feeding by means of esophageal tube of the pooled colostrum to avoid variation/bias in colostrum meal quality at calf level. Blood serum samples were collected as prescribed by the protocol. The pre-colostral evaluation of the neonate bovine serum IgG concentration at $T_0$ was done and all $T_0$ samples (n=24) recorded a negative (neg) result for the test BVDV marker antigen.

#### 3.3.1.6. IgG TITRE

The accepted direct measure for IgG is ELISA or a RID-test. This, however, is time-consuming, very expensive and cannot be executed on-farm (Doepel and Bartier 2014). An alternative procedure for consideration was Brix Refractometry which measures serum total protein (STP) but it is not 100% accurate as it measures Ig and other proteins present and the calf's hydration status has an impact on the final result (Tyler et al., 1996). The Brix test sensitivity, specificity, positive and negative predictive values, and accuracy are 92.9, 65.5, 93.5, 63.3, and 88.5%, respectively (Quigley et al., 2012).

*ELISA* (IgG) - Bovine Viral Diarrhea Virus was used as a titre marker. The ELISA test is developed for a specific antigens i.e. BVDV or *E. coli*. Various other methods exist to titrate antibodies i.e. virus neutralization (VN), complement fixation (CF), hemaglutination (HI) and indirect immuno-fluorescent antibody testing. However, ELISA results correlate very well with these tests and are much quicker and more cost effective (Juntti et al., 1987; Elvander *et al*., 1995). The anti-BVDV titre was determined at IDEXX Laboratories SA in Johannesburg using the BVDV Total Ab ELISA test according to the manufacturer's instructions (IDEXX Laboratories, Georgia, USA). In brief, the ELISA test is an indirect enzyme immunoassay designed to detect BVDV IgG in bovine serum by configuring a micro-titration of immobilized antigen in wells within micro-titre plates. Serum BVDV antibodies are then bound to the antigen after which the samples were incubated. Thereafter, any bound BVDV antibodies may be detected by allowing the binding of anti-bovine antibody to which is conjugated horseradish peroxidase. After washing away unbound conjugate, a substrate solution is added that forms a colored product in the presence of enzyme. Thereafter, a stop solution is added and a positive reaction for BVDV antibodies is confirmed by a change in color from blue to yellow. This is a quantitative test method whereby the absorbance of the well color is measured by a spectrophotometer set to a single (450 nm) or dual wavelength (450 nm and 650 nm). The sample to positive ratio is calculated by using the absorbance of the test sample and the absorbance of a positive control sample, corrected for the absorbance of a negative control sample. Remnant samples were stored at –10°C to –30°C until the following week. Samples were assayed daily.

#### 3.3.1.7. CORTISOL

A cortisol evaluation test was run by IDEXX Laboratories. The test was run on an Immulite 1000 and is a solid-phase, competitive chemiluminescent enzyme immuno-assay. Levels of cortisol (hydrocortisone Compound F) were measured quantitatively in the serum to assess adrenal status.

#### 3.3.1.8. FULL BLOOD COUNT (FBC)

A Full Blood Count was conducted where WBC, RBC, hematocrit, hemoglobin and platelets were measured to (indirectly) correlate any infection present in the animal

during the progress of the study. Thereby, the investigator was able to explain increases in IgG titre due to infection, if such a scenario occurred.

#### 3.3.1.9. HEALTH DATA

Health data (clinical treatment due to disease i.e. diarrhea and respiratory disease) and weight data (D30 and D42 live weight in kg as determined by scale weighing) were kept until forty two days post-partum. Calves were maintained on a milk replacer at 10% of their body weight, determined after each weekly weigh-in, until day forty two post-partum. Normal milk feedings were done twice daily at fifty percent of the daily allocation each. Additionally, calf starter and fresh water were offered *ad libitum*.

#### 3.3.1.10. AVERAGE WEIGHT GAIN AND FEED CONVERSION RATIO

The mass (kg) of each calf was measured at birth, day 30 and 42 days post-partum to determine the average daily gain (ADG – Weight gain over a period / lapsed days i.e. 42) between test groups. Additionally all dry feed offered to a specific study group was weighed to determine the total feed consumption over the study period of 42 days. Total feed wastage was recorded.

The feed conversion ratio (FCR) measures the animals' efficiency to convert feed mass into increased body mass and this ratio was calculated for each group. Calves with a low FCR are more efficient feed users and ultimately leave a reduced carbon footprint compared to calves with a high FCR. Efficient feed converters also imply superior GIT health. To facilitate data interpretation, external factors such as feed moisture, feed composition, environmental conditions, species, age and breed should be specified as all of these factors may impact and/or influence a specific FCR. In this study FCR for the group was determined rather than FCR for an individual calf as the outcome wanted was related to an overall economic parameter and not a selection parameter. When selection of single animals is the aim, single FCR's will be more helpful where in this case the economic impact of the supplement on herd level is sought after.

## 3.4. DATA STORAGE AND INTEGRITY

All the data were collected between 5th and 6th August 2013 by the researcher. Raw data will be stored as an annex to this thesis at UNISA.

All the raw data were recorded on data recording forms in black ink. Each data sheet was identified by the farm name (trial site), the date of the recording and a heading describing the type of data. If a sheet was used to record data on more than one day, each day's data entry was initialled by the recorder. If a single sheet was used for a particular day, that sheet was signed by the recorder. Study data were kept under lock and key when not in use and access to the data was strictly controlled by the investigator.

*Blinding* - Data were only recorded by the researcher or an assistant to the researcher. The analyzing laboratory (IDEXX Labs International) was blinded to the identity of the specimens. Only after all the tests results had been generated was each calf identified and analyzed per group.

## 3.5. STATISTICAL ANALYSIS AND INTERPRETATION

Data were expressed as mean ± SD. Results were considered to be statistically significant for $P < 0.05$.

*IgG, Cortisol, FBC and weight (ADG, FCR)* - Data were firstly analyzed for variance between groups (**ANOVA**) using Minitab version 15. Secondly, Decision Analyst's STATS version 2.0 (2009) and Graphpad software – Quickcalcs (2013), 2-tailed **t-test** was used for determining statistical significance between two study groups as well as for calculating standard means, standard deviation (SD), Standard error of the mean (SEM), CV%, errors, and degrees of freedom (df) and Graphpad was used to determine P-values.

Analysis for variances included treatment group (Control, IVP1, IVP2 and IVP 3), period (Birth, feedings at $T_{<6}$, $T_{12}$, $T_{<24}$, $T_{30}$, $T_{36}$, $T_{42}$) and sample values ($T_3$, $T_{12}$, $T_{24}$, $T_{27}$, $T_{30}$, $T_{33}$, $T_{36}$, $T_{42}$). The data for weight gain and growth were for variance by period (birth day, 42D post-partum), treatment group (Control, IVP1, IVP2 and IVP3)

and gender. Linear regression was done to determine correlations between IgG serum levels, cortisol serum levels and various elements of the FBC. (Minitab® 15. 1. 30. 0, ©2007 Minitab Inc., www.decisionanalyst.com, www.Graphpad.com).

*AEA %* - this was calculated for each calf using the formula described by Quigley (2007) and by Gvozdic et al. 2008.

| % AEA = [(plasma IgG (g/L) x PV (L) x 100) / IgG intake (g)] |
|---|

*Feed conversion ratio (FCR)* and *average daily gain (ADG)* – FCR and ADG was calculated for each group.

All the data were statistically analyzed by the author of this thesis and no additional analyst was sub-contracted to perform analyses. Analytical software was downloaded from the internet before the raw study data was uploaded and the analysis completed.

## 4. CHAPTER 4 - RESULTS

The results of an ELISA test are presented as an optical density reading which is then converted to an SP ratio per sample. The qualitative IgG (ng/ml) can then be read off a typical standard graph curve as indicated in Figure 15 for optical density (OD) by creating an MS Excel trends model (Microsoft Corporation, Redmond, WA USA) for caption of the OD data and presentation of IgG values.

1. ELISA BVDV Serum: Sensitivity = 91%; Specificity = 97%
2. A sample with an S/P ratio greater than 0.30 units is considered a positive result.

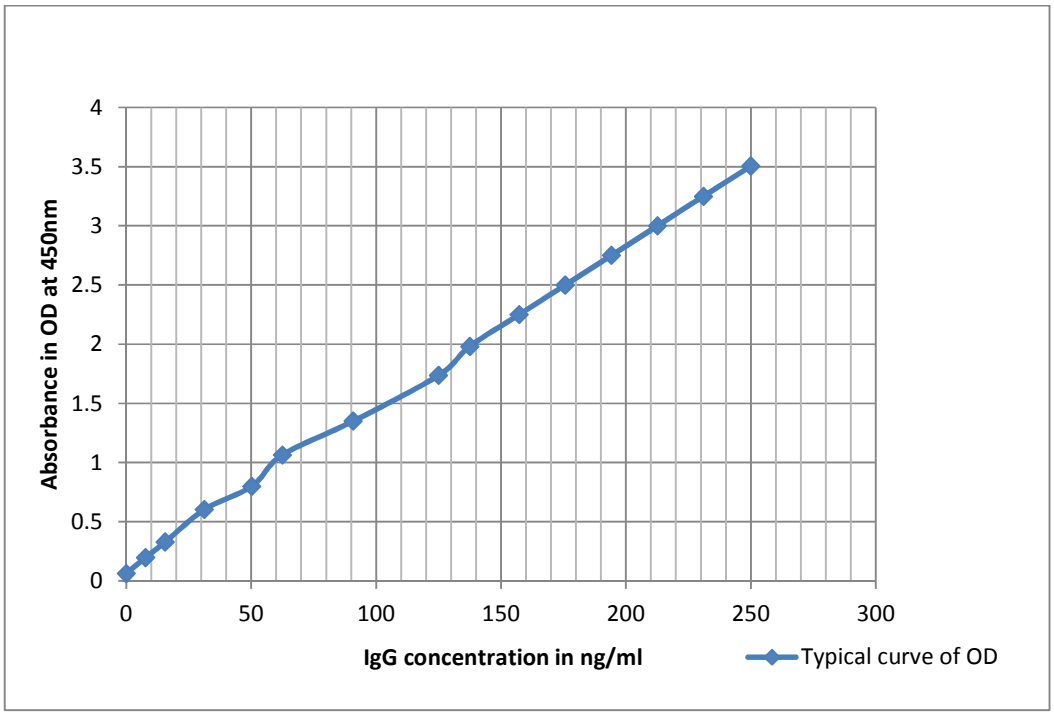


Figure 15: Typical standard curve of density for the conversion of OD to IgG.

*Pre-colostral serum IgG concentration* – Figures 16(a) and 16(b) below indicate the IgG values (ng/ml) of the 24 negative pre-colostral samples evaluated by ELISA testing. There was no statistically significant difference between the groups. Figures 16a and 16b indicate that all calves from the different study groups were uniform for blood serum IgG concentrations (0.000 ng/ml IgG) at the onset of the study ($T_0$) and not significantly different (P=0.91).

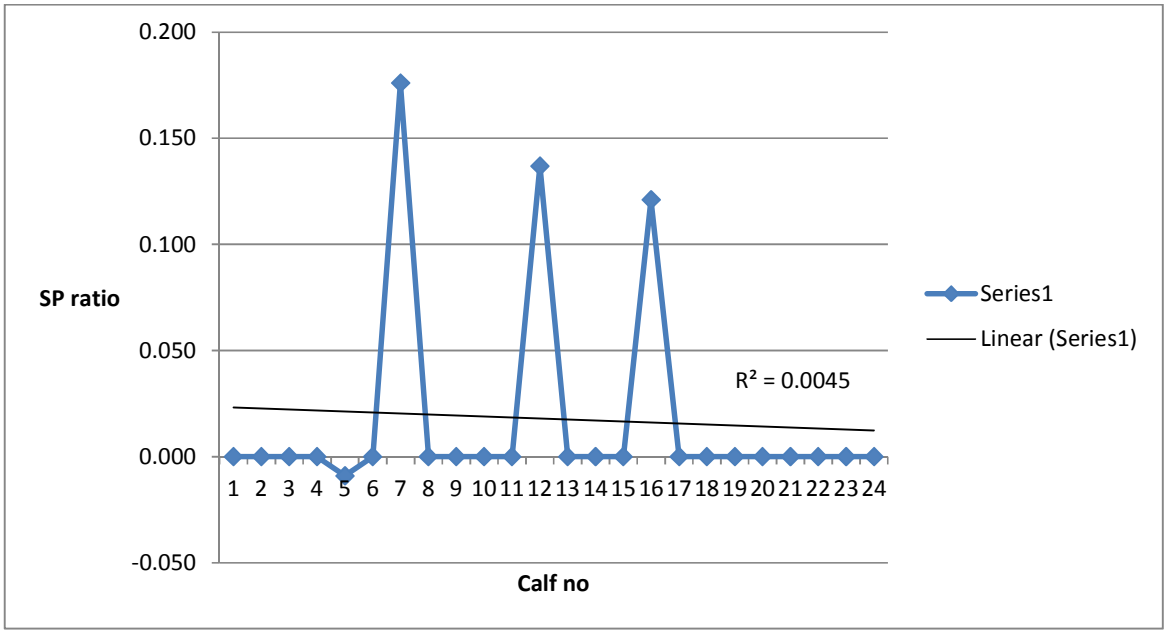


Figure 16a: First bleeding point ($T_0$).

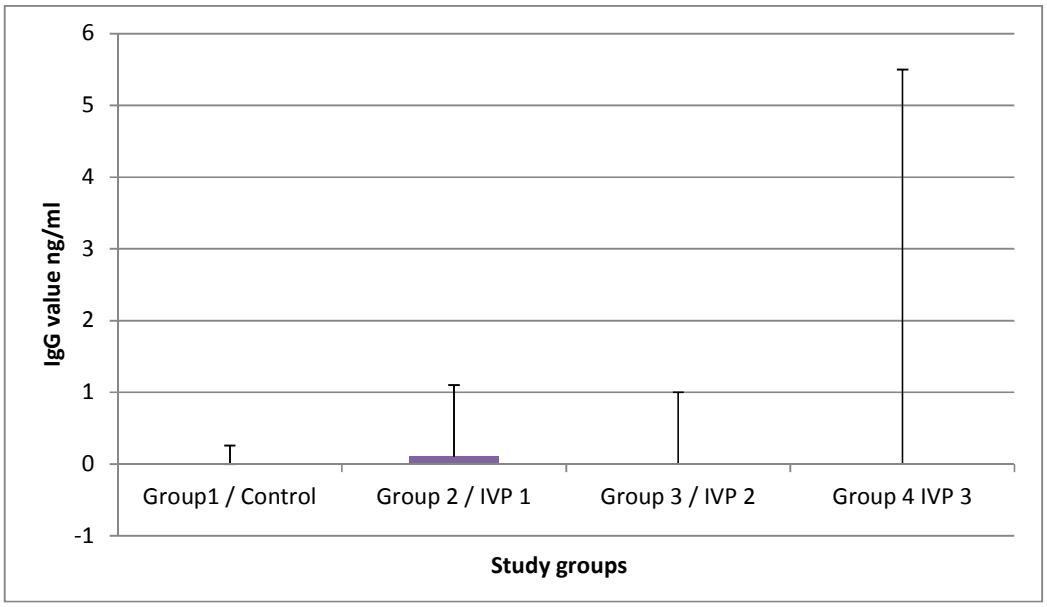


Figure 16 b: Boxplot – Pre-colostral IgG / study group.

*Study animals* - All newborn calves enrolled into the study were removed from their dams, sexed, weighed, marked and penned before their first colostrum meal at $T_0$. The total calf average live weight, n=24 calves, at birth was 37.12 kg ±3.93 kg. At birth, thirteen (n=13) bull calves had an average weight of 37.23 kg ±4.14 kg and 11 heifer calves had an average birth weight of 37.00 kg ±3.87 kg). There were no statistical difference (P=0.490) between the birth weights of the study groups and the groups were uniform. This is indicated in Figure 17 and described in Table 9.

*Mortalities* - (Group 1, calf no: 1) - No official post mortem (PM) was done, however, from the clinical signs it was the opinion of the attending veterinarian that the cause might be related to aspiration pneumonia. This calf was not replaced as the death occurred post gut closure, hence there was no need to replace the test subject as the blood levels pre-gut closure are the significant data needed for this study. These events necessitated a protocol deviation in that each of the study groups had to be reduced to six calves each (n=6). No calves were physically excluded or removed from any group. The groups were merely populated to n=6 from the time of death of calf no.1.

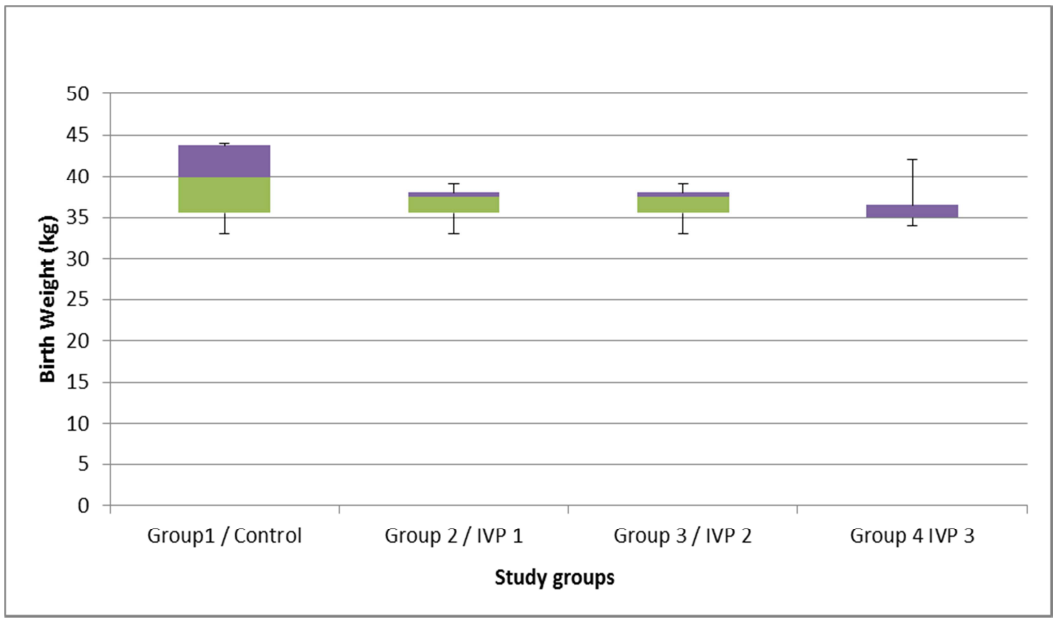


Figure 17: Boxplot - Birth weights / study group (kg)

Table 9: Birth weights (kg) per study group

***Birth weights (kg) per study group***

| | 0hr | |
|---|---|---|
| | Mean | SD |
| Group 1 | 39.333 | ± 4.93 |
| Group 2 | 36.667 | ± 2.25 |
| Group 3 | 36.167 | ± 5.04 |
| Group 4 | 36.333 | ± 2.94 |
| *P-value* | P = 0.490 | |

*Cortisol production* - When the study groups were compared at various time intervals for cortisol production no statistically significant differences (P=0.19) were noted between the groups at $T_0$, the pre-colostral bleeding point. This is indicated in Table 15). However, at the next bleeding point ($T_6$) and at every bleeding point thereafter, a statistically significant difference was noted, between the same sex animals across study groups as well as the groups at different time intervals as can be seen in table 15. This difference was especially obvious when comparing study group 4 that received a parenteral glucocorticoid and the other three study groups. It would appear

that gender did not influence cortisol production of neonates in early life. As shown in Figures 18 and Figure 19, a linear regression evaluation revealed a negative correlation between IgG and cortisol blood serum concentrations at both $T_0$ and $T_{24}$.

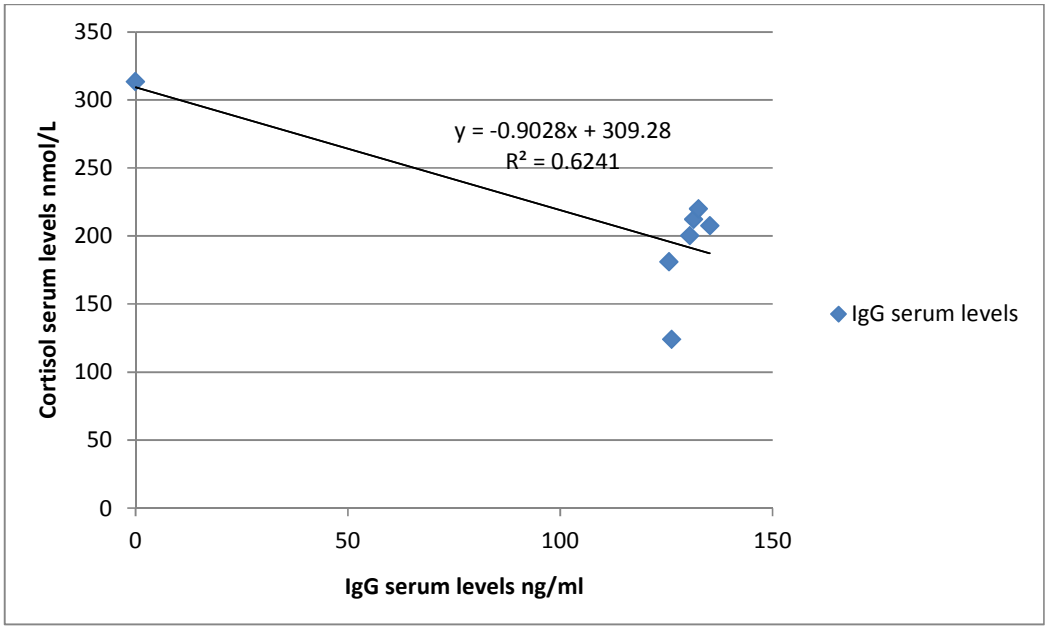


Figure 18: Negative correlation between IgG and cortisol blood serum levels at $T_0$.

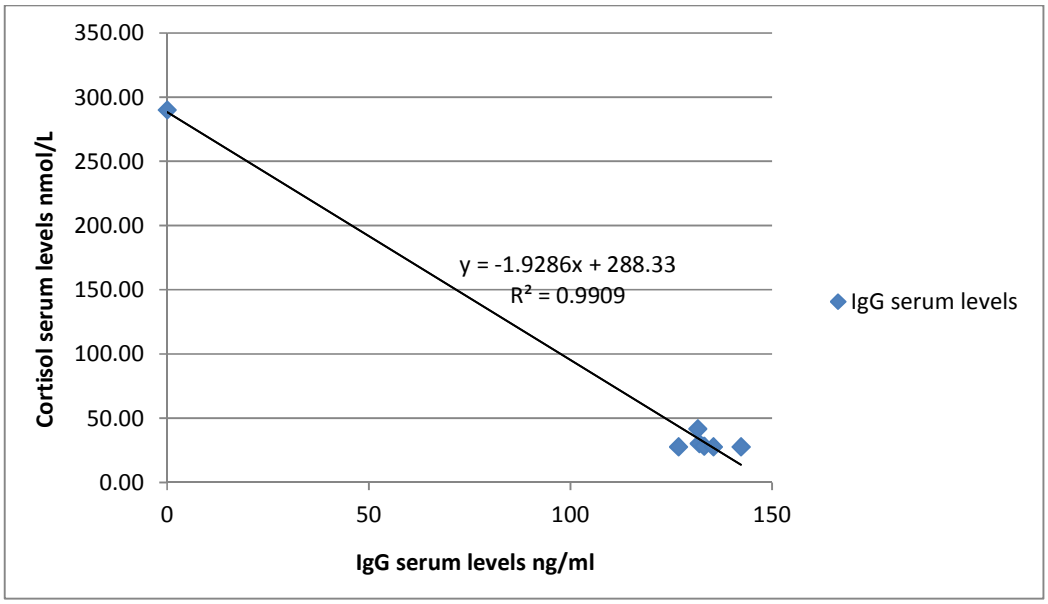


Figure 19: Negative correlation between IgG and cortisol blood serum levels at $T_{24}$.

*Full Blood Count (FBC)* – A FBC was done at $T_0$ and $T_{24}$ to reveal no statistical difference (P=0.100) for WBC between study groups at $T_0$. However, a statistically significant difference (P=0.0006) was recorded between group 4 and the other study groups at $T_{24}$ for WBC (Figure 20).

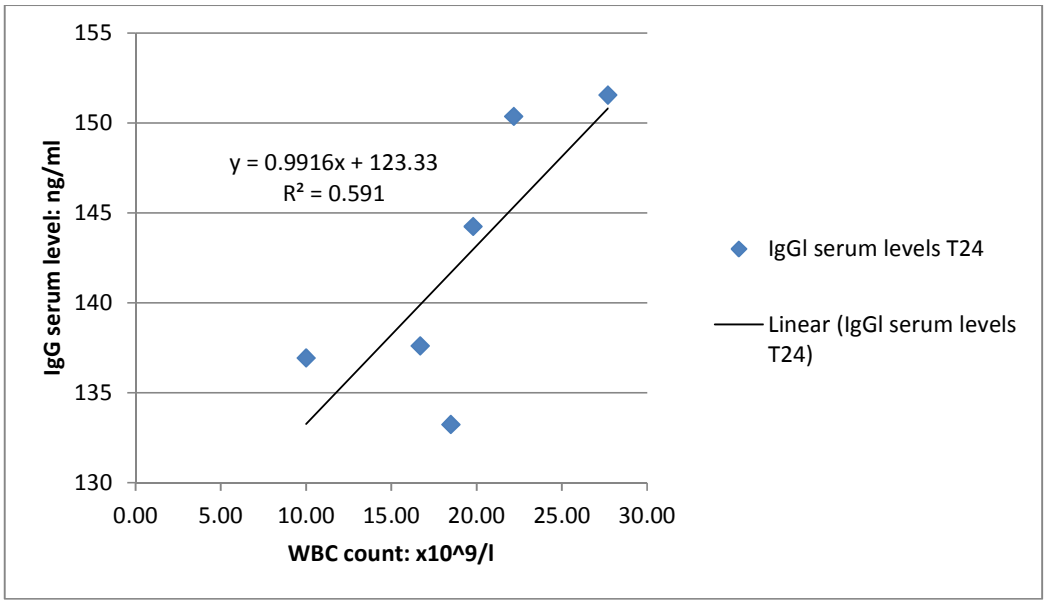


Figure 20: IgG blood serum levels for group 4 at $T_{24}$.

*IgG absorption post-partum (weight and sex)* – There were no statistical significant difference noted for IgG absorption between sexes and their birth weights within the same study group/s nor across study groups when they were evaluated for IgG absorption (Table 14). It would appear that gender and birth weight did not influence IgG absorption by neonates in early life (table 14).

No statistical difference (P = 0.310) were reported between groups for AEA% at Cmax. A positive correlation was noted in Figure 22 between birth weight and IgG absorbed (AEA) and this is supported by the positive correlation between AEA and plasma volume as indicated in Figure 23. A total of seven calves, 1 each in groups 2 and 4, 2 in group 1 and 3 in group 3 suffered from FPT where their blood serum IgG levels fell below 10 g/L IgG at Cmax. Group 1 recorded a mean absorption of 11.579 g IgG ± 1.93 g IgG, group 2 recorded 10.204 g IgG ± 1.16 g IgG, group 3 recorded 10.540 g IgG ± 1.55 g IgG and group 4 recorded 11.392 g IgG ± 1.09 g IgG.

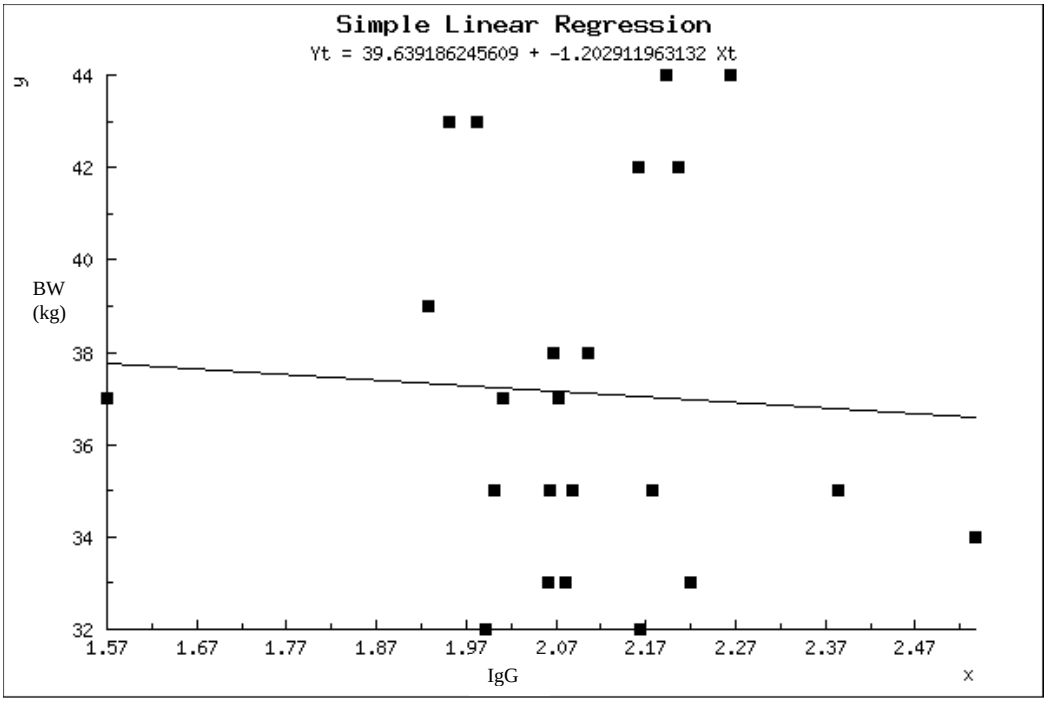


Figure 21: Negative correlation between birth weight and IgG (g/L) at $C_{max}$.

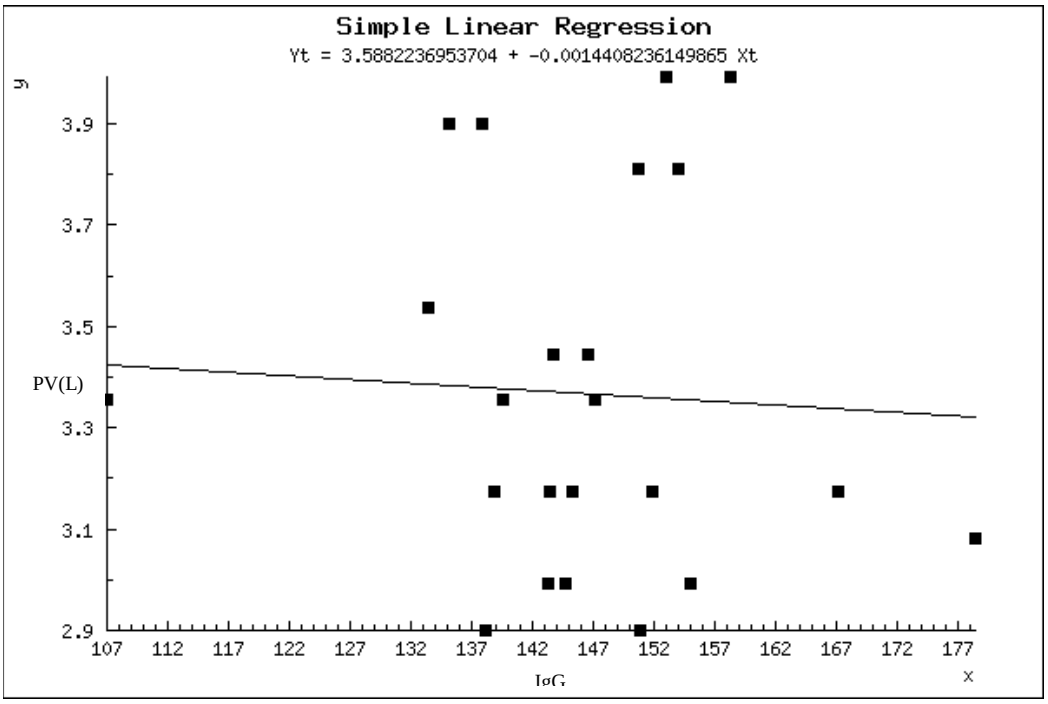


Figure 22: Negative correlation between Plasma Volume (PV) and AEA% at $C_{max}$.

Table 10: AEA - IgG (g/L) / calf / group at $C_{max}$.

***AEA - IgG (g/L) absorption at Cmax***

| | Cmax | | Calf 1 | Calf 2 | Calf 3 | Calf 4 | Calf 5 | Calf 6 |
|---|---|---|---|---|---|---|---|---|
| | Mean | SD | AEA (g/L) | AEA (g/L) | AEA (g/L) | AEA (g/L) | AEA (g/L) | AEA (g/L) |
| Group 1 | 11.579 | ± 1.93 | 11.961 | 10.416 | **9.812**** | 14.055 | 13.589 | **9.64**** |
| Group 2 | 10.204 | ± 1.16 | **7.987**** | 10.502 | 11.03 | 10.135 | 10.33 | 11.243 |
| Group 3 | 10.540 | ± 1.55 | 13.053 | 10.261 | 11.73 | **8.918**** | **9.74**** | **9.542**** |
| Group 4 | 11.39 | ± 1.09 | 12.771 | 10.726 | **9.812**** | 10.991 | 12.246 | 11.807 |
| *P-value* | 0.33 | | | | | | | |

Table 11: AEA% / calf / group at Cmax.

***AEA % / calf / study group at Cmax***

| | Cmax | | Calf 1 | Calf 2 | Calf 3 | Calf 4 | Calf 5 | Calf 6 |
|---|---|---|---|---|---|---|---|---|
| | Mean (%) | SD | AEA (%) | AEA (%) | AEA (%) | AEA (%) | AEA (%) | AEA (%) |
| Group 1 | 25.89 | ± 4.31 | 26.75 | 23.29 | **21.94**** | 31.43 | 30.39 | **21.56**** |
| Group 2 | 22.82 | ± 2.60 | **17.86**** | 23.49 | 24.66 | 22.67 | 23.09 | 25.14 |
| Group 3 | 23.57 | ± 3.47 | 29.19 | 22.95 | 26.22 | **19.94**** | **21.78**** | **21.34**** |
| Group 4 | 25.48 | ± 2.43 | 28.56 | 23.99 | **21.94**** | 24.58 | 27.39 | 26.40 |
| *P-value* | 0.33 | | | | | | | |

* P<0.05

* Statistical significant difference (ANOVA -analysis of variance between groups)

**FPT calves < 10g/L IgG at Cmax

Table 12: Cortisol blood serum levels / study group at different time intervals post-partum.

| | ***Cortisol - Blood serum levels (nmol/L)*** | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Post-partum | 0hr | | 6hr | | 12hr | | 18hr | | 24hr | | 30hr | | 36hr | |
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Group 1 | 313.5 | ± 55.7 | 200.33 | ± 87.5 | 212.33 | ± 84.9 | 207.78 | ± 73.8 | 220.00 | ± 50.1 | 181.20 | ± 52.4 | 124.24 | ± 53.5 |
| Group 2 | 308.67 | ± 65.1 | 154.60 | ± 42.6 | 147.50 | ± 38.7 | 140.50 | ± 52.6 | 167.50 | ± 33.3 | 186.62 | ± 56.3 | 132.18 | ± 34.0 |
| Group 3 | 359.33 | ± 59.3 | 171.83 | ± 73.8 | 149.00 | ± 32.0 | 235.05 | ± 112.0 | 219.15 | ± 118.0 | 164.05 | ± 116.0 | 84.267 | ± 63.3 |
| Group 4 | 290.00 | ± 33.9 | 30.25 | ± 2.95 | 27.60 | ± 3.892 | 28.18 | ± 0.90 | 27.60 | ± 3.892 | 41.667 | ± 34.5 | 27.60 | ± 3.89 |
| *P-value (P=)* | 0.19 | | 0.0005* | | 0.0001* | | 0.0003* | | 0.0002* | | 0.0082* | | 0.0026* | |

* P<0.05

* Statistical significant difference (ANOVA -analysis of variance between groups)

Table 13: Immunoglobulin (IgG) blood serum levels / study group at different intervals post-partum.

| | ***Immunoglobulin (IgG) - Blood serum levels (ng/ml)*** | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Post-partum | 6hr | | 12hr | | 18hr | | 24hr | | 30hr | | 36hr | | Cmax | |
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Group 1 | 130.50 | ± 24.9 | 131.42 | ± 16.9 | 135.22 | ± 7.16 | 132.55 | ± 73.8 | 125.64 | ± 8.36 | 126.28 | ± 15.8 | 145.10 | ± 8.46 |
| Group 2 | 127.05 | ± 20.1 | 121.15 | ± 19.3 | 125.48 | ± 13.9 | 126.95 | ± 52.6 | 117.65 | ± 16.0 | 122.07 | ± 14.7 | 137.94 | ± 16.8 |
| Group 3 | 135.21 | ± 11.2 | 132.94 | ± 1.84 | 130.23 | ± 9.11 | 136.57 | ± 112.0 | 127.9 | ± 7.33 | 131.63 | ± 3.89 | 144.14 | ± 7.21 |
| Group 4 | 131.95 | ± 21.3 | 126.82 | ± 17.0 | 133.15 | ± 7.93 | 142.32 | ± 0.90 | 131.60 | ± 1.08 | 135.45 | ± 8.94 | 155.40 | ± 14.4 |
| *P-value (P=)* | 0.91 | | 0.56 | | 0.37 | | 0.066 | | 0.13 | | 0.25 | | 0.14 | |

* P<0.05

* Statistical significant difference (ANOVA -analysis of variance between groups

.

Table 14: 2 Tailed t-test and ANOVA results to compare immunoglobulins (sexes) within and between study groups.

***Immunoglobulins (IgG) - Blood serum levels (ng/ml): t-test comparison between sexes***

| | 0hr | | 6hr | | 12hr | | 18hr | | 24hr | | 30hr | | 36hr | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sexes | M | F | M | F | M | F | M | F | M | F | M | F | M | F |
| Group 1 | 0 | 0 | 88.071 | 118.110 | 114.789 | 118.922 | 137.151 | 138.775 | 139.218 | 130.804 | 126.819 | 128.221 | 129.254 | 124.309 |
| | 0 | 0 | 129.476 | 157.964 | 116.043 | 147.410 | 138.627 | 141.727 | 135.011 | 121.874 | 134.125 | 111.615 | 114.493 | 135.232 |
| | -8.096 | 0 | 144.901 | 144.458 | 152.724 | 138.627 | 121.726 | 133.313 | 135.823 | 106.744 | | 96.485 | | 133.609 |
| *P-value (P=)* | | | 0.562 | | 0.660 | | 0.412 | | 0.069 | | 0.145 | | 0.324 | |
| Group 2 | 4.230 | 0 | 104.677 | 109.032 | 86.595 | 116.191 | 103.496 | 118.626 | 133.166 | 135.159 | 122.021 | 138.554 | 75.229 | 123.055 |
| | 0 | 0 | 119.660 | 127.926 | 143.203 | 126.523 | 124.826 | 143.498 | 135.970 | 129.771 | 131.764 | 111.172 | 117.815 | 128.000 |
| | 0 | 1.351 | 154.717 | 146.303 | 132.280 | 122.095 | 136.192 | 126.228 | 120.914 | 153.683 | 105.932 | 136.708 | 135.454 | 105.342 |
| *P-value (P=)* | | | 0.725 | | 0.978 | | 0.547 | | 0.723 | | 0.748 | | 0.539 | |
| Group 3 | 0 | 0 | 119.291 | 126.745 | 132.059 | 132.723 | 117.372 | 139.144 | 144.975 | 123.571 | 124.605 | 127.261 | 127.040 | 127.261 |
| | 0 | 0 | 133.904 | 137.816 | 134.863 | 129.845 | 134.199 | 128.885 | 123.719 | 117.150 | 132.575 | 110.213 | 131.616 | 110.213 |
| | 0 | 0 | 142.982 | 150.510 | 133.756 | 134.421 | 139.587 | 122.169 | 150.362 | 131.026 | 132.797 | 128.885 | 136.118 | 128.885 |
| *P-value (P=)* | | | 0.983 | | 0.227 | | 0.727 | | 0.171 | | 0.218 | | 0.172 | |
| Group 4 | 0 | 0 | 111.172 | 104.604 | 100.913 | 109.548 | 132.723 | 120.176 | 133.240 | 151.543 | 133.240 | 132.280 | 134.790 | 124.974 |
| | 0 | 0 | 125.638 | 146.893 | 138.627 | 135.159 | 132.133 | 144.975 | 137.594 | 144.237 | 131.616 | 131.395 | 129.845 | 143.720 |
| | 0 | | 146.746 | | 138.554 | | 135.306 | | 136.930 | | 130.066 | | 146.524 | |
| | 0 | | 156.636 | | 138.111 | | 133.609 | | 133.609 | | 133.609 | | 134.273 | |
| *P-value (P=)* | | | 0.899 | | 0.443 | | 0.505 | | 0.055 | | 0.825 | | 0.479 | |

* P<0.05

* Statistical significant difference (2 Tailed t-test to analize variance between sexes wihtin the group)

***Immunoglobulins (IgG) - Blood serum levels (ng/ml): ANOVA comparison between sexes***

| | 6hr | | 12hr | | 18hr | | 24hr | | 30hr | | 36hr | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | M | F | M | F | M | F | M | F | M | F | M | F |
| *P-value (P=)* | 0.850 | 0.660 | 0.89 | 0.47 | 0.500 | 0.690 | 0.52 | 0.027* | 0.160 | 0.280 | 0.190 | 0.170 |

* P<0.05

* Statistical significant difference (ANOVA -analysis of variance between groups)

.

Table 15: 2 Tailed t-test and ANOVA results to compare cortisol (sexes) within and between study groups.

***Cortisol - Blood serum levels (nmol/L): t-test comparison between sexes***

| Post-partum | 0hr | | 6hr | | 12hr | | 18hr | | 24hr | | 30hr | | 36hr | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sexes | M | F | M | F | M | F | M | F | M | F | M | F | M | F |
| Group 1 | 263 | 247 | 356 | 142 | 364 | 247 | 309 | 256 | 215 | 298 | 150 | 234 | 151 | 191 |
| | 359 | 367 | 144 | 120 | 154 | 145 | 170 | 97.7 | 172 | 182 | 119 | 166 | 106 | 47.2 |
| | 281 | 364 | 209 | 231 | 150 | 214 | 183 | 231 | | 233 | | 237 | | 126 |
| *P-value (P=)* | 0.639 | | 0.370 | | 0.801 | | 0.717 | | 0.410 | | 0.094 | | 0.908 | |
| Group 2 | 255 | 403 | 129 | 154 | 147 | 175 | 105 | 222 | 163 | 202 | 183 | 229 | 102 | 174 |
| | 331 | 315 | 214 | 88.6 | 173 | 167 | 172 | 126 | 193 | 191 | 261 | 187 | 143 | 146 |
| | 217 | 331 | 173 | 169 | 72 | 151 | 72 | 146 | 130 | 126 | 97.7 | 162 | 81.1 | 147 |
| *P-value (P=)* | 0.117 | | 0.374 | | 0.340 | | 0.309 | | 0.732 | | 0.824 | | 0.082 | |
| Group 3 | 411 | 342 | 188 | 162 | 201 | 113 | 233 | 240 | 284 | 83.9 | 155 | 104 | 110 | 35.3 |
| | 323 | 244 | 143 | 244 | 126 | 147 | 264 | 414 | 154 | 419 | 164 | 389 | 27.6 | 198 |
| | 266 | 246 | 48 | 246 | 170 | 137 | 190 | 69.3 | 175 | 199 | 103 | 69.3 | 55.5 | 79.2 |
| *P-value (P=)* | 0.352 | | 0.141 | | 0.237 | | 0.911 | | 0.794 | | 0.674 | | 0.504 | |
| Group 4 | 274 | 287 | 32 | 28.1 | 27.6 | 27.6 | 27.6 | 27.6 | 27.6 | 27.6 | 27.6 | 27.6 | 27.6 | 200 |
| | 252 | 350 | 30.1 | 28.4 | 27.6 | 27.6 | 27.6 | 27.6 | 27.6 | 27.6 | 27.6 | 27.6 | 27.6 | 27.6 |
| | 274 | | 35.3 | | 27.7 | | 29.2 | | 27.7 | | 27.6 | | 27.7 | |
| | 303 | | 27.6 | | 27.6 | | 29.5 | | 27.6 | | 112 | | 27.6 | |
| *P-value (P=)* | 0.161 | | 0.286 | | | | 0.316 | | | | 0.542 | | 0.178 | |

* P<0.05

* Statistical significant difference (2 Tailed t-test to analize variance between sexes wihtin the group)

***Cortisol - Blood serum levels (nmol/L): ANOVA comparison between sexes***

| | 0hr | | 6hr | | 12hr | | 18hr | | 24hr | | 30hr | | 36hr | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | M | F | M | F | M | F | M | F | M | F | M | F | M | F |
| *P-value (P=)* | 0.430 | 0.360 | 0.014* | 0.0043* | 0.019* | 0.0004* | 0.0009* | 0.130 | 0.0017* | 0.074 | 0.037* | 0.120 | 0.020* | 0.700 |

* P<0.05

* Statistical significant difference (ANOVA -analysis of variance between groups)

*Gut closure* – compared to the other groups in the study, Group 4 was bled 7 times with, an additional bleed at $T_{42}$ to illustrate whether or not a delay in gut-closure was experienced in this group of calves. The control group 1 recorded a Cmax; OD 1.951 (IgG 135.232 ng/ml) at 18 hours post-partum after which a clear decline in IgG blood serum level was detected. This is indicated in Figure 23. Group 4 recorded an initial IgG blood serum peak at $T_{24}$ of OD 2.047 (IgG 142.318 ng/ml), after which a second peak or C-max was recorded at $T_{42,}$ OD 2.104 (IgG 146.524 ng/ml). When group 4, at $T_{42}$, is compared to the control group 1 at $T_{30}$ (P=0.023) and $T_{36}$ (P=0.029) statistically significant results are recorded. It is, therefore, clear that the absorption of IgG continued post-24 hr. in the glucocorticoid group 4 (Table 24).

A white blood count was conducted as a part of the FBC. At birth there were no statistical difference (P=0.100) between the WBC of the study groups. However, at 24 hours post-partum there was a statistically significant difference (P=0.0008) between the study groups. A 2 tailed t-test revealed a statistically significant difference (P=0.0006) between the group 4 measure at birth (mean 7.08 x $10^9$/l ±SD 1.4782 x $10^9$/l) compared to the same group's count (mean 19.14 x $10^9$/l ±SD 5.8766 x $10^9$/l) at 24 hours post-partum (Tables 3 and 4 – annexure – FBC lab report). Linear regression confirmed a positive correlation between WBC and IgG titre at 24 hours post-partum as indicated in Figure 20.

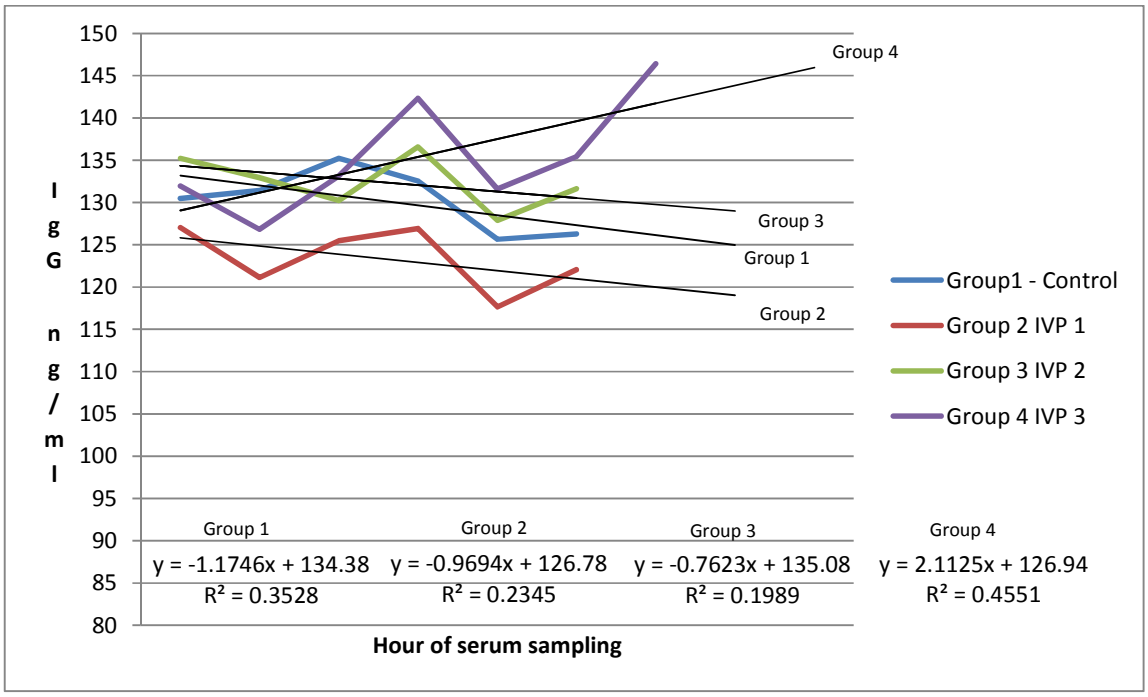


Figure 23: Gut closure – IgG absorption / study group at different time intervals.

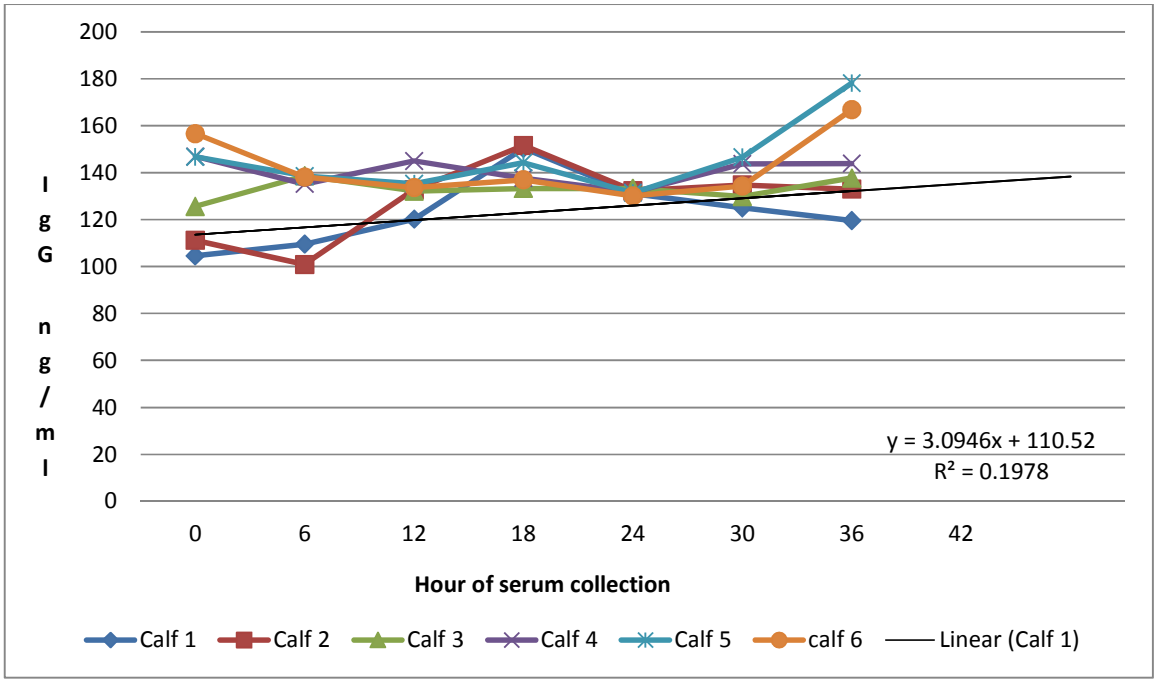


Figure 24: IgG serum levels of study group 4.

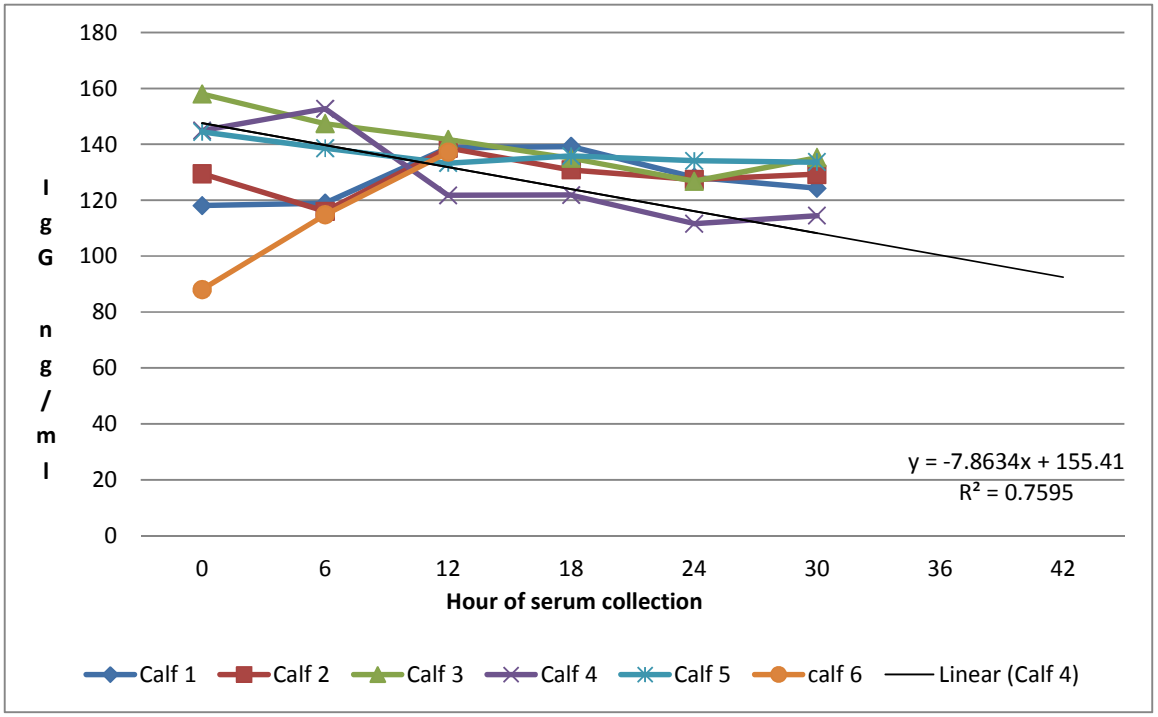


Figure 25: IgG serum levels of the control group.

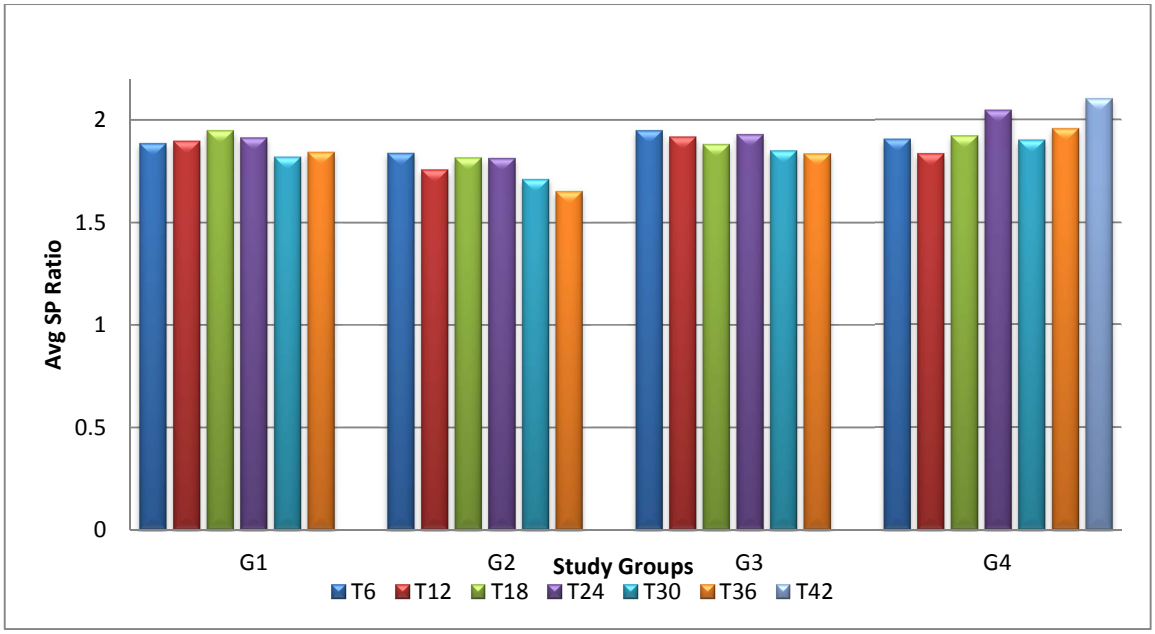


Figure 26: Mean SP Ratio / group / bleeding point ($G_1$-$G_3$ six times; $G_4$ 7 times)

Table 16: Growth profile / study group over the trial period.

***Body weights (kg) per study group***

| | Day 1 (Birth) | | Day 30 | | Day 42 | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD |
| Group 1 | 39.333 | ± 4.93 | 46.400 | ± 4.39 | 48.600 | ± 8.68 |
| Group 2 | 36.667 | ± 2.25 | 52.333 | ± 3.98 | 59.167 | ± 3.54 |
| Group 3 | 36.167 | ± 5.04 | 47.667 | ± 6.19 | 48.500 | ± 5.61 |
| Group 4 | 36.333 | ± 2.94 | 47.333 | ± 2.07 | 50.167 | 2.32 |
| *P-value (P=)* | 0.49 | | 0.13 | | 0.0042* | |

* P<0.05

* Statistical significant difference (ANOVA -analysis of variance between groups)

Table 17: Growth profile (dry feed consumption) / study group

| Post-partum | ***Growth profile (Dry feed consumption - kg) / study group*** kg | kg | ADG (kg) | | | FCR (feed: meat) |
|---|---|---|---|---|---|---|
| | Total feed | Waste | Mean | SD | t-test** | Mean |
| Group 1 | 200 | 0.350 | 0.238 | ± 0.238 | | 4.000 |
| Group 2 | 350 | 0.500 | 0.535 | ± 0.111 | P=0.022** | 2.593 |
| Group 3 | 200 | 0.450 | 0.293 | ± 0.132 | | 2.703 |
| Group 4 | 250 | 0.600 | 0.329 | ± 6.6E-02 | | 3.012 |
| *P-value (P=)* | | | 0.014* | | | 0.0001* |

* P<0.05

* Statistical significant difference (ANOVA -analysis of variance between groups)

** 2 Tailed t-test, compared to Group 1 - Control

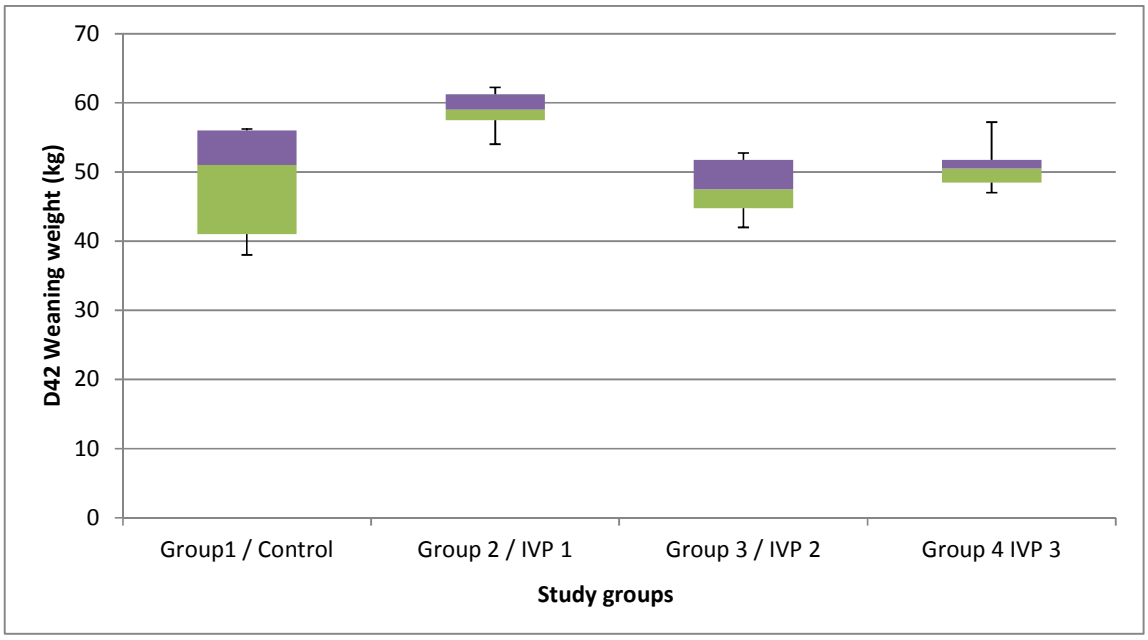


Figure 27 a: Boxplot – D42 weight/study group.

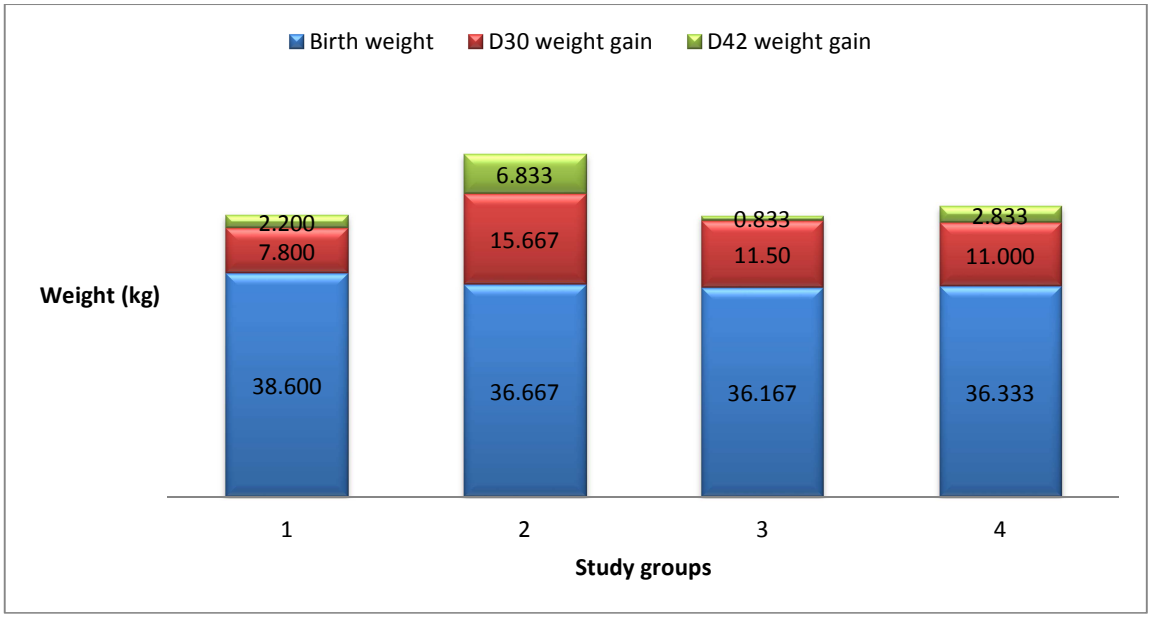


Figure 27 b: Mean growth (kg) per study group / weigh point (days)

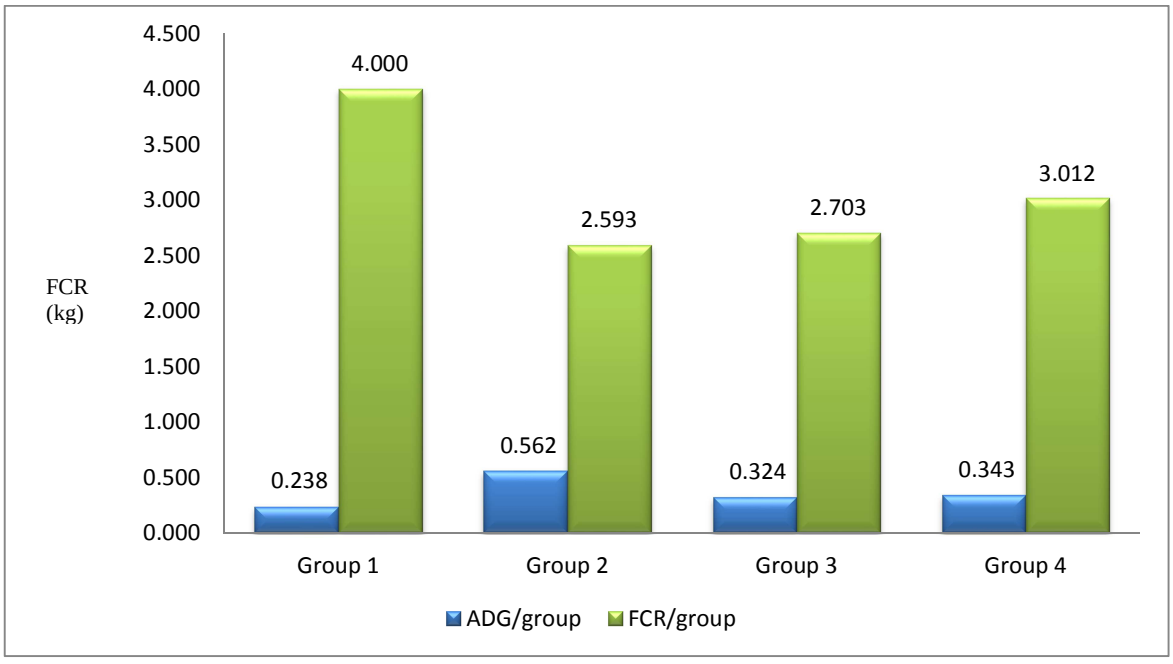


Figure 28: FCR (kg) and ADG (kg/day) / study group

## 5. CHAPTER 5 – DISCUSSION

*Elisa test* – The serum sensitivity of the test is 91% + and the specificity is 97% + (as indicated in manufacturers package insert). The qualitative IgG (ng/ml) can then be read off a typical standard graph curve for optical density (OD). This measure was necessary to confirm that no study subject was nursed by its mother before the trial started as it would skew the results. The detection of any anti-BVDV IgG (marker) in the serum collected from a calf before the ingestion of colostrum indicates transplacental infection i.e. *de novo* production of IgG by the calf. All blood samples with a SP ratios $\leq$ 0.200 OD were considered to be negative as per the ELISA-test specifications and so were fit for enrollment in the study. The reason for assaying Ig is as a result of its high titre occurrence in colostrum and in calf serum at 48 hours of age, as well as its importance in the further development of the neonate. Normally, the titre of blood serum IgG of un-suckled calves is very low (<0.1 g/L) (Arthington et al., 2000). The 0.000 ng/ml pre-colostral blood serum levels result recorded from all study calves confirmed that the dams did not nurse any of the calves before the first bleeding point. Bovine fetuses become immunocompetent at between 145 to 200 days of gestation (Dean et al., 2003), thus, the results of the current study suggests that all the calves born during this study were agammaglobulinemic at birth. As all test subjects were reported to be negative, entry to this study was compliant.

*Colostrum quality / serum evaluation (IgG)* – The best quality colostrum that was produced by the test subjects' cows were 44.72 g/L IgG, as collected and then measured by KRUUSE colostrometer. This is below the ideal of 50 g/L IgG that was required for this study but similar to that reported by Arthington (2000). No bacterial counts were done for the colostrum samples. The IgG blood serum results from this study reported no statistical significance between the negative control group and treated study groups with regards to improved IgG absorption within the first 36 hours of life (Table 13). The absorption rate may be attributed to the method by which the colostrum was fed. Research has shown that administration by esophageal tube reduces the uptake of IgG because of the remnant colostrum remaining in the reticulo-rumen after the feeding (Quigley, 2007; Doepel and Bartier, 2014). It would, hence,

appear that the nutritional supplement (IVP 1) made no significant impact on the IgG absorption by the calves in this time period.

*Birth weight and sexes* – It was necessary to confirm that the groups were uniform as to weight (Figure 17) and that there was no bias for weight and/or sex advantage that might influence the IgG absorption / cortisol production. It is clear from the results that neither gender (Table 14) nor birth weight (P = 0.49) impacted directly on the IgG absorption / cortisol production results. This is consistent with findings by Vann and co-workers (1995). However, Roy found in 1990 that gender may influence AEA as heifer calves are known to have greater blood serum IgG concentrations than bull calves (Roy, 1990; Vann et al., 1995).

*Cortisol production* – The reason that there was no statistically significant difference (P=0.19) between groups when compared for cortisol concentration at $T_0$ is that the group 4 blood samples were taken before the parenteral administration of the glucocorticoid to establish the base stress level that the group 4 calves were at when enrolled. All other bleed points showed a statistical significant difference between group 4 and the other study groups at different bleed points (Table 12) as a result of the negative feedback effect of glucocorticoids on the production of cortisol. Figures 18 and 19 illustrate the negative correlation between cortisol blood serum levels and IgG blood serum levels. This would be advantageous to a neonate because whilst its cortisol levels are reduced by the parenteral glucocorticoids its IgG serum concentration is higher due to the negative correlation. This is consistent with findings from Wooley, (2010).

*Full blood count (FBC)* – a Full Blood Panel consists of a blood serum evaluation. There is a positive correlation between WBC and IgG blood serum levels (Figure 20). A statistically significant difference was recorded for group 4 when comparing its $T_0$ and $T_{24}$ (P=0006) bleed point results. This is due to prolonged gut maturation caused by the parenteral glucocorticoids. Although the period for IgG absorption is prolonged the same happens for the absorption of bacteria hence the increased WBC activity. This may also allow the absorption of more resident gut *E.coli* spp. and/or *Salmonella* spp. pathogens. However, the greater volumes of colostrum, containing as it does various maternal leukocytes, oligosaccharides and peptides with antimicrobial

properties, that is ingested by the calf should kill these pathogens (Quigley, 2002; Progressive Dairyman, 2013). This phenomenon is related to the aim of this study to determine a delay in the natural gut closure by parenteral glucocorticoids. Figure 21 indicates a positive correlation between IgG and WBC at $T_{24}$ for group 4 and this linear regression analysis supports the statement that natural gut closure was delayed in this study group.

*Gut closure* – Group 4 was bled at an additional time point at $T_{42}$ compared to the control group 1 to illustrate whether or not a delay in gut-closure was experienced in this group of calves compared to the control group 1. The decline in group 1 of the IgG serum concentration from 18 hours post-partum supports previous research that normal gut closure occurs at between 12-24 hours post-partum (Stott *et al*., 1979; Arthington *et al*., 2000; Weaver *et al*., 2008). The increasing trend in IgG blood serum levels in group 4 at 24 hours post-partum to and including 42 hours post-partum is indicative of a prolonged gut-closure window (Figures 24, 25). This is supported by the increase in WBC's of group 4 at 24 hours post-partum.

*AEA%* - this is a measurement of the aptitude of an individual calf to efficiently absorb IgG rather than the measure of the amount of IgG absorbed. AEA% values typically vary between 20% - 35%, however this may be influenced by the colostral IgG titre (Quigley *et al.,* 1998). In this study, pooled colostrum of quality measuring 44.72 g/L was fed. Pooled colostrum was utilized to remove inter-calf dissimilarities. A fixed amount of colostrum was fed to each calf during this study. Although the AEA% in this study was overall satisfactory, 7 from 24 calves (29%) still suffered from FPT (blood serum levels of <10mg/ml at Cmax). This is consistent with findings from Alley and co-workers in 2012 that between 25% and 30% of all dairy calves have neonate blood serum IgG levels of <10mg/ml (Alley *et al*., 2012). This might be attributed to the negative correlation between birth weights and Plasma volume to IgG at Cmax (Table 21-22). All but one of the seven calves had a birth weight of ≥35kg. What remains a perplexity is that all the characteristics of an improved immune system i.e. reduced mortalities, no clinical or sub-clinical cases of diarrhea, no signs of respiratory stress, improved production parameters were clearly noticed, however, it is not supported by a statistical significant improved IgG absorption. Additional reasoning for the reduced AEA% or non-significant IgG absorption may

be a high bacteria count of the colostrum, the feeding of larger than normal colostrum feeding such as this, under experimental conditions and the pooling of colostrum which impacts on the Ig concentration (Jones and Heinrichs, 2009; Doepel and Bartier, 2014).

Results pertaining to weight performance (kg gain, ADG and FCR) from this study suggested that the addition of nutritional nucleotides and other necessary supplements as in IVP 1significantly improved the post-$T_{42}$ to weaning performance and/or production of the study group -2 calves. This may be critical to the successful rearing of replacement heifers in a dairy operation. The improved growth rate makes it possible to reach the breed critical mating mass (CMM) earlier and together with an improved FCR reduces the dairy carbon footprint as well as one of the largest (feed cost is approximately 60%) dairy input costs associated with replacing production cows. A healthier animal suggests a stronger immune system to further defend itself against opportunistic pathogens. Furthermore, healthier dairy animals which achieve target mass pre-production produce more milk per lactation and they are more likely to extend lactations (Quigley 2002).

*Glucocorticoids* – The results from this study showed that the parenteral addition of glucocorticoids immediately post-partum, prolonged IgG absorption of the neonate dairy calf post 24 hours when compared to the other study groups and other untreated groups and extended the time before "gut closure". This is consistent with findings by Hough and co-workers (1990) and may be attributed to the gut maturing properties of glucocorticoids, as the addition of glucocorticoids has a negative feedback on cortisol production. This is further supported by the statistically significant lower serum cortisol levels of the group 4 calves compared to the other study groups at various bleed points (Table 12).

*Growth profile (weight, ADG)* –Neonatal calf diarrhea is a persistent challenge at the study site. No additional cases of mortality were reported from $T_{42}$ until weaning at day 42 in any of the four study groups of this study. No calf diarrhea and or respiratory related diseases were reported during the 42 days pre-weaning. Compared to the study area results this is an improvement and might be attributed to improved colostrum management. The recorded birth weights (Table 9) suggested uniform

study groups as no statistically significant differences were found (P = 0.490). At day 30 the IVP1 group 2 recorded a statistically significantly difference (P = 0.043) when compared to the other study groups. The day 42 growth profile of Group 2 showed a continuing statistically significant growth when compared to the Negative control Group (P = 0.0227). However, the growth profile of Groups 3 and 4 did not differ significantly from the control group 1, at (P = 0.982) and (P = 0.6783), respectively. The primary advantage of improved growth would be reaching the breed critical mating mass (CCM) earlier than expected and with lower feed costs. Calf 4 of group 1 suffered from a mild case of joint ill for 5 days while calf 5 of this group appeared morbid from D32 - D36 post-partum. Both calves were treated with a broad spectrum antibiotic after which they recovered. This occurrence as well as the major calf syndromes i.e. diarrhoea and respiratory disease is the norm both for calves born at this farm as well as calves from other dairies in the area. The negative control is a fair reflection of the dairy reality. A consistent increase in weight of each of the calves in IVP group 2 from birth through until day 42 was noted. These results indicate a positive effect on the economical and/or growth parameters measured in this study reflecting the positive effect of an additional nutri-supplement such as nucleotides and essential vitamins, minerals, fatty acids, amino-acids and pre-biotics. The addition of vitamins and minerals in the injectable form seems to be normal practice in neonate care (Arthington et al., 2000; McGuirk and Collins, 2004).

*Feed conversion ratio (FCR)* – The procurement cost of balanced feed in most intensive operations represents the greatest proportion of the farming expenses. Case studies have shown that this cost can be as high as 43%-67% of the total cost per annum (Ho *et al.*, 2005). It is, hence, important that the animal is very efficient in its conversion of feed to milk and or beef to maintain a profitable business. An additional benefit of efficient production is, on the other hand a reduction in the methane production on the farm (Hagerty *et al.,* 2007) When compared to the negative control group and study groups 3 and 4, study Group 2 had the lowest feed to mass conversion ratio (Table 17 and Figure 32). In addition, study group 2 also produced the greatest mass gain over the 42 day period which is indicative of improved production when compared to other study groups. Calves that grow at an improved rate are healthier animals that consequently require less pharmaceutical interventions. This not only contributes favorably to the environment and the economics of calf

rearing but also the carbon footprint left by the dairy (EPA, 1993; Gibbs and Johnson, 1994).

*Carbon footprint* – Animal health management in relation to agriculture is in an exciting phase of change as the interest in natural practices to preserve the integrity of the food chain increases. Most of the world's vegetation biomass is very rich in fiber and it is well known that only ruminants can convert this into quality protein for human consumption, hence the importance of ruminants to mankind (Scholtz *et al*., 2012). This, ultimately, contributes to the emission of greenhouse gases (GHG) which impacts negatively on the carbon footprint left by dairies (Steinfeld *et al*., 2006). The livestock industry must, thus, be cognizant of the fact that it influences climate change and, therefore, it is very importance to put mechanisms in place to mitigate this effect. However, the improvement of production efficiency i.e. improved FCR is a cost effective and permanent way of reducing the carbon footprint left by dairies (Scholtz *et al*., 2012). This study measured FCR and growth rates of neonate dairy calves up to and including weaning at 42 days of age. Improved FCR is deemed indicative of increased production efficiency and a net decrease in the carbon footprint of the production phase as less resources are required for achieving the same results.

In this study it would appear that study group 2 of the investigated calves which received a nutritional supplement containing nucleotides, essential fatty acids, essential amino acids, vitamins, trace-minerals and pro-biotics had a subsequent improved production rate when compared to the negative control group 1 and study group 3 containing nucleotides only. The addition of the two limiting amino acids (AA) (Lysine and Methionine) enhances the food supplement as it is a key regulator of various pathological and physiological processes including that of nucleotides (Schwab *et al.,* 2009). Essential Fatty Acids (EFA) acts in a similar way as an immune and inflammatory modulators in the body for it to function optimally (Fritsche, 2006). Adequate intakes of vitamins and minerals are essential for the efficient functionality of the immune system (Wintergerts *et al.,* 2007). The gut immune response is influenced and manipulated by prebiotics (Vieira *et al*., 2013). It would, hence, appear that the addition of the five nutrients to nucleotides seems to have rendered group 2 healthier with an improved immune response and growth potential compared with group 3 that received a supplement containing nucleotides

alone. This appears to have resulted in more efficient feed converters and leaving less of a carbon footprint than the calves of the other study groups. This result, therefore, supports the prediction (page 54) as well as the hypotheses H1.

*Practical and economic justification* – Logistically, it might be challenging for large scale commercial dairies to fully comply with the strict requirements for good colostrum management, although to the detriment of both the neonate and the farming enterprise. While delivery of quality colostrum in the desired quantities to reduce FTP is manageable, quick and frequent delivery until "gut closure" seems to present logistical challenges to the larger dairies' workforce as birthing is not always witnessed and this problem is exacerbated by the sheer number of cows calving down in seasonal calving systems. This study has indicated that the addition of a colostrum supplement i.e. IVP 1 as well as the parenteral treatment used as a once off treatment and/or application was well accepted by the study site and its workforce.

The use of both nucleotides with vitamins and minerals and parenteral glucocorticoids resulted in significant growth by the IVP 1- group 2 and extremely low levels of cortisol with prolonged gut closure in the glucocorticoid IVP group 4, respectively. Although the results of this study are promising, the concurrent use of these two products within early life of the neonate requires further investigation.

According to in-house dairy records and site history this study site is continuously challenged by calf scours. At the time of this study a comprehensive treatment for a single case of calf scours amounted to >ZAR150 / calf (RSA local currency 2013). When compared to the investment into a colostrum supplement and/or a parenteral treatment to reduce stress, enhance the neonate immune system, stimulate growth and improve FCR at a cost of ZAR25 / calf (RSA local currency 2013) it is economically justified and well accepted on commercial farmer level. In the author's experience in the industry over 17 years it is clear that emerging small-scale farmers are open to experimenting and implementing any regime that might increase production and/or reproduction of their herd. It, therefore, is expected that little resistance will come from this farming segment towards the nutritional regime. At emerging farmer level, one might expect some resistance towards the proposed nutritional regime as milk and/or herd quality and production rate is rarely the first priority.

# 6. CHAPTER 6 - CONCLUSION

Early life events experienced by the calf appear to have long-term effects on the performance of the cow (Wittum et al., 1995; Quigley, 2002). A greater understanding of the multi-factorial issues affecting the occurrence of FPT is required if meaningful advances are to be made to progress neonatal calf mortality and morbidity. The research presented in this thesis signifies an ongoing effort to discover techniques to improve the serum Ig titre of the neonate calf as well to attempt to prolong the period of macromolecular uptake from the small intestine. Innovative new technologies i.e. colostral supplements, may provide the dairy farmer with the improved capability to manage his/her colostrum feeding program optimally.

Neonatal mortality in dairy animals worldwide is unacceptably high and resistance by the calf to diseases is highly dependent on the efficient transfer of passive immunity. A major management tool to assist in reducing health problems is the feeding of the dam's colostrum to the neonate calf. Although the ability to absorb macromolecules such as IgG across the undeveloped intestinal wall of the ruminant neonate is a unique characteristic, it remains a poorly understood process (Sangild, 2003). In spite of this, there is a great demand for high value replacement heifers in the dairy industry, and hence the high priority for exceptional management. Many bovine neonate and late life health challenges can be controlled by good quality nutrition and herd management. Nucleotides are necessary supplements to a dairy animal's normal diet as this normal diet does not support the levels of nucleotides required for growth, development (Koppel, 2003) and immunity (Carver and Walker, 1995).

Some of the most challenging calf syndromes that pre-weaned calves face are diarrhea and respiratory infections. Worldwide, these two syndromes are aggravated by poor colostrum and poor passive immunity management and, consequently, producers constantly seek alternative ways to improve the absorption of IgG and the transfer of passive immunity. The IVPs used in this study are designed to supplement colostrum and it has the potential to aid in increasing the immune response of newborn dairy calves.

According to this study it can be concluded that by treating neonate calves with glucocorticoids immediately post-partum you may delay “gut closure”, whilst feeding them good quality colostrum (<40 g - 50 g IgG/L) at a rate of at least 10% of BW plus a colostral supplement such as IVP 1 a commercial dairy farmer may contribute to improving the production parameters i.e. growth, ADG and FCR. It was concluded by Chen and co-workers (1999) that cortisol and immunoglobulin absorption is mainly affected by prematurity and that elevated cortisol serum concentrations in the neonate calf actually enhances immunoglobulin absorption.

The use of antibiotics to prevent disease and the use of sub-therapeutic levels of antibiotics to enhance animal production appears to be common practice. Unfortunately the extensive use of antibiotics leads to antibiotic resistance which in turn results in limited treatment options for both animal and man (Scott *et al.*, 2002). Treating animals with natural feed supplements together with improved colostrum management practices, achieving similar growth and immunity parameters, may well reduce the general use of antibiotics in commercial dairy herds. There is a case to be made for the economic, environmental and production efficiency welfare of animal production. There is little confirmation that the use of antibiotics in animal production influences humans until now, however, it may not be ignored. A little extra effort to implement the findings and/or recommendations of this study might result in sustainable dairy calf rearing for dairies worldwide. These sentiments echo research findings by Scott et al., (2002) who concluded that “alternatives to growth-promoting and prophylactic uses of antimicrobials in agriculture include improved management practices, wider use of vaccines, and introduction of pro-biotics”.

Table 18: Results and advantages of the addition of nutritional nucleotides

| Action | Result | Advantage | Carbon footprint (CF) |
|---|---|---|---|
| Addition of a feeding supplement i.e. Byboost Calf containing nucleotides, vitamins, trace minerals, fatty acids, amino acids and probiotics | Less morbid sick calves | Reduced antibiotic usage<br>More healthy calves | Reduced |
| | Improved FCR | Reduced production period<br>Reduced production costs | Reduced |
| | Improved growth rate | Reduced replacement rate<br>Reduced production costs | Reduced |
| | Less mortality compared to the farm average | Improved production ratio | Reduced |

*Effect on humans* - Antibiotics are injected, dosed orally or added to animal drinking water or feed to treat or prevent disease and to improve growth and production. The principal concern relates to the probability that the indiscriminate use of such antibiotics in domestic food animals could contribute to a rise in the occurrence of antibiotic resistance among bacteria that causes foodborne ailments in humans. For a portion of the human population, particularly children, the aged, and those with compromised immune systems antibiotics may be required to resolve the ailment. In such instances it could increase the severity of the disease, prolong cure or even be fatal if the specific bacteria is resistant to the antibiotic used for treatment (Barton, 2000). There is, thus, a need to ensure that antimicrobials important in human medicine are not used therapeutically or prophylactically in animal production. The findings in this study may well contribute to reduced use of antibiotics at farm level and to improved "natural" production without the use of antibiotics.

*Further research* - Obviously we have much to learn about the consistency of the response and the mechanisms that affect the early life immune system of a calf. However, it appears that there is some potential profit in spending more time and resources on the neonate at an early stage of life. The process of gut closure and the factors that regulate the process of macromolecular uptake by the enterocytes in neonate ruminants as well as the role of glucocorticoids in the mechanism of gut closure is still not fully understood and more research in this regard is necessary. An increased understanding of maternally derived immunity will enable commercial farmers to develop a superior disease prevention strategy by better "preparing colostrum" i.e. through improved vaccination programs there is an increase in the resulting antibodies available to enter the neonate calf.

Additionally, the use of nutritional additives i.e. nucleotides, probiotics etc. to increase the host *in vivo* immune response is a new approach and is likely to produce interesting data into the future (Ballou, 2012; Hanson, 2012; Soberon and Van Amburgh, 2013). The use and diverse functionality of nucleotides as a management tool allows them to be used to control stress and enhance performance of livestock. Besides enhancing the development of regulatory systems and/or organs they contribute to cellular and molecular repair mechanisms.

Reducing the carbon footprint is about doing more with less. Improving production with the same resources reduces the production of greenhouse gasses, recycling and waste management ensures sustainability whilst reducing the carbon footprint (Sheane et al., 2011). Carbon foot printing in the dairy industry is a fairly new concept and additional work is required to develop the methodology and reduce the assumptions used to calculate a carbon footprint of a dairy farm. Dairy farming systems and operations influence the environment, animal welfare, and the nutritional quality and safety of milk and dairy products in many ways (Sheane et al., 2011). It is important that the commercial dairy industry recognizes the potentially negative effect of livestock metabolism on climate change and this industry should act timeously and responsibly to mitigate this effect. Increased production efficiency and improved animal genetics may contribute to a permanent and cost effective solution to factors contributing significantly to climate change. Moreover, the most important measures to reduce greenhouse gases and improve livestock production include meeting the mineral, vitamin and essential nutrient requirements of producing dairy animals of all ages as it is crucial to achieving optimal production, maintaining herd health, improving immunity and the overall herd reproductive performance. Longevity of a dairy animal is critical to the reduction of the carbon footprint of a farm as this reduces the farmer's expenditure on feeding for young cows at a time when methane and manure is produced without the benefit of any production in terms of milk and/or meat. Thus, it is critically important to contribute actively to successful rearing of healthy calves.

The specific objectives of the study were:

1) To evaluate the effect of the addition of nutritional nucleotides, to colostrum, on immunoglobulin (Ig) absorption of the newborn calf post-partum; the results indicated that no statistically significant difference in IgG serum levels could be reported between the study groups to support this objective. This objective was not fully met (figures 10, 11, 14).

(2) To evaluate the influence of serum cortisol concentrations on the absorption of Ig in neonatal calves; a negative correlation was reported between serum cortisol levels and serum IgG levels. This objective was met (figure 19).

3) To evaluate the effect of parenteral cortisol on the rate of maturation of intestinal epithelial (natural gut closure at 24 hours post-partum). IVP 3 group 4 showed a statistically significant difference when compared to the other study groups. The IVP 3 group illustrated a positive absorption trend post 24 hours whilst the other study group illustrated a negative absorption trend post-24 hours (figures 24, 25, 26) and,

4) To determine/note any improvement in gut health (reduced neonate calf mortality and use of antibiotics to treat one of the major calf syndromes i.e. diarrhea at the study site). Calf growth (Greater kg weight gain during the study period compared to the control group 1) and in the feed conversion ratio (FCR – ratio of the amount of dry feed ration (kg) consumed to gain 1kg bodyweight). Only 1 calf from the control group died during the trial. No calf syndrome diseases were reported until weaning. Good statistical significant growth (P=0.0042) was reported from the IVP 2 group (table 1).

The author concludes that these objectives were met.

## 7. CHAPTER 7 - RECOMMENDATIONS

*Colostrum supplementation* - The term "colostrum supplement" refers to a nutritional remedy and/or treatment that is intended to enhance an IgG dose to >100 g IgG / meal and is not formulated to replace colostrum (Quigley *et al*., 2001). It is evident from this study report that commercial farmers would benefit from feeding an oral nutritional supplement such as the IVP 1, in conjunction with colostrum. This colostrum additive is formulated to provide nutrients such as vitamin E that are needed by the calf but whose concentration is inherently variable, and may be sub-optimal, in maternal colostrum. Additionally, during the early life period, energy is needed for the calf to thermoregulate, digest protein sources i.e. amino acids for protein synthesis and gluconeogenesis, as well as vitamins (water and fat soluble), chelated trace minerals (MAAC) to sustain growth and development and nucleotides to promote immunity, growth and development of all ruminants (Quigley, 2007).

*Trio Q* - As part of an effective colostrum management program commercial and all operators alike should ensure the **"Trio Q" principle** (Schoombee, 2011 – Accepted MSc dissertation - UNISA). This is, briefly:

A **Quick** colostrum supply is essential. The first colostrum meal should be within the first hour of life, thereafter followed up at < $T_6$ and a third meal at <$T_{12}$. Ensure a sufficient mass of colostrum (quality (g Ig/L) x quantity (L)). Good **Quality** colostrum, measured by KRUUSE colostrometer, containing <50 g Ig/L should be fed. A **Quantity** of at least 8%-10% of BW should be fed per meal.

*Dry cow vaccination program* (Quigley, 2002) – A comprehensive vaccination program that is representative of the areas diseases and ailments is required to "prepare" the colostrum for an optimal immune response from the calf. It is important to realize that colostrum can only contain what has been put into the cow timeously i.e. by means of comprehensive vaccination protocol.

*Clean calving environment,* (James *et al.,* 1981) – The calf GIT is sterile until birth, where after it is colonized by the local resident bacteria. An abundant overgrowth may

affect the gut integrity and secondary to that affect IgG absorption and shorten the period to gut closure.

*Addition of glucocorticoids* - It may be advantageous to calves, especially weak and premature calves, calves associated with difficult birth and/or dystocia and neonates with a high morbidity count immediately after birth, be treated with a single parenteral glucocorticoid injection at a rate of 4ml-5ml/calf, deep IM route. This management intervention could be an effective method to enhance IgG absorption (negative correlation between cortisol and IgG), reduce stress levels and assist in gut and essential organ maturation and to delay "gut closure" in such vulnerable neonates.

*Measurement of colostrum quality using a colostrometer* - Commercial operators will benefit from the use of technologies i.e. the pre-feeding measurement of colostrum quality by KRUUSE colostrometer and the calculation of AEA% of a calf to optimize their colostrum management and the efficiency of transfer of passive immunity in their farming enterprise.

*Reducing the carbon footprint of the dairy industry* - Commercial dairy farms may reduce their carbon footprint by increased production efficiency where:

- Improved FCR requires less feed for a better financial return resulting from reduced expenditure on feed while showing a concomitant increase in neonate calf weight gain and also resulting in reduced animal waste and methanogen production
- Crossbreeding where improved heterosis results in a higher slaughter weight of the progeny relative to the weight of the dam and improved genetics where a smaller yet more efficient cow is bred (Schotz *et al*., 2012)
- Improving the tempo of reaching a heifer's critical mating mass (CMM) will result in a shorter "non-productive" period hence reducing the carbon footprint left by the calf rearing phase of dairy production.

All of this is only possible when an optimally functional immune system (healthy animal) and growth potential (pre-determines genetic potential) is put in place during

the early life period by supplementing colostrum with a proven nutritional supplement.

Finally it may be concluded that the results obtained from this study support the research hypothesis that the addition of a nutritional supplement i.e. IVP 1 will reduce the need for antibiotics to prevent or cure disease and, by so doing, naturally improve the growth and feed conversion ratio without the use of growth promoters.

## 8. CONFLICT OF INTEREST

The author of this thesis is employed by Bayer Healthcare in South Africa. Bayer Healthcare is the legal distributor of the IVP 2 product which was commercialized after the onset of the clinical trials required for this study. The IVP 2 product is manufactured by a third party supplier to Bayer Healthcare, Ashkan Consulting and Animal Health. Bayer Healthcare funded the PhD research studies of the author. Ashkan Consulting and Animal Health; the manufacturers of both the products kindly donated the IVP 1 & 2 for the purpose of this study. The participation of either company did not influence or bias the performance, presentation and or integrity of the research described in this paper in any way. No other authors were involved in this work at all.

## 9. AUTHORS CONTRIBUTION

WS Schoombee designed and presented the protocol for authorization after which he conducted the study collected the data wrote the first paper draft and wrote the paper.

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## ANNEXURE 1 – Raw data

Table1: Study group orientation and Cortisol serum concentrations / bleeding point.

| | | | | | | | Blood serum cortisol concentrations (Lab Values) - nmol/l | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Calf no.** | **IVP** | **Tag** | Group | **Calf no** | **Calf gender** | **BM (Kg)** | **Bleed $T_1$** | **Bleed $T_2$** | **Bleed $T_3$** | **Bleed $T_4$** | **Bleed $T_5$** | **Bleed $T_6$** | **Bleed $T_7$** | **Bleed $T_8$** | **Avg / calf** |
| 1 | Negative control | green | 1 | 1 | m | 43 | 263.0 | 356.0 | 364.0 | 309.0 | - | - | - | | 184.6 |
| 2 | | green | 1 | 2 | f | 37 | 247.0 | 142.0 | 247.0 | 256.0 | 298.0 | 234.0 | 191.0 | | 230.7 |
| 3 | | green | 1 | 3 | m | 35 | 359.0 | 144.0 | 154.0 | 170.0 | 215.0 | 150.0 | 151.0 | | 191.9 |
| 4 | | green | 1 | 4 | f | 44 | 367.0 | 120.0 | 145.0 | 97.7 | 182.0 | 166.0 | 47.2 | | 160.7 |
| 5 | | green | 1 | 5 | m | 44 | 281.0 | 209.0 | 150.0 | 183.0 | 172.0 | 119.0 | 106.0 | | 174.3 |
| 6 | | green | 1 | 6 | f | 33 | 364.0 | 231.0 | 214.0 | 231.0 | 233.0 | 237.0 | 126.0 | | 233.7 |
| 8 | Nucleotides | yellow | 2 | 8 | m | 37 | 255.0 | 129.0 | 147.0 | 105.0 | 163.0 | 183.0 | 102.0 | | 154.9 |
| 9 | Vitamins | yellow | 2 | 9 | f | 39 | 403.0 | 154.0 | 175.0 | 222.0 | 202.0 | 229.0 | 174.0 | | 222.7 |
| 10 | Minerals | yellow | 2 | 10 | f | 38 | 315.0 | 88.6 | 167.0 | 126.0 | 191.0 | 187.0 | 146.0 | | 174.4 |
| 11 | Pre-biotics | yellow | 2 | 11 | m | 35 | 331.0 | 214.0 | 173.0 | 172.0 | 193.0 | 261.0 | 143.0 | | 212.4 |
| 12 | EFA | yellow | 2 | 12 | m | 33 | 217.0 | 173.0 | 72.0 | 72.0 | 130.0 | 97.7 | 81.1 | | 120.4 |
| 13 | EAA | yellow | 2 | 13 | f | 38 | 331.0 | 169.0 | 151.0 | 146.0 | 126.0 | 162.0 | 147.0 | | 176.0 |
| 15 | Nucleotides | orange | 3 | 15 | m | 42 | 411.0 | 188.0 | 201.0 | 233.0 | 284.0 | 155.0 | 110.0 | | 226.0 |
| 16 | | orange | 3 | 16 | f | 35 | 342.0 | 162.0 | 113.0 | 240.0 | 83.9 | 104.0 | 35.3 | | 154.3 |
| 17 | | orange | 3 | 17 | m | 43 | 323.0 | 143.0 | 126.0 | 264.0 | 154.0 | 164.0 | 27.6 | | 171.7 |
| 18 | | orange | 3 | 18 | f | 32 | 403.0 | 244.0 | 147.0 | 414.0 | 419.0 | 389.0 | 198.0 | | 316.3 |
| 19 | | orange | 3 | 19 | f | 32 | 411.0 | 246.0 | 137.0 | 69.3 | 199.0 | 69.3 | 79.2 | | 173.0 |
| 20 | | orange | 3 | 20 | m | 33 | 266.0 | 48.0 | 170.0 | 190.0 | 175.0 | 103.0 | 55.5 | | 143.9 |
| 22 | Glucocorticoids | white | 4 | 22 | f | 42 | 287.0 | 28.1 | 27.6 | 27.6 | 27.6 | 27.6 | 200.0 | 27.6 | 81.6 |
| 23 | | white | 4 | 23 | m | 35 | 274.0 | 32.0 | 27.6 | 27.6 | 27.6 | 27.6 | 27.6 | 27.6 | 59.0 |
| 24 | | white | 4 | 24 | m | 35 | 252.0 | 30.1 | 27.6 | 27.6 | 27.6 | 112.0 | 27.6 | 27.6 | 66.5 |
| 25 | | white | 4 | 25 | f | 37 | 350.0 | 28.4 | 27.6 | 27.6 | 27.6 | 27.6 | 27.6 | 27.6 | 68.0 |
| 26 | | white | 4 | 26 | m | 34 | 274.0 | 35.3 | 27.6 | 29.2 | 27.6 | 27.6 | 27.6 | 27.6 | 59.6 |
| 27 | | white | 4 | 27 | m | 35 | 303.0 | 27.6 | 27.6 | 29.5 | 27.6 | 27.6 | 27.6 | 53.5 | 65.5 |

Table 2: SP ratio, IgG serum concentrations and Cmax / bleeding point.

**Blood serum IgG (SP ratio) concentrations (Lab Values) - ng/ml**

| Calf no. | $T_{<1}$ | IgG ng/ml | $T_6$ | IgG ng/ml | $T_{12}$ | IgG ng/ml | $T_{18}$ | IgG ng/ml | $T_{24}$ | IgG ng/ml | $T_{30}$ | IgG ng/ml | $T_{36}$ | IgG ng/ml | $T_{42}$ | IgG ng/ml | $C^{max}$ SP Ratio | $C^{max}$ IgG |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.000 | 0.000 | 1.312 | 88.071 | 1.674 | 114.789 | 1.977 | 137.151 | | | | | | | | | 1.977 | 137.560 |
| 2 | 0.000 | 0.000 | 1.719 | 118.11 | 1.730 | 118.922 | 1.999 | 138.775 | 2.005 | 139.218 | 1.856 | 128.221 | 1.803 | 124.309 | | | 2.005 | 139.222 |
| 3 | 0.000 | 0.000 | 1.873 | 129.476 | 1.691 | 116.043 | 1.997 | 138.627 | 1.891 | 130.804 | 1.845 | 127.409 | 1.870 | 129.254 | | | 1.997 | 138.632 |
| 4 | 0.000 | 0.000 | 2.259 | 157.964 | 2.116 | 147.41 | 2.039 | 141.727 | 1.948 | 135.011 | 1.837 | 126.819 | 1.951 | 135.232 | | | 2.259 | 157.969 |
| 5 | -0.009 | -8.096 | 2.082 | 144.901 | 2.188 | 152.724 | 1.768 | 121.726 | 1.770 | 121.874 | 1.631 | 111.615 | 1.670 | 114.493 | | | 2.188 | 152.729 |
| 6 | 0.000 | 0.000 | 2.076 | 144.458 | 1.997 | 138.627 | 1.925 | 133.313 | 1.959 | 135.823 | 1.936 | 134.125 | 1.929 | 133.609 | | | 2.076 | 144.463 |
| 8 | 0.176 | 4.230 | 1.537 | 104.677 | 1.292 | 86.595 | 1.521 | 103.496 | 1.565 | 106.744 | 1.426 | 96.485 | 1.138 | 75.229 | | | 1.565 | 106.747 |
| 9 | 0.000 | 0.000 | 1.596 | 109.032 | 1.693 | 116.191 | 1.726 | 118.626 | 1.923 | 133.166 | 1.772 | 122.021 | 1.786 | 123.055 | | | 1.923 | 133.170 |
| 10 | 0.000 | 0.000 | 1.852 | 127.926 | 1.833 | 126.523 | 2.063 | 143.498 | 1.950 | 135.159 | 1.996 | 138.554 | 1.853 | 128.000 | | | 2.063 | 143.503 |
| 11 | 0.000 | 0.000 | 1.740 | 119.66 | 2.059 | 143.203 | 1.810 | 124.826 | 1.877 | 129.771 | 1.625 | 111.172 | 1.715 | 117.815 | | | 2.059 | 143.208 |
| 12 | 0.000 | 0.000 | 2.215 | 154.717 | 1.911 | 132.28 | 1.964 | 136.192 | 1.961 | 135.970 | 1.904 | 131.764 | 1.954 | 135.454 | | | 2.215 | 154.722 |
| 13 | 0.137 | 1.351 | 2.101 | 146.303 | 1.773 | 122.095 | 1.829 | 126.228 | 1.757 | 120.914 | 1.554 | 105.932 | 1.546 | 105.342 | | | 2.101 | 146.308 |
| 15 | 0.000 | 0.000 | 1.735 | 119.291 | 1.908 | 132.059 | 1.709 | 117.372 | 2.201 | 153.683 | 1.971 | 136.708 | 1.840 | 127.040 | | | 2.201 | 153.688 |
| 16 | 0.000 | 0.000 | 1.836 | 126.745 | 1.917 | 132.723 | 2.004 | 139.144 | 2.083 | 144.975 | 1.793 | 123.571 | 1.843 | 127.261 | | | 2.083 | 144.979 |
| 17 | 0.000 | 0.000 | 1.933 | 133.904 | 1.946 | 134.863 | 1.937 | 134.199 | 1.904 | 131.764 | 1.807 | 124.605 | 1.902 | 131.616 | | | 1.946 | 134.868 |
| 18 | 0.121 | 0.170 | 1.986 | 137.816 | 1.878 | 129.845 | 1.865 | 128.885 | 1.795 | 123.719 | 1.706 | 117.150 | 1.612 | 110.213 | | | 1.986 | 137.820 |
| 19 | 0.000 | 0.000 | 2.158 | 150.51 | 1.940 | 134.421 | 1.774 | 122.169 | 1.923 | 133.166 | 1.915 | 132.575 | 1.865 | 128.885 | | | 2.158 | 150.515 |
| 20 | 0.000 | 0.000 | 2.056 | 142.982 | 1.931 | 133.756 | 2.010 | 139.587 | 1.909 | 132.133 | 1.918 | 132.797 | 1.963 | 136.118 | | | 2.056 | 142.987 |
| 22 | 0.000 | 0.000 | 1.536 | 104.604 | 1.603 | 109.548 | 1.747 | 120.176 | 2.156 | 150.362 | 1.894 | 131.026 | 1.812 | 124.974 | 1.739 | 119.586 | 2.156 | 150.367 |
| 23 | 0.000 | 0.000 | 1.625 | 111.172 | 1.486 | 100.913 | 1.917 | 132.723 | 2.172 | 151.543 | 1.911 | 132.280 | 1.945 | 134.790 | 1.920 | 132.944 | 2.172 | 151.548 |
| 24 | 0.000 | 0.000 | 1.821 | 125.638 | 1.997 | 138.627 | 1.909 | 132.133 | 1.924 | 133.240 | 1.924 | 133.240 | 1.878 | 129.845 | 1.984 | 137.668 | 1.997 | 138.632 |
| 25 | 0.000 | 0.000 | 2.109 | 146.893 | 1.950 | 135.159 | 2.083 | 144.975 | 1.983 | 137.594 | 1.902 | 131.616 | 2.068 | 143.720 | 2.068 | 143.867 | 2.109 | 146.898 |
| 26 | 0.000 | 0.000 | 2.107 | 146.746 | 1.996 | 138.554 | 1.952 | 135.306 | 2.073 | 144.237 | 1.899 | 131.395 | 2.104 | 146.524 | 2.532 | 178.113 | 2.532 | 178.118 |
| 27 | 0.000 | 0.000 | 2.241 | 156.636 | 1.990 | 138.111 | 1.929 | 133.609 | 1.974 | 136.930 | 1.881 | 130.066 | 1.938 | 134.273 | 2.379 | 166.821 | 2.379 | 166.826 |

Table 3: FBC at $T_0$.

| | | | FBC (First bleed) - birth | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Range | | | 5.00 -9.00 | 8.00 - 14.00 | 24.00 - 40.00 | 40.00 - 60.00 | 30.00 - 36.00 | 4.00 - 10.00 | 200.00 - 600.00 | |
| | | | x10^12/l | g/dl | % | fl | g/dl | x10^9/l | x10^9/l | |
| Group | Calf | No. | RBC | Haemoglobin | Haematocrit | Mean cell volume | Mean cell haem. | WBC | Platelet count | Test value |
| 1 | 1 | 1 | 8.67 | 11.00 | 36.00 | 41.50 | 30.60 | **9.10** | 416.00 | Satisfactory |
| 1 | 2 | 2 | 8.47 | 11.20 | 37.90 | 44.70 | 29.60 | **7.92** | 326.00 | Satisfactory |
| 1 | 3 | 3 | 6.98 | 9.90 | 33.10 | 47.40 | 29.90 | **9.42** | 343.00 | Satisfactory |
| 1 | 4 | 4 | 9.41 | 12.80 | 43.80 | 46.50 | 29.20 | **8.44** | 620.00 | Satisfactory |
| 1 | 5 | 5 | 7.60 | 9.80 | 34.10 | 44.90 | 28.70 | **7.41** | 370.00 | Satisfactory |
| 1 | 6 | 6 | 6.73 | 9.00 | 31.40 | 46.70 | 28.70 | **4.71** | 313.00 | Satisfactory |
| 2 | 8 | 8 | 9.94 | 12.10 | 42.20 | 42.50 | 28.70 | **10.86** | 378.00 | Satisfactory |
| 2 | 9 | 9 | 7.51 | 9.90 | 32.80 | 43.70 | 30.20 | **8.48** | 386.00 | Satisfactory |
| 2 | 10 | 10 | 9.13 | 11.60 | 38.00 | 41.60 | 30.50 | **10.66** | 460.00 | Satisfactory |
| 2 | 11 | 11 | 8.11 | 10.30 | 37.70 | 46.50 | 27.30 | **15.80** | 343.00 | Satisfactory |
| 2 | 12 | 12 | 8.36 | 10.90 | 35.70 | 42.70 | 30.50 | **8.78** | 200.00 | Satisfactory |
| 2 | 13 | 13 | 7.07 | 8.30 | 28.80 | 40.70 | 28.80 | **15.50** | 413.00 | Satisfactory |
| 3 | 15 | 15 | 8.16 | 9.90 | 34.80 | 42.60 | 28.40 | **5.70** | 483.00 | Satisfactory |
| 3 | 16 | 16 | 11.72 | 13.50 | 44.40 | 37.90 | 30.40 | **8.99** | 596.00 | Satisfactory |
| 3 | 17 | 17 | 8.85 | 12.20 | 42.80 | 48.40 | 28.50 | **16.81** | 197.00 | Satisfactory |
| 3 | 18 | 18 | 8.49 | 12.30 | 41.20 | 48.50 | 29.90 | **14.13** | 325.00 | Satisfactory |
| 3 | 19 | 19 | 8.69 | 11.40 | 38.50 | 44.30 | 29.60 | **7.59** | 146.00 | Satisfactory |
| 3 | 20 | 20 | 8.92 | 11.00 | 35.70 | 40.00 | 30.80 | **3.27** | 569.00 | Satisfactory |
| 4 | 22 | 22 | 9.55 | 12.70 | 43.20 | 45.20 | 29.40 | **8.93** | 362.00 | Satisfactory |
| 4 | 23 | 23 | 8.31 | 10.90 | 36.80 | 44.30 | 29.60 | **6.99** | 849.00 | Satisfactory |
| 4 | 24 | 24 | 8.14 | 10.30 | 34.50 | 42.40 | 29.90 | **7.90** | 348.00 | Satisfactory |
| 4 | 25 | 25 | 9.80 | 12.10 | 39.10 | 39.90 | 30.90 | **7.49** | 487.00 | Satisfactory |
| 4 | 26 | 26 | 8.43 | 10.10 | 33.90 | 40.20 | 29.80 | **6.63** | 789.00 | Satisfactory |
| 4 | 27 | 27 | 6.40 | 8.40 | 28.40 | 44.40 | 29.60 | **4.54** | 559.00 | Satisfactory |

Table 4: FBC at $T_{24.}$

| | | | FBC (Second bleed) - T24 | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Range | | | 5.00 -9.00 | 8.00 - 14.00 | 24.00 - 40.00 | 40.00 - 60.00 | 30.00 - 36.00 | 4.00 - 10.00 | 200.00 - 600.00 | |
| | | | x10^12/l | g/dl | % | fl | g/dl | x10^9/l | x10^9/l | |
| Group | Calf | No. | RBC | Haemoglobin | Haematocrit | Mean cell volume | Mean cell haem. | WBC | Platelet count | Test value |
| 1 | 1 | 113 | | | | | | | | Satisfactory |
| 1 | 2 | 114 | 7.99 | 10.50 | 34.60 | 43.30 | 30.30 | 9.25 | 215.00 | Satisfactory |
| 1 | 3 | 115 | 6.97 | 9.90 | 32.00 | 45.90 | 30.90 | 13.19 | 473.00 | Satisfactory |
| 1 | 4 | 116 | 8.35 | 11.00 | 37.30 | 44.70 | 29.50 | 9.99 | 294.00 | Satisfactory |
| 1 | 5 | 117 | 7.35 | 9.50 | 32.10 | 43.70 | 29.60 | 10.82 | 296.00 | Satisfactory |
| 1 | 6 | 118 | 5.69 | 7.60 | 24.90 | 43.80 | 30.50 | 5.31 | 232.00 | Satisfactory |
| 2 | 8 | 120 | 8.63 | 10.50 | 34.40 | 39.90 | 30.50 | 9.78 | 382.00 | Satisfactory |
| 2 | 9 | 121 | 6.62 | 8.60 | 28.00 | 42.30 | 30.70 | 9.36 | 465.00 | Satisfactory |
| 2 | 10 | 122 | 9.72 | 12.30 | 38.80 | 39.90 | 31.70 | 8.29 | 342.00 | Satisfactory |
| 2 | 11 | 123 | 7.82 | 9.70 | 33.10 | 42.30 | 29.30 | 10.19 | 304.00 | Satisfactory |
| 2 | 12 | 124 | 7.53 | 9.50 | 30.20 | 40.10 | 31.50 | 9.03 | 341.00 | Satisfactory |
| 2 | 13 | 125 | 6.85 | 7.80 | 26.60 | 38.80 | 29.30 | 11.70 | 261.00 | Satisfactory |
| 3 | 15 | 127 | 7.42 | 8.90 | 30.50 | 41.10 | 29.20 | 6.96 | 454.00 | Satisfactory |
| 3 | 16 | 128 | 10.61 | 12.40 | 39.00 | 36.80 | 31.80 | 10.57 | 403.00 | Satisfactory |
| 3 | 17 | 129 | 8.10 | 11.20 | 37.30 | 46.00 | 30.00 | 11.56 | 234.00 | Satisfactory |
| 3 | 18 | 130 | 7.92 | 11.10 | 37.70 | 47.60 | 29.40 | 16.60 | 193.00 | Satisfactory |
| 3 | 19 | 131 | 7.43 | 9.40 | 31.90 | 42.90 | 29.50 | 8.78 | 210.00 | Satisfactory |
| 3 | 20 | 132 | 8.62 | 10.40 | 33.00 | 38.30 | 31.50 | 6.35 | 105.00 | Satisfactory |
| 4 | 22 | 134 | 7.79 | 10.30 | 34.30 | 44.00 | 30.00 | 22.18 | 441.00 | Satisfactory |
| 4 | 23 | 135 | 7.41 | 9.60 | 31.80 | 42.90 | 30.20 | 27.70 | 514.00 | Satisfactory |
| 4 | 24 | 136 | 7.49 | 9.10 | 30.10 | 40.20 | 30.20 | 18.50 | 632.00 | Satisfactory |
| 4 | 25 | 137 | 9.12 | 11.00 | 34.70 | 38.00 | 31.70 | 16.70 | 235.00 | Satisfactory |
| 4 | 26 | 138 | 8.14 | 9.50 | 31.60 | 38.80 | 30.10 | 19.80 | 363.00 | Satisfactory |
| 4 | 27 | 139 | 6.09 | 7.70 | 25.90 | 42.50 | 29.70 | 10.01 | 380.00 | Satisfactory |

Table 5: Growth profile / calf / group.

| Group | Calf no. | Gender | BW D0 | W 30D | W 42D | Gain1 | ADG (kg) | Gain 2 | ADG (kg) | Feed (Kg) | Waste kg | FCR (kg) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | m | 43 | 0 | 0 | 0 | - | 0 | 0 | 0 | 0 | 0 |
| 1 | 2 | f | 37 | 51 | 57 | 14 | 0.333 | 6 | 0.476 | 72.000 | 0 | 0 |
| 1 | 3 | m | 35 | 49 | 51 | 14 | 0.333 | 2 | 0.381 | 64.000 | 0 | 0 |
| 1 | 4 | f | 44 | 48 | 56 | 4 | 0.095 | 8 | 0.286 | 32.000 | 0 | 0 |
| 1 | 5 | m | 44 | 40 | 38 | -4 | - 0.095 | -2 | -0.143 | 12.000 | 0 | 0 |
| 1 | 6 | f | 33 | 44 | 41 | 11 | 0.262 | -3 | 0.190 | 20.000 | 0 | 0 |
| | | | **193** | **232** | **243** | **39** | **0.155** | **11** | **0.238** | **200.00** | **0.35** | **4.000** |
| 2 | 8 | m | 37 | 48 | 54 | 11 | 0.262 | 6 | 0.405 | 44.074 | 0 | 0 |
| 2 | 9 | f | 39 | 53 | 57 | 14 | 0.333 | 4 | 0.429 | 46.667 | 0 | 0 |
| 2 | 10 | f | 38 | 49 | 59 | 11 | 0.262 | 10 | 0.500 | 54.444 | 0 | 0 |
| 2 | 11 | m | 35 | 51 | 59 | 16 | 0.381 | 8 | 0.571 | 62.222 | 0 | 0 |
| 2 | 12 | m | 33 | 54 | 62 | 21 | 0.500 | 8 | 0.690 | 75.185 | 0 | 0 |
| 2 | 13 | f | 38 | 59 | 64 | 21 | 0.500 | 5 | 0.619 | 67.407 | 0 | 0 |
| | | | **220** | **314** | **355** | **94** | **0.373** | **41** | **0.562** | **350.00** | **0.50** | **2.593** |
| 3 | 15 | m | 42 | 50 | 48 | 8 | 0.190 | -2 | 0.143 | 16.216 | 0 | 0 |
| 3 | 16 | f | 35 | 51 | 57 | 16 | 0.381 | 6 | 0.524 | 59.459 | 0 | 0 |
| 3 | 17 | m | 43 | 57 | 53 | 14 | 0.333 | -4 | 0.238 | 27.027 | 0 | 0 |
| 3 | 18 | f | 32 | 40 | 42 | 8 | 0.190 | 2 | 0.238 | 27.027 | 0 | 0 |
| 3 | 19 | f | 32 | 45 | 47 | 13 | 0.310 | 2 | 0.357 | 40.541 | 0 | 0 |
| 3 | 20 | m | 33 | 43 | 44 | 10 | 0.238 | 1 | 0.262 | 29.730 | 0 | 0 |
| | | | **217** | **286** | **291** | **69** | **0.274** | **5** | **0.324** | **200.00** | **0.45** | **2.703** |
| 4 | 22 | f | 42 | 51 | 53 | 9 | 0.214 | 2 | 0.262 | 33.133 | 0 | 0 |
| 4 | 23 | m | 35 | 47 | 51 | 12 | 0.286 | 4 | 0.381 | 48.193 | 0 | 0 |
| 4 | 24 | m | 35 | 47 | 47 | 12 | 0.286 | 0 | 0.286 | 36.145 | 0 | 0 |
| 4 | 25 | f | 37 | 45 | 48 | 8 | 0.190 | 3 | 0.262 | 33.133 | 0 | 0 |
| 4 | 26 | m | 34 | 46 | 50 | 12 | 0.286 | 4 | 0.381 | 48.193 | 0 | 0 |
| 4 | 27 | m | 35 | 48 | 52 | 13 | 0.310 | 4 | 0.405 | 51.205 | 0 | 0 |
| | | | **218** | **284** | **301** | **66** | **0.262** | **17** | **0.343** | **250.00** | **0.60** | **3.012** |

Table 6: AEA, AEA%, Mass of IgG / calf/ group.

| | | Cmax | | Avg T36 | | | | Colostrum | | T36 | Cmax | Mass | AEA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Group | Calf | IgG (ng/ml) | IgG (mg/L) | IgG (ng/ml) | IgG (mg/L) | BW (kg) | Act. PV (L) | Meals (L) | IgG (g/L) | AEA% | AEA% | g | g/L |
| 1 | 1 | 137.56 | 0.138 | 95.898 | 0.096 | 43 | 3.913 | 4.5 | 44.72 | 18.65% | 26.75% | 53.83 | 11.9616 |
| 1 | 2 | 139.22 | 0.139 | 127.930 | 0.128 | 37 | 3.367 | 4.5 | 44.72 | 21.40% | 23.29% | 46.88 | 10.4169 |
| 1 | 3 | 138.63 | 0.139 | 128.594 | 0.129 | 35 | 3.185 | 4.5 | 44.72 | 20.35% | 21.94% | 44.15 | 9.8121 |
| 1 | 4 | 157.97 | 0.158 | 140.699 | 0.141 | 44 | 4.004 | 4.5 | 44.72 | 27.99% | 31.43% | 63.25 | 14.0557 |
| 1 | 5 | 152.73 | 0.153 | 127.930 | 0.128 | 44 | 4.004 | 4.5 | 44.72 | 25.45% | 30.39% | 61.15 | 13.5895 |
| 1 | 6 | 144.46 | 0.144 | 136.639 | 0.137 | 33 | 3.003 | 4.5 | 44.72 | 20.39% | 21.56% | 43.38 | 9.6405 |
| 2 | 8 | 106.75 | 0.107 | 95.529 | 0.096 | 37 | 3.367 | 4.5 | 44.72 | 15.98% | 17.86% | 35.94 | 7.9870 |
| 2 | 9 | 133.17 | 0.133 | 120.328 | 0.120 | 39 | 3.549 | 4.5 | 44.72 | 21.22% | 23.49% | 47.26 | 10.5027 |
| 2 | 10 | 143.50 | 0.144 | 133.318 | 0.133 | 38 | 3.458 | 4.5 | 44.72 | 22.91% | 24.66% | 49.62 | 11.0274 |
| 2 | 11 | 143.21 | 0.143 | 124.387 | 0.124 | 35 | 3.185 | 4.5 | 44.72 | 19.69% | 22.67% | 45.61 | 10.1359 |
| 2 | 12 | 154.72 | 0.155 | 137.746 | 0.138 | 33 | 3.003 | 4.5 | 44.72 | 20.56% | 23.09% | 46.46 | 10.3251 |
| 2 | 13 | 146.31 | 0.146 | 121.140 | 0.121 | 38 | 3.458 | 4.5 | 44.72 | 20.82% | 25.14% | 50.59 | 11.2430 |
| 3 | 15 | 153.69 | 0.154 | 131.030 | 0.131 | 42 | 3.822 | 4.5 | 44.72 | 24.89% | 29.19% | 58.74 | 13.0532 |
| 3 | 16 | 144.98 | 0.145 | 132.432 | 0.132 | 35 | 3.185 | 4.5 | 44.72 | 20.96% | 22.95% | 46.18 | 10.2613 |
| 3 | 17 | 134.87 | 0.135 | 131.842 | 0.132 | 43 | 3.913 | 4.5 | 44.72 | 25.64% | 26.22% | 52.77 | 11.7275 |
| 3 | 18 | 137.82 | 0.138 | 124.609 | 0.125 | 32 | 2.912 | 4.5 | 44.72 | 18.03% | 19.94% | 40.13 | 8.9185 |
| 3 | 19 | 150.52 | 0.151 | 133.613 | 0.134 | 32 | 2.912 | 4.5 | 44.72 | 19.33% | 21.78% | 43.83 | 9.7400 |
| 3 | 20 | 142.99 | 0.143 | 136.270 | 0.136 | 33 | 3.003 | 4.5 | 44.72 | 20.33% | 21.34% | 42.94 | 9.5420 |
| 4 | 22 | 150.37 | 0.150 | 122.911 | 0.123 | 42 | 3.822 | 4.5 | 44.72 | 23.34% | 28.56% | 57.47 | 12.7712 |
| 4 | 23 | 151.55 | 0.152 | 128.078 | 0.128 | 35 | 3.185 | 4.5 | 44.72 | 20.27% | 23.99% | 48.27 | 10.7262 |
| 4 | 24 | 138.63 | 0.139 | 132.949 | 0.133 | 35 | 3.185 | 4.5 | 44.72 | 21.04% | 21.94% | 44.15 | 9.8121 |
| 4 | 25 | 146.90 | 0.147 | 140.551 | 0.141 | 37 | 3.367 | 4.5 | 44.72 | 23.52% | 24.58% | 49.46 | 10.9912 |
| 4 | 26 | 178.12 | 0.178 | 145.865 | 0.146 | 34 | 3.094 | 4.5 | 44.72 | 22.43% | 27.39% | 55.11 | 12.2466 |
| 4 | 27 | 166.83 | 0.167 | 142.322 | 0.142 | 35 | 3.185 | 4.5 | 44.72 | 22.53% | 26.40% | 53.13 | 11.8076 |