

**EVALUATION OF SODIUM BUTYRATE AND  
CONCENTRATE CRUDE PROTEIN LEVEL ON THE  
HEALTH OF ARTIFICIALLY REARED BEEF CALVES TO 12  
WEEKS**

by

PETER WILLIAMS

being an Honours Research Project submitted in partial fulfilment  
of the requirements for the  
BSC (Honours) Degree in  
Agriculture

2015

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## **List of Abbreviations**

BRD: Bovine respiratory disease

CMR: Calf milk replacer

CP: Crude protein

DLWG: Daily live weight gain

Ig: Immunoglobulin

RDP: Rumen degradable protein

SB: Sodium butyrate

UDP: Rumen undegradable protein

VFA: Volatile fatty acid



1<sup>st</sup> Author: Peter Williams  
BSc Honours Agriculture

2<sup>nd</sup> Author: Dr. Emma Bleach

Sponsored by: Bonanza Calf Nutrition

## Introduction

- In order to artificially rear calves successfully it is important to achieve optimum growth rates without compromising calf health, whilst remaining cost effective to ensure profitability.
- It has been shown that supplementing sodium butyrate can enhance small intestine development in calves (Górka *et al.*, 2014), and improve growth rates (Kelly *et al.*, 2014). Reduced crude protein levels in concentrates can be fed without having negative effects on calf health (Hanson, 2014).
- However, most of these studies focus on calf performance, rather than the effect on health. Moreover the interaction between the effects on calves fed lower crude protein levels and supplementing sodium butyrate has not been investigated.

## Objectives:

The study will determine whether the use of lower protein concentrate, costing less than regular concentrate, can be effectively combined with supplemented sodium butyrate without having any negative impact on calf health.

## Research Hypothesis:

Sodium butyrate can be combined with a lower protein concentrate to improve performance without having a negative effect on calf health.

## Benefits:

Sodium butyrate can enhance pre-weaning performance; if it can be achieved with lower crude protein levels in concentrate without compromising the health of the calf then it will establish a more cost effective way to artificially rear calves whilst achieving improved performance.

**References:** Górka, P., Pietrzak, P., Kotunia, A., Zabielski, R. and Kowlaski, Z. M., 2014. Effect of method of delivery of sodium butyrate on maturation of the small intestine in newborn calves. *Journal of Dairy Science*, 97. pp. 1026-1035.

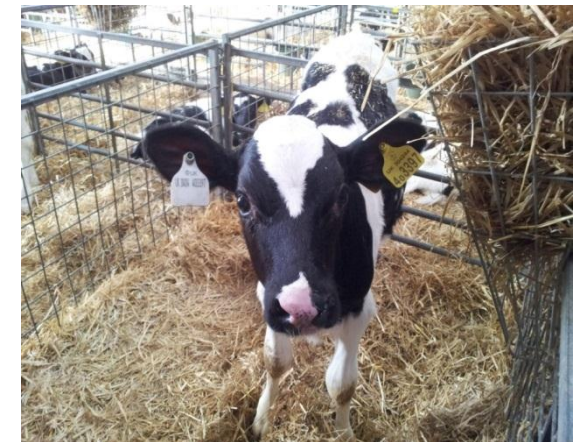
Kelly, A., O'Doherty, J., Kenny, D., Boland, T. and Pierce, K., 2014. *Performance and rumen development of artificially reared calves to dietary butyrate supplementation. 2014 ADSA-ASAS-CSAS Joint Annual Meeting. [On-line]. Asas. Abstract only. Available from: <https://asas.confex.com/asas/jam2014/webprogram/Paper6511.html> [Accessed 15/11/2014].*

Hanson, J. R. 2014. *Evaluation of early weaning concentrate quality on the health of artificially reared beef calves to 12 weeks.* Newport: Harper Adams University.

## Methodology

- 48 Holstein (n=24) and continental cross (n=24) calves will assigned onto one of four treatments groups based on live weight, breed and age in a 2x2 factorial design.
- The calves will be housed in individual pens with water and straw *ad lib*.
- They will be fed Shine milk replacer twice daily at 175g per 825ml water per feed, either with or without sodium butyrate, and *ad lib*. concentrates with a crude protein level of either 16% or 18%. The treatments are as follows:
  - Na butyrate + 16% crude protein (n=12)
  - Na butyrate + 18% crude protein (n=12)
  - Control + 16% crude protein (n=12)
  - Control + 18% crude protein (n=12)
- The calves will then be weaned after 6 weeks, and continue to be fed the same concentrates for a subsequent 6 weeks.
- Individual calf health will be monitored 3x per week while on milk, at weaning and again at the end of the trial using the following criteria:

- Dehydration score 1-5
- Cough score 0-3
- Nasal discharge 0-3
- Eye discharge score 0-3
- Ear score 0-3
- Faecal score 0-3
- Coat bloom score -1-5



- Results will be analysed through Genstat using ANOVA and X<sup>2</sup>.

## **Acknowledgements**

This project would not have been possible without the help of the following people, and I would like to thank them for their contribution:

Dr Emma Bleach, my supervisor who provided guidance and advice throughout;

Simon Marsh, responsible for organising the trial;

Jak Ramsey, for undertaking this trial with me and sharing the workload;

Giles Vince, Nicola Naylor and all the staff on Harper beef unit for all their help in running the trial;

Bonanza Calf Nutrition for kindly sponsoring the trial;

My family and friends, for their help and advice in writing this project.

## Evaluation of sodium butyrate and concentrate crude protein level on the health of artificially reared beef calves to 12 weeks

P. Williams and E. Bleach.  
*BSc Agriculture*

**Introduction:** Sodium butyrate supplemented within the calf's diet can enhance growth of the calf and immunological response (Kelly *et al.*, 2014). It can also improve rumen development, enhancing rumen size and papillae development, as well as reducing diarrhoea incidence, reducing treatment costs and production losses (Górka *et al.*, 2011). Whilst the effect of sodium butyrate on pre-weaning calf health and performance is documented, the long term effects are less well understood (Górka *et al.*, 2011). Crude protein is an expensive component of the calf's diet, and a reduction of crude protein usage would reduce rearing costs. Calves fed reduced crude protein have been shown to achieve more efficient and cost effective growth (Hill *et al.*, 2008). The objective of this trial is to study the effects of sodium butyrate supplementation and reduced crude protein on the health of artificially reared calves in both the pre and post-weaning stages.

**Materials and Methods:** The study was conducted at Harper Adams University, Shropshire, used 48 Holstein-Friesian and Continental-cross bull calves. Calves were randomly allocated to one of four treatments. Calves were fed Shine whey, skim and buttermilk based calf milk replacer (CMR) containing 20% crude protein (CP) and 17% oil (Bonanza Calf Nutrition, Dundalk) with 15g/kg sodium butyrate (SB) or without as a control and concentrates with 16% CP content or 18% CP content as a control. The treatment groups were: control CMR and 18% CP concentrate; control CMR and 16% CP concentrate; CMR with sodium butyrate and 18% CP concentrate; CMR with SB and 16% CP concentrate. Calves were penned individually and provided straw, water and concentrate *ad libitum*. Calves were fed 1.7 litres of CMR twice daily at a rate of 175g per 825ml water twice a day at 37°C. Calves were vaccinated against respiratory disease after 3 weeks. After 6 weeks, once consuming 1kg concentrates for 3 consecutive days calves were weaned and moved to group housing and fed treatment concentrate for a further 6 weeks. Health scores were taken for 7 criteria: dehydration, coughing, nasal discharge, ocular discharge, ear droop, coat bloom and faecal consistency. Scores were taken 3 times a week pre-weaning, once at the point of weaning and again at the conclusion of the trial (week 12). Clinical incidence of diarrhoea, respiratory disease and mortality were recorded.

**Results:** There was no effect of either SB or CP on calf health. Of the health indicators measured associated with enteric disease, faecal consistency ( $P=0.96$ ), dehydration ( $P=0.38$ ), coat bloom ( $P=0.9$ ) and clinical diarrhoea incidence ( $P=0.48$ ) there were no significant interactions between SB and CP. Of the health indicators associated with respiratory disease cough score ( $P=0.36$ ), nasal discharge ( $P=0.29$ ), ocular discharge ( $P=0.62$ ) and ear droop ( $P=0.95$ ) were not significantly different. Interaction between time and treatment had a tendency to significance on pre-weaning faecal score ( $P=0.053$ ). There was a tendency ( $P=0.08$ ) for higher incidence of respiratory disease among calves fed SB. Treatments did not significantly affect calf mortality (4%;  $P=0.55$ ).

**Conclusion:** This study indicates that the health of artificially reared dairy-bred beef calves was not significantly affected by supplementing CMR with sodium butyrate or by feeding concentrate with reduced crude protein content (16%), but that SB may reduce diarrhoea and increase BRD incidence. Both CMR with added sodium butyrate and concentrate with 16% crude protein content can be fed with no significant negative impact on calf health, but further investigation is required.

**References:** Górka, P., Kowalski, Z. M., Pietrzak, P., Kotunia, A., Jagusiak, W., Holst, J. J., Guilloteau, P and Zabielski, R. 2011. Effect of method of delivery of sodium butyrate on rumen development in newborn calves. *Journal of Dairy Science*, 94, pp. 5578-5588.  
Hill, T. M., Bateman II, H. G., Aldrich, J. M and Schlotterbeck, R. L. 2008. Crude protein for diets fed to weaned dairy calves. *Professional Animal Scientist*, 24 (6), pp. 596-603.  
Kelly, A., O'Doherty, J., Kenny, D., Boland, T. and Pierce, K. 2014. Performance and rumen development of artificially reared calves to dietary butyrate supplementation. 2014 ADSA-ASAS-CSAS Joint Annual Meeting. [On-line]. ASAS. Abstract only. Available from:

<https://asas.confex.com/asas/jam2014/webprogram/Paper6511.html> [Accessed 15 November 2014].

## CHAPTER 1: Introduction

In recent years there has been an increase in the number of dairy-bred bull calves being reared for beef production. 86% of male dairy calves born in 2013 were reared for beef, having risen from 50% since 2006 (CHAWG, 2014). Use of dairy-bred calves for beef can be favourable because the costs associated with feeding, housing and calving the dam fall upon the dairy enterprise, and being a surplus product, calves are readily available across much of the country. Two thirds of UK beef farmers in the UK currently receive a negative margin on each animal (EBLEX, 2014); a source of livestock with lower associated costs is highly desirable.

Artificial calf rearing is an essential process for many dairy and beef systems. Although labour input is higher, increasing costs (Nix, 2014); the potential gains are greater as it enables greater influence over calf performance and disease control. Therefore any improvements in calf management or nutrition that can improve efficiency and profitability will benefit both the dairy and beef industries.

The effect of sodium butyrate has become a key area of research, with trials linking its use to improved growth, rumen development (Górka *et al.*, 2011) and intestinal development and efficiency (Górka *et al.*, 2014). Enhanced rumen development may allow earlier weaning (Kehoe *et al.*, 2007). There are also claims of improved immune response (Kelly *et al.*, 2014), possibly as a result of improved digestive development (Fratric, 2013).

Dietary protein, particularly in the form of soymeal, is commonly one of the most expensive fractions of any formulated diet (DairyCo, 2015). Therefore reduced protein use could reduce cost of production. Although current recommendations are to feed concentrates containing 18% crude protein (NRC, 2001) it is possible to feed calves reduced crude protein whilst maintaining optimal, cost effective growth (Hill *et al.*, 2008), however the effect on immune function is less well understood (Nonnecke *et al.*, 2000).

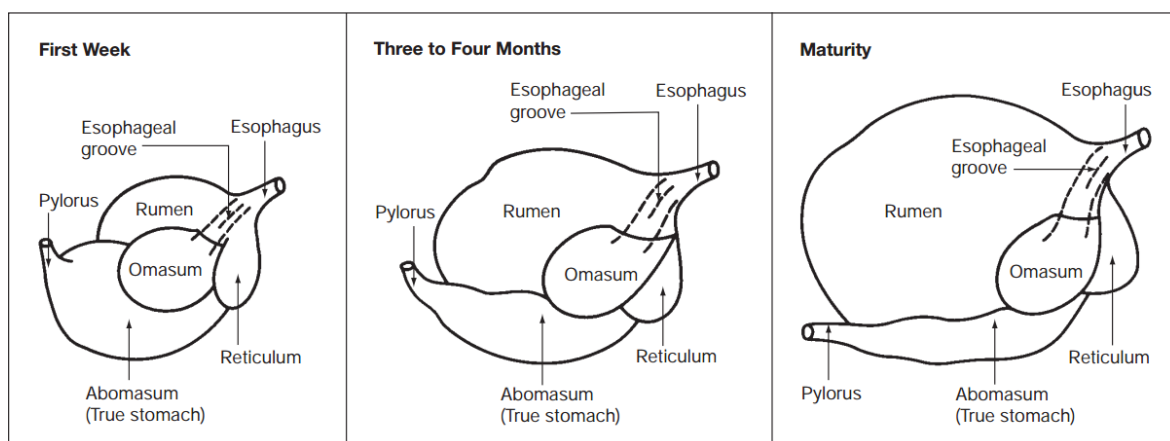
The objective of this trial is to further investigate the effect on the health of artificially reared beef calves when fed calf milk replacer supplemented with sodium butyrate and concentrate with reduced crude protein content.

## CHAPTER 2: Literature review

### 2.1 Digestive physiology of the calf

Cattle are ruminants, and as such have four stomach chambers. These consist of the abomasum or true stomach, which performs the same function as the stomach in monogastrics, and the three forestomachs: the rumen, reticulum and omasum (Frandsen *et al.*, 2009). The rumen contains a microbial population with the role of breaking down carbohydrates through fermentation, particularly lignin found in plants, which cannot otherwise be broken down by the animal. The rumen and reticulum are similar in function and structure, although the rumen is much larger. Both contain large volumes of fluid required for the fermentation of feed and have epithelial linings covered in papillae, microscopic folds that increase surface area and aid in the absorption of nutrients. The role of the omasum is to remove excess water from, and further grind and break down digesta through mechanical action (Moran, 2005). Papillae length, width and density directly affect absorption capability, and development of papillae within the calf is crucial for future rumen function (Heinrichs, 2005). The abomasum is a glandular stomach chamber that digests feed through acidic break down, in the same manner as a monogastric, allowing the small intestine to absorb any nutrients (Frandsen *et al.*, 2009).

The digestive system of the new born calf functions in much the same way as a non-ruminant, rendering the calf effectively a monogastric at birth (Drackley, 2008). At birth the abomasum is the principal stomach chamber and is used for the digestion of milk, the sole source of nutrition for the neonate. The enzyme rennin coagulates the milk into clots in order to aid digestion (Ohnstad, 2015). The rumen, whilst present, does not begin to develop and function until around two weeks of age (Heinrichs and Jones, 2003). Figure 1 illustrates the development of the digestive system of the calf over time.



**Figure 1: Development of bovine stomach compartments from birth to maturity. (Source: Heinrichs and Jones, 2003).**

At birth, the abomasum is the largest stomach; the rumen is not fully mature and must develop both physically and metabolically to achieve full mature functionality (Baldwin *et al.*, 2004). The neonatal rumen has a relatively small volume with an undeveloped epithelium, lacking muscularisation, vascularisation and papillae (Heinrichs and Lesmeister, 2005). As the calf matures the rumen volume greatly increases in proportion to the other stomach chambers. Table 1 demonstrates the proportion of total stomach capacity of each chamber at the ages illustrated in Figure 1.



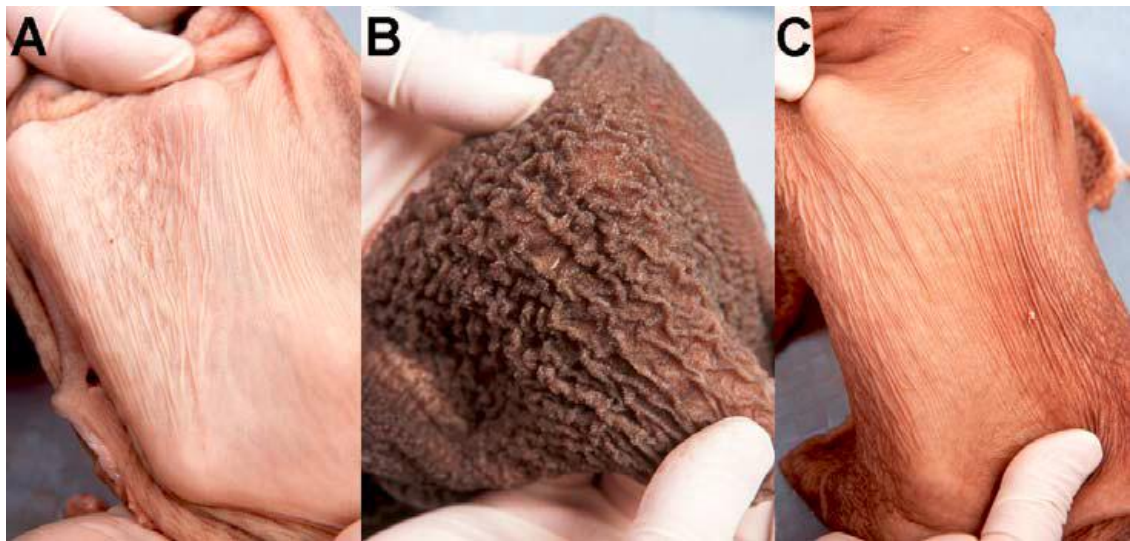
**Table 1: Relative size of stomach compartments from birth to maturity.**

Age	Proportion of total stomach capacity (%)			
	Rumen	Reticulum	Omasum	Abomasum
First week	25	5	10	60
3 to 4 months	65	5	10	20
Mature	80	5	7-8	7-8

(Source: Adapted from Heinrichs and Jones, 2003).

Although Table 1 describes the rumen developing as the calf ages, it cannot do so without sufficient stimulation from volatile fatty acids, derived from digestion of certain feedstuffs (McDonald *et al.*, 2011). Fibrous foods such as straw and hay will stimulate muscular development of the reticulo-rumen (Tamate *et al.*, 1962); development of the rumen papillae (Flatt *et al.*, 1958) and expansion of the reticulum (McDonald *et al.*, 2011).

Figure 2 depicts rumen papillae development at 6 weeks when fed different diets.



**Figure 2: Comparative rumen papillae development at 6 weeks in calves fed different diets. (Source: Heinrichs, 2005).**

These calves were fed milk only (image A); milk and grain (image B) and milk and hay (image C). Image B depicts ideal rumen epithelium at 6 weeks, with visible papillae and dark pigmentation. Milk alone is insufficient to stimulate ruminal development. Hay causes some papillae development and pigmentation but optimal results are obtained when fed concentrates (Heinrichs, 2005).

As the calf suckles, a reflex causes muscular contraction of the oesophageal groove to form a closed tube (Frandsen *et al.*, 2009). While the calf is reliant on milk for its nutrition it uses the oesophageal groove to channel milk toward the abomasum, bypassing the rumen (McDonald *et al.*, 2011). Although the presence of milk in the rumen can have a limited effect on rumen papillae development (Tamate *et al.*, 1962), it cannot be digested within the rumen, but must be broken down in the abomasum. Excessive milk within the rumen can ferment and cause rumen bloat (Roy, 1990).

### 2.1.1 Volatile fatty acids

Microbial fermentation of carbohydrates within the rumen produces volatile fatty acids (VFAs), namely acetic, propionic and butyric acid (McDonald *et al.*, 2011).

VFAs stimulate the formation of rumen papillae, whether present due to microbial fermentation or supplementation (Tamate *et al.*, 1962). Butyric acid, formed by ruminal-

degradation of concentrates and cereals, has a greater stimulatory effect than acetic or propionic acid (Heinrichs and Lesmeister, 2005). The calf, given sufficient stimulus, can utilise VFAs within three weeks of life which potentially allows weaning at four weeks of age (Kehoe *et al.*, 2007), eliminating costs associated with feeding milk.

### 2.1.2 Protein digestion

Crude protein (CP) can be considered to consist of either rumen degradable protein (RDP) or rumen undegradable protein (UDP). RDP is broken down in the rumen by the rumen microbes into amino acids, converted to microbial protein and absorbed in the abomasum and small intestine whereas UDP is directly absorbed by the animal (Chamberlain and Wilkinson, 1998). Excess protein from excessive feeding of RDP and non-protein nitrogen is converted into ammonia in the rumen. The ammonia is absorbed into the blood, converted to urea by the liver and excreted within the urine (McDonald *et al.*, 2011). Surplus protein is excreted as waste in the urine.

However, insufficient protein can have negative impacts on cattle health and production. Cows and heifers with inadequate nutritional protein have poor reproductive capabilities compared to cattle with adequate protein supply (Randel, 1990) and insufficient protein can negatively influence coat bloom, a key indicator of health status (Anon., 2008).

## 2.2 Calf health

### 2.2.1 Calf immunity

The immune system can be broadly divided into two interlinked systems: innate and acquired. The innate system is effective at destroying invading microbes with macrophages, dendritic cells, neutrophils and eosinophils but can take days to remove infection and can be overwhelmed by viruses or high volumes of bacteria. Acquired immunity utilises B cells and T cells, or antibodies, which contain protein receptors which bind to corresponding antigens on specific invading microbes, enabling the innate system to destroy the pathogen more quickly. These antibodies, or immunoglobulins, must have previously encountered a pathogen in order to recognise and bind with the antigen (Sompayrac, 2012). Therefore the neonatal calf with no previous exposure to pathogens, although immunocompetent, is immunonaïve. It can mount an innate immune response (Barrington and Parish, 2001) but lacks antibodies required for an adaptive immune response (Larson *et al.*, 1980), so is initially reliant on passive immunity acquired from the dam.

Whilst some mammals can transfer passive immunity from dam to neonate in utero (Israel *et al.*, 1993) others, such as cattle and pigs, cannot (Rooke and Bland, 2002). The calf cannot receive maternal antibodies in utero as ruminants have a cotyledonary synepitheliochorial placenta, meaning the uterine epithelial structure changes during pregnancy (Peter, 2013). This forms a barrier between the blood supplies of dam and neonate (Noakes, 2009) preventing prepartum transfer of maternal immunoglobulins through blood serum (Barrington and Parish, 2001). These maternal immunoglobulins must transfer from serum to colostrum via the mammary gland to be consumed by the calf (Korhonen *et al.*, 2000), along with a quantity of B cells, macrophages and neutrophils, all of which are fully functional following absorption (Cortese, 2009). Absorbed through the small intestine, the immunoglobulins provide the calf with passive immunity until its own acquired immune system matures and the calf is able to produce its own antibodies (Godden, 2008). The primary form of immunoglobulin found within bovine colostrum is



IgG<sub>1</sub>, forming approximately 80% of total immunoglobulins present, whilst IgG<sub>2</sub>, IgA and IgM form the remaining 20% (Stelwagen *et al.*, 2009).

External factors, such as nutrition, are linked to and can influence the immune function of the calf (Quigley, 2013), as is development of the digestive system (Fratric, 2013). Stress also can have a major impact on immunological performance within the neonate (Reinhardt, 2002). By placing the animal under stress, for example at weaning or following prolonged transport, the calf's immune system is negatively impacted (Blecha, 2001). Reducing stress will reduce negative impacts on the calf's immune system, reducing chance of clinical disease incidence.

A good coat bloom is seen in calves with adequate nutrition (Anon., 2008), and is a key indicator of calf health (Rogers, 2001) as a rough coat with poor bloom is a common symptom of disease and poor nutrition (Ward and Lardy, 2005).

### 2.2.2 Enteric disease

Diarrhoea, or scours, is an increase in the frequency of defecation, as well as a reduction in the dry matter content of the faeces due to increased loss of fluid, and is the most common manifestation of enteric disease (Radostits *et al.*, 2007). The extent to which the faecal consistency has been reduced (i.e. how watery the faeces is) can indicate the severity of the illness. Dehydration is associated with scouring; increased fluid loss will dehydrate the calf. A skin pinch test will indicate the degree of calf dehydration (Kehoe and Heinrichs, 2005).

Scouring is one of the most common afflictions of young calves, and is estimated to be the cause for around 50% of total calf mortality in the UK (Ohnstad, 2015). The cost of an outbreak of calf diarrhoea within the herd is estimated to be over £50 per cow (ADAS, 2013), including cost of treatment, calf mortality and loss of production due to restricted growth.

Poor nutrition is a common cause of calf diarrhoea, whether due to a change in diet or period of high stress (Kehoe and Heinrichs, 2005), but certain pathogens can cause enteric disease; poor calf nutrition, inadequate supply of colostrum, overstocking and stress are all influential factors in diarrhoea incidence (Lorenz *et al.*, 2011). Table 2 lists some common causal organisms. Scouring calves must always be treated with oral rehydration therapy, typically in the form of electrolytes, in order to replace lost fluids (Kehoe and Heinrichs, 2005). Oral rehydration, as well as replacing fluids, will replace electrolytes and provide nutritional support, primarily in the form of glucose to increase energy levels (Smith, 2009). Milk should continue to be fed during incidence of diarrhoea in order to maintain energy and adequate nutrition (Lorenz *et al.*, 2011).

**Table 2: Table of common scour causing infectious agents.**

Infectious agent	
Category	Name
Bacterial	<i>Escherichia coli (E. coli)</i>
	Salmonella
Viral	Rotavirus
	Coronavirus
Protozoal	<i>Cryptosporidium parvum</i>
	Coccidiosis

(Source: Adapted from Blowey, 1999).

*E. coli*, *Cryptosporidium parvum*, rotavirus and coronavirus are commonly present within the environment and therefore the faeces of healthy as well as ill calves. Infection is determined by environmental factors increasing infection pressure and the calf's own immune status (Lorenz *et al.*, 2011). *E. coli* is particularly prevalent within farm manure and is impossible to remove entirely (Smith *et al.*, 2009). Constable (2004) states that, regardless of the cause of scour, *E. coli* will become a threat to the affected calf; approximately 30% of systematically ill calves are found to have bacteraemia, where bacteria is present within the bloodstream. Therefore any antimicrobial treatment should focus on controlling *E. coli* rather than solely controlling the causal organism. However causal organisms should not be overlooked and appropriate antimicrobial treatment should be given. Salmonella can cause severe infections across the entire herd, but can be treated with antimicrobial therapy. Caused by ingestion of bacteria, it can soon become endemic. Salmonella can be introduced by external pests, so biosecurity is vital to control the disease (McGuirk and Peek, 2003).

Viral diarrhoea, caused by rotavirus and coronavirus, will cause sudden onset of severe diarrhoea and typically affects calves aged between 5 and 14 days. Being viral, antimicrobial treatments are ineffective; therefore antibodies provided through colostrum and oral rehydration therapy are vital. Viral damage to intestinal villi will recover after 7 days, but it can take up to 21 days for the calf to recover to its previous growth rate (Radostits *et al.*, 2007).

Diarrhoea incidence can be greatly reduced by providing adequate nutrition, reducing stress and maintaining good environmental hygiene (Lorenz *et al.*, 2011).

### 2.2.3 Bovine respiratory disease

Coughing is the most common symptom of bovine respiratory disease (BRD), and can indicate either primary or secondary respiratory disease. BRD not only causes illness at the time of infection, but will damage lung tissue, having repercussive effects on the future health and productivity of the animal and leaving damaged tissue prone to reinfection (Blowey, 1999).

BRD will cause the animal to cough; the frequency of the cough will increase as the severity of inflammation increases, therefore severity of infection increases but it is important to note that absence of coughing does not necessarily indicate absence of BRD (Radostits, 2007). Other indicators of BRD include mucosal nasal discharge; watery or purulent discharge from the eyes and noticeable ear droop and head tilting. Ear droop is particularly associated with *Mycobacterium bovis* (Maeda *et al.*, 2003). Ear droop, along with eye discharge, can often be an indicator of otitis media, an infection of the middle ear, which is also linked with BRD (Walz *et al.*, 1997).

BRD most commonly occurs during the winter months, from October to February, with greater levels of clinical diagnoses during this period (AHVLA, 2013) due to the housing of stock which coincides with colder, wetter weather. Svensson *et al.* (2006) found season and the size and location of the housing to be influential factors on the incidence of BRD. The calf has a thermal neutral zone between 15°C and 25°C; below which the calf must divert energy away from other bodily functions to maintain body temperature, placing the calf under stress (Stull and Reynolds, 2008). Calves exposed to cold temperatures have been shown to be more susceptible to BRD (Nonnecke *et al.*, 2009); the calf should not be exposed to high winds or draughts.

The housing of stock within enclosed environments and in close proximity to others will also have an effect on BRD. Viral pathogens, such as parainfluenza virus 3 or bovine respiratory syncytial virus, are transmissible via aerosol in the form of exhalation from animals. Larger groups will result in greater incidence of BRD, as the increased level of direct contact between calves is likely to be more effective at spreading pathogens than aerosol transmission (Svensson and Liberg, 2006), although aerosol transmission remains a risk to stock.

In a well ventilated building, the 'stack effect' removes warm, pathogen-laden air and replaces it with fresh air (DairyCo, 2012a) whereas a poorly ventilated building will lead to the formation of condensation overhead and form droplets, returning any aerosol transmissible pathogens to the calves (Blowey, 2005). If animals of differing ages share the same air space there is a risk that disease will spread from the older animals to the younger, more immunologically naïve animals (DairyCo, 2012b).

## 2.3 Calf rearing methods

### 2.3.1 Artificial calf rearing

Any system of calf rearing utilised must minimise welfare challenges and health risks as much as possible whilst remaining both practical and economically viable.

Artificially rearing calves allows monitoring of individual intake, enabling control over intake and the potential to optimise growth, albeit with greater labour inputs (Garnsworthy, 2005) of approximately 2.3 hours per head per month, compared with 0.9 hours for suckler production (Nix, 2014).

A common problem seen amongst artificially reared calves is that of cross-sucking; the non-nutritive sucking of the body of another calf, leading to potential hair loss and inflammation where sucked, particularly around the navel (Jensen, 2003). Such areas are vulnerable to infection, and cross-sucking can increase this risk. It is a problem exclusive to artificial rearing systems, as calves allowed to naturally suckle from a cow do not display this behaviour (Lidfors *et al.*, 1994).

Artificial rearing of calves can be necessary to reduce disease risk. Johne's disease (*Mycobacterium avium* subspecies *paratuberculosis*) for example is spread from the infected dam to the calf via ingestion of colostrum or faecal matter (Radostits, 2007). Therefore calves should be removed from dam at birth to reduce risk of infection (Cutler, 2012), ideally within 12 hours of birth (Windsor and Whittington, 2010).

### 2.3.2 Colostrum management

When the calf is born it is vital that it receives adequate colostrum within the first few hours of life because it is the sole form of nutrition available to the neonate and provides immunological protection for 48 hours postpartum (Stelwagen *et al.*, 2009). The neonatal small intestine is highly permeable to allow effective absorption of immunoglobulins, but permeability of the small intestine decreases within 12 hours, reducing absorption capability (Quigley *et al.*, 2005).

By law, calves must receive colostrum within the first 6 hours of life (The Welfare of Farmed Animals (England) Regulations 2007), and should receive a minimum of 5% of bodyweight within this time (Ohnstad, 2015). The quality and quantity of colostrum intake should be monitored to optimise calf health. The quality can be determined by measuring

IgG content with a hydrometer or refractometer (Heinrichs and Jones, 2011). IgG levels within blood serum can be increased with commercially available supplements, but IgG absorption is not very efficient (Quigley *et al.*, 2001), highlighting the importance of timing and quality of colostrum fed, rather than volume.

### 2.3.3 Basic requirements of the calf

Basic nutritional requirements are provided through milk, water and dry feeds, such as concentrates and fibrous straw or hay. Milk is the main source of nutrition for the neonatal calf and dry feeds, although not necessary for survival, are required to stimulate reticulo-ruminal development (Heinrichs and Jones, 2003).

Calves must be provided with an adequate supply of clean drinking water daily (The Welfare of Farmed Animals (England) Regulations 2007). Water intake does not affect milk intake (Gottardo *et al.*, 2002), and has been linked to dry feed intake, thereby affecting weight gain (Kertz *et al.*, 1984). Water palatability affects intake, and can be improved by adding flavouring, as well as ensuring water is clean and uncontaminated (Thomas *et al.*, 2007).

### 2.3.4 Frequency of milk feeding

The volume and timing of milk feeding can affect calf performance, as well as production costs. Three common practices in the UK are to feed calves restricted volumes twice a day; feed a restricted volume once a day, albeit at higher volume per feed than twice a day, or to feed milk *ad libitum*, allowing the calf to dictate intake. Legislation requires the calf be fed at least twice a day (The Welfare of Farmed Animals (England) Regulations 2007), but dry feed can be fed in lieu of milk to meet requirements; a system providing *ad libitum* concentrate is adequate.

Feeding calves once a day reduces labour costs but increases feed costs compared to twice a day feeding. Whilst once a day feeding reduces costs, reduced daily live-weight gain compared to twice a day (approximately 4kg) may reduce total margins per animal (Marsh and Collinson, 2008).

Uys *et al.* (2011) studied the effect on Jersey calves of high milk volumes, either as *ad libitum* or twice a day, compared to restricted volumes, and concluded that high milk volumes can increase live-weight gain and reduce cross-sucking whilst having no negative effects on health. However, calves fed restricted volumes of milk consumed more dry feed than those on high milk volumes. This corresponds with similar findings that *ad libitum* milk can delay initial consumption of dry feed (Kehoe *et al.*, 2007), and that *ad libitum* feeding results in higher pre-weaning live-weight gains, despite reducing dry feed intake (Appleby *et al.*, 2001). Kehoe *et al.* (2007) also found that *ad libitum* milk can delay development post weaning, whereas Jasper and Weary (2002) concluded that the benefit to growth rates of increased milk intake balanced the reduced intake of dry feed, and that growth benefits could be influential beyond milk feeding and into the post-weaning period.

### 2.3.5 Calf housing

There are advantages and disadvantages to individual and group housing. Individual pens allow individual monitoring of feed intake and calf health, reduce disease transmission by restricting contact and eliminate opportunity for cross-sucking of other calves (Svensson and Liberg, 2006) whereas group housing enables expression of more natural behaviour (Babu *et al.*, 2004), and can have lower labour requirements. Individual pens can be used

for pre weaned calves, but any calf older than 8 weeks must be group housed (The Welfare of Farmed Animals (England) Regulations 2007).

Group housing encourages calves to display more social behaviours and begin earlier consumption of dry feed and rumination (Babu *et al.*, 2004). This was attributed to calves being able to observe and imitate behaviour of other calves, stimulating early learning amongst the group.

Calf groups should be small and stable, containing calves of similar age and size. Cases of BRD are reduced in smaller groups due to reduced opportunity for disease spread (Svensson and Liberg, 2006). Differences in size and age result in bullying of smaller calves, affecting calf health (Hindhede *et al.*, 1999). Stable groups reduce stress and lower risk of disease transmission, reducing incidence of clinical respiratory and enteric disease, and improving calf welfare (Pedersen *et al.*, 2009).

### 2.3.6 Method of milk feeding

Bucket feeding, where calves drink milk from buckets as they would water provides no opportunity to satiate the calf's need to suck, thus increasing opportunity to cross-suck (Webster, 1984). Teat sucking stimulates the oesophageal groove reflex, enabling deposition of the milk into the abomasum therefore allowing more rapid digestion and absorption of nutrients. Teat feeding reduces level of cross-sucking but does not alleviate competition for milk (Jensen and Budde, 2006). Appleby *et al.* (2001) found increased performance of calves fed *ad libitum* via teat rather than by bucket, as well a reduction in cross-sucking.

Nielsen *et al.* (2008) concluded that group housed calves fed with a milk bar should be fed with a shared compartment teat feeder, in which the milk is pooled into one compartment, rather than a compartmentalised teat feeder. Despite compartmentalised feeders allowing monitoring of individual intake, no effect on milk intake was incurred with the different types of feeder. The shared teat feeder was found to reduce cross-sucking and encourage earlier consumption of concentrates than the compartmentalised feeder.

### 2.3.7 Weaning management

Gradual weaning is more beneficial than abrupt weaning; resulting in reduced feed costs per kilogram of DLWG (Marsh, 2008). Sweeney *et al.* (2010) found gradual preferable to abrupt weaning as it reduces stress, therefore reducing growth checks and improving immune function.

To achieve this, milk volume should be reduced gradually over a period of time to acclimatise the calf to a solid feed diet. Whilst a similar effect is achieved by diluting milk with water and keeping total liquid volume consistent, concentrate intake is lowered compared to reduced volume (Nielsen *et al.*, 2008).

Weaning should only be initiated once the calf's rumen is sufficiently developed to allow proper digestion of feed. Although the calf is capable of utilising VFAs at four weeks of age (Kehoe *et al.*, 2007), weaning should be delayed until the calf is consuming at least 1 kilogram of concentrate daily (Stamey *et al.*, 2012).

## 2.4 Sodium butyrate

The effect of volatile fatty acids on cattle has long been understood. Flatt *et al.* (1958) established a link between the presence of these fatty acids and stimulation of rumen papillae development, and Sander *et al.* (1959) demonstrated an improvement in rumen

growth of calves when supplemented with sodium butyrate (SB) and sodium propionate, sodium salts of butyric and propionic acid respectively.

More recently there has been renewed interest in the effect of SB on a variety of species. Le Gall *et al.* (2009) linked dietary SB to enhanced growth and maturation of the porcine gastro-intestinal tract, increasing digestibility of nutrients and stimulating growth of the animal. Dietary SB has been shown to moderate the immune response in stressed broiler chickens, mitigating negative effects on growth (Zhang *et al.*, 2011). Butyrate has also been linked with anti-inflammatory effects in humans (Meijer *et al.*, 2010).

A trial by Kelly *et al.* (2014) demonstrated a tendency for SB within CMR to improve calf growth rates, which was reinforced by previous studies. Guilloteau *et al.* (2009) found a pre-weaning increase in DLWG when calves were fed SB within CMR. Dietary SB was shown to increase pancreatic secretion, enhancing digestibility of feed (Guilloteau *et al.*, 2010) which is likely to contribute to an improved DLWG. However Wanat *et al.* (2015) found that, when SB is fed via concentrates, DLWG decreases as quantity of supplementary SB is increased, suggesting that the form in which SB is supplied is influential on its effect.

Kelly *et al.* (2014) found no effect from SB on rumen papillae but other trials have found SB to have positive effect on rumen development and papillae growth. Górká *et al.* (2011) provided calves with SB in both CMR and concentrate. Calves were euthanised at 26 days and SB was found to have increased rumen papillae in both length and width, along with an increased reticulo-rumen weight. In a similar study Górká *et al.* (2014) found SB enhanced development of the small intestine. SB was found to be more effective in stimulating small intestine development when fed via CMR, rather than via concentrate.

Calves fed SB were found by Górká *et al.* (2011) to have reduced incidence of diarrhoea, but Kelly *et al.* (2014) found no effect of SB on faecal consistency. Findings by Wanat *et al.* (2015), however, show that when SB is fed within concentrates diarrhoea incidence increases. Current literature suggests that CMR is the preferred form of supplementation (Górká *et al.*, 2011), and that there may be an optimum level of supplementation of SB (Wanat *et al.*, 2015).

## 2.5 Crude protein

Protein typically forms the more expensive individual components of concentrates. In February 2015 high protein soymeal Hipro cost £327 per tonne compared to feed wheat with lower levels of protein at £133.5 per tonne (DairyCo. 2015). The high costs, combined with the environmental impact have resulted in efforts to reduce soymeal use without affecting production (DEFRA, 2012). In cattle approximately 75% of protein fed is excreted as waste; excess feeding is wasteful both economically and environmentally (Froidmont *et al.*, 2010).

Current recommendations are that calf starter concentrates contain 18% CP (NRC, 2001). Historic opinion recommended between a maximum of 16% CP (Morrill and Dayton, 1978; NRC, 1978), but standards were more recently revised (NRC, 1989).

When calves were fed between 15% and 22.4% CP within concentrate calf DLWG increased as CP content increased, but post-weaning DLWG did not improve beyond 18% CP (Akayezu *et al.*, 1994). Therefore, whilst improvements to growth can be achieved,

there is a limited response beyond a threshold amount of CP. This corresponds with findings from Hill *et al.* (2008) that, when fed varied CP content in concentrates, optimum calf performance to 8 weeks was seen with 18% CP, but just 16% CP from 8 to 12 weeks. However, feed conversion efficiency was maximised at 16.5% CP.

Stamey *et al.* (2012) demonstrated improved DLWG in calves fed both CMR and CP containing increased levels of CP. The CMR had a notable effect on DLWG, but the concentrate had only minor effects. Despite the lesser effect of enhanced concentrate its provision increased intake, which was seen to improve rumen development and reduce stress at weaning.

Nonnecke *et al.* (2000) studied the immunological effects on calves of increased protein and energy in CMR. Results showed no significant alteration of immunoglobulin and leukocyte levels with increased nutrition, and any increases in immunological status were likely due to maturation of the calf's immune system rather than treatments administered.

## 2.6 Research Gap

The primary focus of research into the effects of SB on calves has been on calf performance. Efforts to study the effect of SB on calf health, whilst undertaken, have considered on immunological response (Kelly *et al.*, 2014), but not investigated gross health or disease incidence. Many trials on SB have euthanised calves at an early age in order to determine effects of the gastro-intestinal tract, such as Górká *et al.* (2014), who established positive effects on calf growth up to 26 days, but identified the need to study the longer term effects of SB on the calf.

Past trials studying dietary CP concentration have focussed on calf performance, and not on calf health (Hill *et al.*, 2008). Nonnecke *et al.* (2000) recommended further research into the link between varied nutrition, particularly varied protein levels, and immunity after an inconclusive study.

Furthermore, there have been no studies conducted on the effects of interaction between dietary sodium butyrate and crude protein concentration, either on calf performance or health.

## CHAPTER 3: Materials and Methods

### 3.1 Experimental design

The experiment, designed to evaluate the effect of altered diets on the health of 48 artificially reared beef calves, was conducted at Harper Adams University, Shropshire. The objectives were to ascertain any effects on calf health from feeding supplementary sodium butyrate (SB) in calf milk replacer (CMR) and differing crude protein (CP) levels in concentrate for 12 weeks.

CMR used for the trial was Shine whey, skim and buttermilk based milk replacer, produced by Bonanza Calf Nutrition. Calves were offered either standard CMR as a control or treatment CMR with added SB. The concentrates, produced by Carrs Billington, were based on typical early weaning concentrate, and contained either a control 18% CP or a treatment 16% CP fresh weight.

There were four treatments in total:

- Control CMR and 18% (control) CP concentrate (C18)
- Control CMR and 16% (treatment) CP concentrate (C16)
- CMR with treatment SB and 18% (control) CP concentrate (SB18)
- CMR with treatment SB and 16% (treatment) CP concentrate (SB16).

The experiment took place over 12 weeks. Calves were fed CMR for 42 days, weaned, and then continued to be fed either 16% or 18% CP concentrate for a subsequent 42 days. This allowed for both pre-weaning and post-weaning effects to be observed.

Calves were allocated by randomised block design, based on the breed and start weight of each calf. The calves were weighed upon arrival and assigned to one of the four treatments, with 12 calves per treatment. In order to remove bias each breed was evenly allocated between each treatment and start weights within each breed were distributed evenly between treatments, resulting in similar average start weights across each treatment.

The calves were Holstein-Friesian (n=24) and continental cross-bred (n=24) bull calves with Belgian Blue cross-bred (n=22) and Simmental cross-bred (n=2) calves. The calves were sourced from the Harper Adams dairy unit and two other farms in the area and were aged between 4 and 35 days old on arrival, with a mean age of 19 days. A list of calf ages and breeds can be seen in appendix 1.

A parallel experiment was conducted on the calves to investigate the effect of SB and CP levels in concentrate on calf growth and performance. Both trials began on the 1<sup>st</sup> October 2014 with the arrival of the first batch of calves, and concluded on the 6<sup>th</sup> February 2015 with the weaning of the final batch of calves. Due to limited available space calves arrived in batches; the final batch began the trial on the 14<sup>th</sup> November after the first batch had been weaned.

### 3.2 Management of calves

Whilst being fed CMR the calves were kept in individual pens and bedded on wheat straw. Pen design separated calves but still allowed nose to nose contact. Calves were fed CMR



twice a day and were offered concentrate, wheat straw and water *ad libitum*. Straw was offered in racks and water and concentrate were offered in individual buckets.

Calves were weaned 6 weeks into the trial, providing that concentrate consumption per calf was recorded at 1kg per day for at least three days. For three days prior to weaning CMR was fed once a day, morning feed only. This is in order to acclimatise the calves and make weaning less abrupt, reducing stress and growth checks on calves (Sweeney *et al.*, 2010).

At weaning, the calves were moved into groups, according to treatment, and loose housed in straw pens. There the calves continued to be fed respective concentrates, as well as *ad libitum* straw in racks for a further 6 weeks.

Any calves showing signs of diarrhoea were given electrolytes via Life-Aid Xtra and any other illnesses were treated according to veterinary advice. All medicine use during the trial was recorded and the full list can be seen in appendix 2. All calf deaths during the trial were recorded, as well as cause of death and are available in appendix 3. Clinical incidences were recorded for both respiratory disease and diarrhoea, and can be seen in appendices 4 and 5 respectively.

The calves were dehorned after three weeks on trial using Metacam and Lignocaine, and were simultaneously vaccinated with 5ml Bovilis ® Bovipast RSP, with a subsequent dose two weeks later. Dehorning and vaccination was carried out by staff at Harper Adams.

Calf coats were not used as standard on the calves, but any calves unwell or with low body temperature were given coats when it was deemed necessary.

### 3.2.1 Calf milk replacer

Calves were fed 1.7 litres of Shine milk replacer twice daily at a rate of 175g per 825ml water, totalling 600g of CMR per calf per day. CMR was fed at 37°C. Both the control CMR and the treatment CMR contained 20% CP and 17% oil. The treatment CMR contained 15kg per tonne of SB. SB was supplied via CMR rather than any other means as studies have demonstrated that effect on calves is greatest with CMR (Górka *et al.*, 2014).

CMR was fed via compartmentalised teat feeders, enabling accurate monitoring of individual intake. After each feed, all teat feeders were cleaned with soap and warm water to maintain good hygiene and reduce infection risk (Lorenz *et al.*, 2011). Any CMR refusals were recorded, and a full list is available in appendix 6.

### 3.2.2 Concentrate

Concentrate was offered from the start *ad libitum*. Weekly intake was monitored. Daily input was recorded, with any uneaten concentrate weighed weekly and total weekly intake per calf was calculated. Any concentrate refused was discarded and discounted from total intake. The main difference between the control concentrate (18% CP) and treatment concentrate (16% CP) is the quantity of protein but other constituents vary slightly between the two feeds. Laboratory analyses were conducted on 3<sup>rd</sup> and 22<sup>nd</sup> October; results of which were very similar to the stated analysis. See appendix 7 for full concentrate analysis.

### 3.3 Measurements

Health measurements for each calf were taken three times each week on Mondays, Wednesdays and Fridays whilst on CMR, once at weaning and again at the conclusion of the trial. Measurements were not taken during the post-weaning period because the post-weaning group housing made individual assessment impractical.

Each measurement scored the calf on 7 individual criteria allowing quarter scores for greater accuracy (see Table 3). In order to ensure consistency, all health measurements were conducted by one person.

For full description and illustrated examples of each score see appendix 8.

**Table 3: Table of measurement criteria for calves.**

<b>Criteria</b>	<b>Dehydration</b>	<b>Cough</b>	<b>Nasal discharge</b>	<b>Ocular discharge</b>	<b>Ear droop</b>	<b>Coat bloom</b>	<b>Faecal consistency</b>
<b>Score range</b>	1-5	0-3	0-3	0-3	0-3	1-5	0-3
<b>Normal score (on a healthy calf)</b>	1	0	0	0	0	3	0

### 3.4 Data analysis

Data was analysed using a repeated measures Anova in Genstat (16<sup>th</sup> Edition) and Chi<sup>2</sup> analysis. The repeated measures Anova was used to compare the measurements of the same criteria over time. Significance was defined as  $P < 0.05$ , and a tendency at  $P < 0.1$ . Anovas compared effect of each measurement criteria during the pre-weaning phase (weeks 1-6) and across the entire trial (weeks 1-12). Due to the nature of the scoring system data was skewed. Log 10 transformation was performed, but it did not remove the skew. Therefore the results presented are from Anovas run with untransformed data. Calves that died during the trial were entered as missing values.

Chi<sup>2</sup> analysis was used to compare clinical incidence of pneumonia, diarrhoea and mortality and determine any influence of treatment, similar to previous trials on calf health (Earley *et al.*, 2004). Any repeat treatments on calves were considered as one continued illness rather than separate occurrence of disease, due to the likelihood of failure of treatment rather than re-infection.

Following the Chi<sup>2</sup> analysis hypothetical analyses were performed by using the same percentage of pneumonia and diarrhoea incidence within a larger population, in order to determine whether any significant effect of treatment would be apparent with a larger trial. Calculations can be seen in appendix 9.

Weekly mean scores for each calf are available in appendix 10.

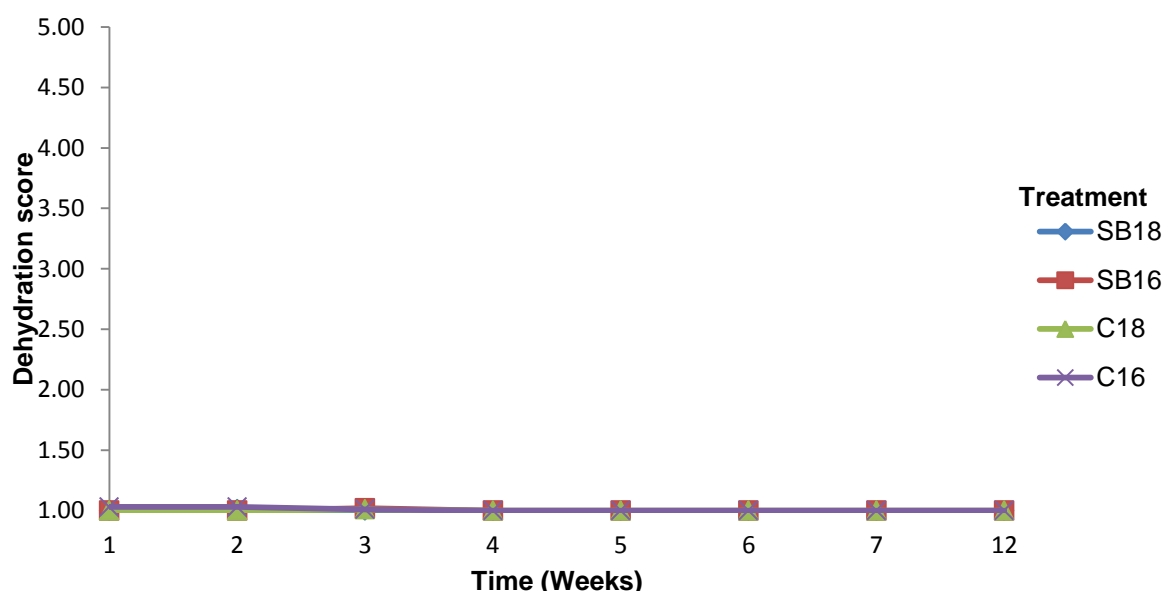
## CHAPTER 4: Results

Although the trial was planned to study each calf for 84 days, with 42 days pre-weaning and 42 days post weaning, timing was altered due to limited staff availability during the Christmas period; there was insufficient available labour to wean calves at the necessary time. Therefore in order to eliminate bias all calves were weaned after 38 days, spending 46 days in post-weaning group housing.

Due to insufficient quantity of treatment concentrates the final 12 calves (3 per treatment group) were fed standard concentrate for the final week of the trial. The final health scores for these calves were not included in analysis. These calves, and those that died during the trial, were entered as missing values.

### 4.1 Dehydration score

As Figure 3 demonstrates, mean dehydration score remained low for the duration of the trial for each treatment group, with almost no variation between groups.



**Figure 3: Weekly mean dehydration score of calves (n=48) fed calf milk replacer (CMR) with added sodium butyrate (SB) and concentrate with reduced crude protein (CP) content per treatment group.**

**SB18 (n=12) = CMR with SB and control concentrate (18% CP).**

**SB16 (n=12) = CMR with SB and concentrate with reduced CP (16% CP).**

**C18 (n=12) = Control CMR and control concentrate (18% CP).**

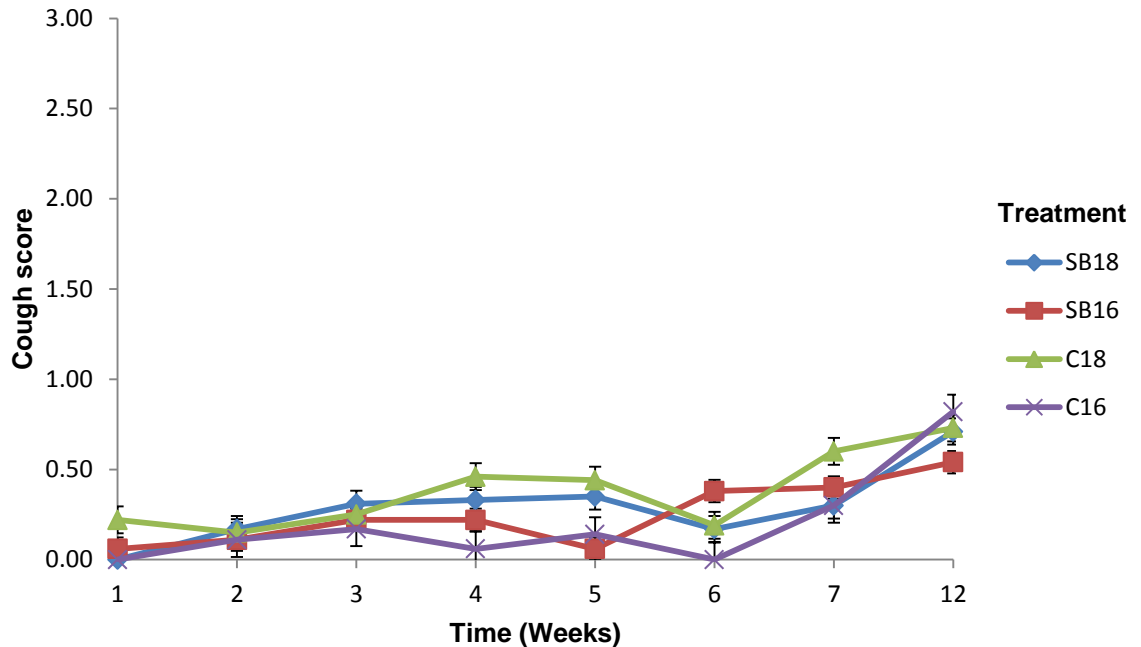
**C16 (n=12) = Control CMR and concentrate with reduced CP (16% CP).**

Pre-weaning (weeks 1-6) there was no significant effect on mean dehydration score of either CMR ( $P=0.39$ ) or concentrate ( $P=0.28$ ), nor was there a significant interaction between CMR and concentrate ( $P=0.39$ ). Time did not affect dehydration score ( $P=0.21$ ) and there was no interaction between time and CMR ( $P=0.66$ ), time and concentrate ( $P=0.62$ ) or time, CMR and concentrate ( $P=0.19$ ).

For the entire trial (weeks 1-12) there was no significant effect of CMR ( $P=0.38$ ), concentrate ( $P=0.3$ ) or interaction between CMR and concentrate ( $P=0.4$ ). There was no significant effect of time ( $P=0.18$ ), or interaction between time and CMR ( $P=0.63$ ), time and concentrate ( $P=0.57$ ) or time, CMR and concentrate ( $P=0.22$ ).

## 4.2 Cough frequency score

As Figure 4 demonstrates, mean cough frequency score remained low for each treatment group, with a combined mean score of 0.28 for all calves throughout the trial. Combined mean score for all groups increased slightly over time from 0.07 in week 1 to 0.27 in week 4, reduced in the final week pre-weaning (week 6) to 0.19 and then increased again after week 7 to 0.7 in week 12.



**Figure 4: Weekly mean cough frequency score of calves (n=48) fed calf milk replacer (CMR) with added sodium butyrate (SB) and concentrate with reduced crude protein (CP) content per treatment group.**

**SB18 (n=12) = CMR with SB and control concentrate (18% CP).**

**SB16 (n=12) = CMR with SB and concentrate with reduced CP (16% CP).**

**C18 (n=12) = Control CMR and control concentrate (18% CP).**

**C16 (n=12) = Control CMR and concentrate with reduced CP (16% CP).**

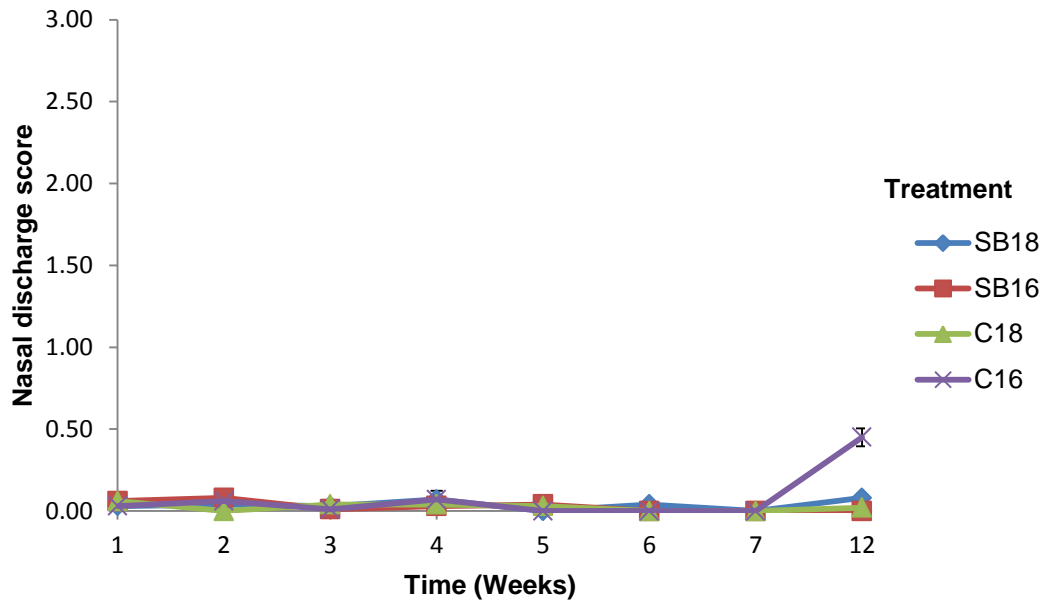
Pre-weaning (weeks 1-6) there was no significant effect on mean cough score of CMR ( $P=0.85$ ) or concentrate ( $P=0.1$ ). There was no significant interaction between CMR and concentrate ( $P=0.3$ ) or between time and CMR ( $P=0.43$ ), time and concentrate ( $P=0.2$ ) or time, CMR and concentrate ( $P=0.52$ ).

Throughout the entire trial (weeks 1-12) there was no significant effect on mean cough score from CMR ( $P=0.96$ ) or concentrate ( $P=0.18$ ). There was no significant interaction between CMR and concentrate ( $P=0.17$ ) or between time and CMR ( $P=0.67$ ), time and concentrate ( $P=0.57$ ) or time, CMR and concentrate ( $P=0.73$ ).

Time had a tendency of influence pre-weaning ( $P=0.06$ ), and a highly significant influence during weeks 1-12 ( $P<0.01$ ).

### 4.3 Nasal discharge score

Figure 5 shows very little variation between mean nasal discharge score amongst treatment groups; combined mean scores remained low (0.04) throughout the trial.



**Figure 5: Weekly mean nasal discharge score of calves (n=48) fed calf milk replacer (CMR) with added sodium butyrate (SB) and concentrate with reduced crude protein (CP) content per treatment group. SB18 (n=12) = CMR with SB and control concentrate (18% CP).**

**SB16 (n=12) = CMR with SB and concentrate with reduced CP (16% CP).**

**C18 (n=12) = Control CMR and control concentrate (18% CP).**

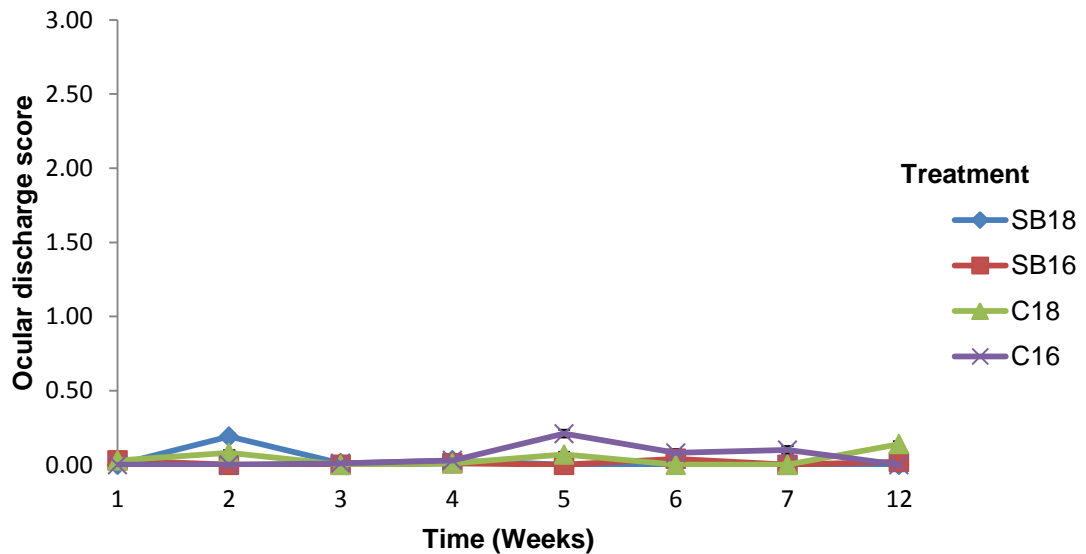
**C16 (n=12) = Control CMR and concentrate with reduced CP (16% CP).**

Pre-weaning (weeks 1-6) nasal discharge score was unaffected by CMR ( $P=0.61$ ), concentrate ( $P=0.94$ ) or time ( $P=0.34$ ). Nor was there any significant interaction between CMR and concentrate ( $P=0.94$ ), time and CMR ( $P=0.86$ ), time and concentrate ( $P=0.61$ ) or time, CMR and concentrate ( $P=0.56$ ).

During weeks 1-12 mean nasal discharge scores were unaffected by CMR ( $P=0.42$ ), concentrate ( $P=0.71$ ) or time ( $P=0.28$ ). There was no significant interaction between CMR and concentrate ( $P=0.68$ ), time and CMR ( $P=0.77$ ), time and concentrate ( $P=0.5$ ) or time, CMR and concentrate ( $P=0.43$ ).

## 4.4 Ocular discharge score

As Figure 6 demonstrates, there was little variation between treatments for mean ocular discharge score; overall combined mean scores remained low (0.03) during the trial.



**Figure 6: Weekly mean ocular discharge score of calves (n=48) fed calf milk replacer (CMR) with added sodium butyrate (SB) and concentrate with reduced crude protein (CP) content per treatment group. SB18 (n=12) = CMR with SB and control concentrate (18% CP).**

**SB16 (n=12) = CMR with SB and concentrate with reduced CP (16% CP).**

**C18 (n=12) = Control CMR and control concentrate (18% CP).**

**C16 (n=12) = Control CMR and concentrate with reduced CP (16% CP).**

There was no significant effect on mean ocular discharge score from CMR ( $P=0.44$ ), concentrate ( $P=0.98$ ), time ( $P=0.26$ ) or interaction between CMR and concentrate ( $P=0.3$ ), time and CMR ( $P=0.14$ ) or time, CMR and concentrate ( $P=0.62$ ) during the pre-weaning period (weeks 1-6).

For the duration of the trial (weeks 1-12) mean ocular discharge scores were not significantly affected by CMR ( $P=0.16$ ), concentrate ( $P=0.64$ ), time ( $P=0.3$ ) or the interaction between CMR and concentrate ( $P=0.67$ ), time and CMR ( $P=0.14$ ) or time, CMR and concentrate ( $P=0.37$ ).

There was a tendency of influence from interaction between time and concentrate during weeks 1-6 ( $P=0.07$ ) and weeks 1-12 ( $P=0.07$ ).

## 4.5 Ear droop score

Mean ear droop score was low throughout the trial for all treatment groups; decreasing from a combined mean for all treatment groups of 0.13 in week 1 to 0 in week 12, as demonstrated in Figure 7.

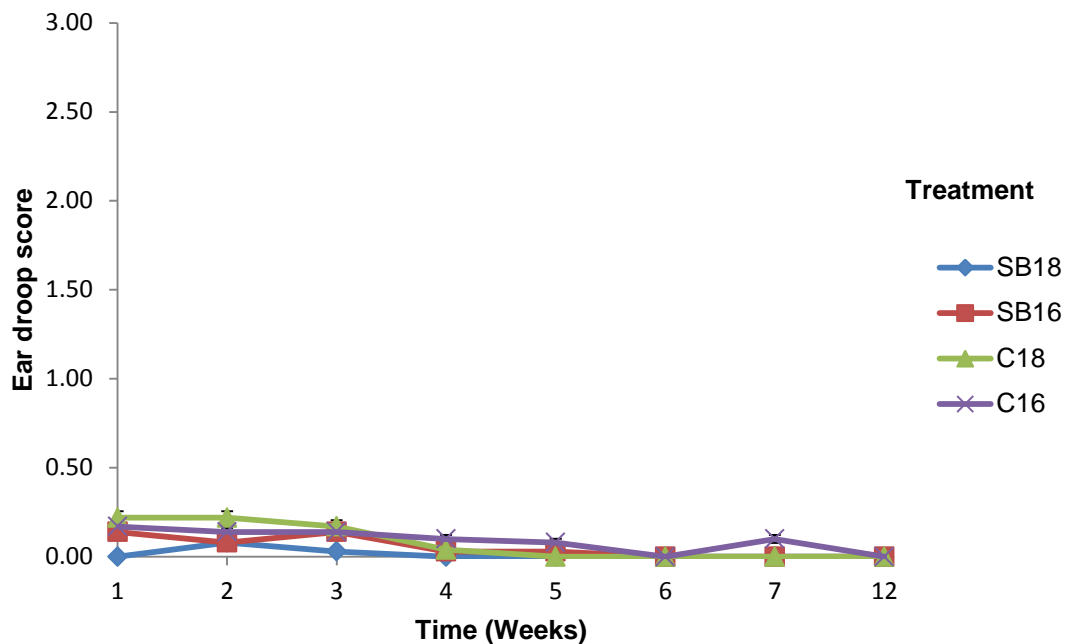


Figure 7: Weekly mean ear droop score of calves (n=48) fed calf milk replacer (CMR) with added sodium butyrate (SB) and concentrate with reduced crude protein (CP) content per treatment group.

SB18 (n=12) = CMR with SB and control concentrate (18% CP).

SB16 (n=12) = CMR with SB and concentrate with reduced CP (16% CP).

C18 (n=12) = Control CMR and control concentrate (18% CP).

C16 (n=12) = Control CMR and concentrate with reduced CP (16% CP).

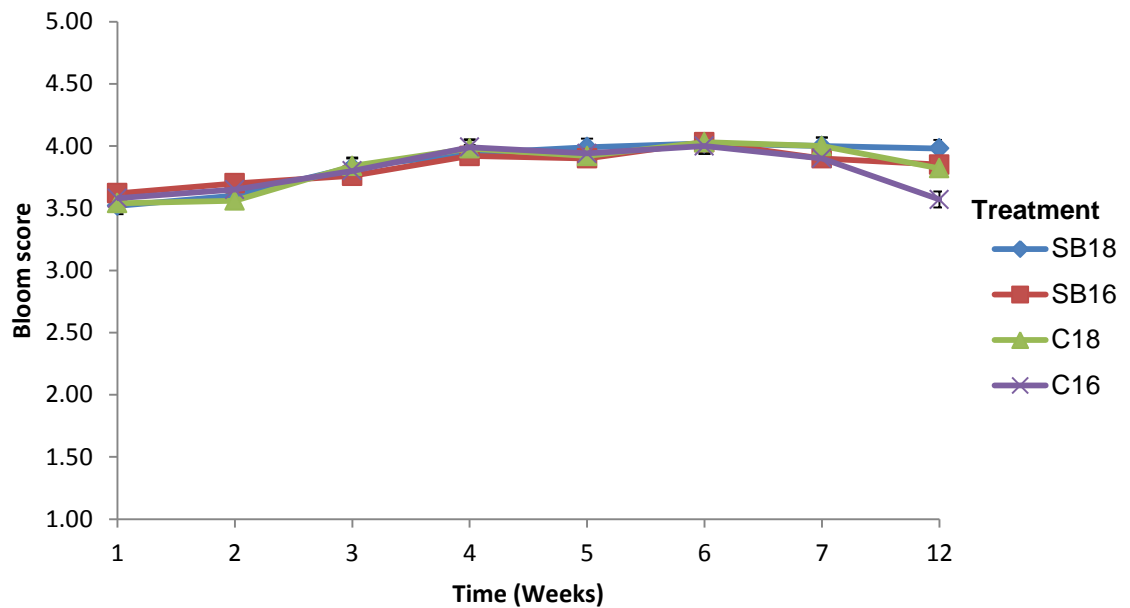
Pre-weaning (weeks 1-6) there was no significant effect of CMR ( $P=0.035$ ), of concentrate ( $P=0.68$ ) or of interaction between CMR and concentrate ( $P=0.79$ ), time and CMR ( $P=0.63$ ), time and concentrate ( $P=0.64$ ) or time, CMR and concentrate ( $P=0.34$ ) on mean ocular discharge score.

Across weeks 1-12 mean ocular discharge score was unaffected by CMR ( $P=0.33$ ), concentrate ( $P=0.6$ ) or by interaction between CMR and concentrate ( $P=0.99$ ), time and CMR ( $P=0.62$ ), time and concentrate ( $P=0.64$ ) or time, CMR and concentrate ( $P=0.26$ ).

Time had a significant effect during weeks 1-6 ( $P=0.03$ ) and weeks 1-12 ( $P=0.03$ ).

## 4.6 Coat bloom score

Figure 8 demonstrates the mean coat bloom scores for each treatment group. The combined mean bloom scores for all groups increased from 3.57 in week 1, reaching a peak of 4.02 at week 6 and reduced slightly to 3.81 in week 12 but retaining the net increase from the start of the trial.



**Figure 8: Weekly mean coat bloom score of calves (n=48) fed calf milk replacer (CMR) with added sodium butyrate (SB) and concentrate with reduced crude protein (CP) content per treatment group.**

**SB18 (n=12) = CMR with SB and control concentrate (18% CP).**

**SB16 (n=12) = CMR with SB and concentrate with reduced CP (16% CP).**

**C18 (n=12) = Control CMR and control concentrate (18% CP).**

**C16 (n=12) = Control CMR and concentrate with reduced CP (16% CP).**

Mean bloom scores were not significantly affected pre-weaning (weeks 1-6) by CMR ( $P=0.97$ ), concentrate ( $P=0.9$ ) or by interaction between CMR and concentrate ( $P=0.95$ ), time and CMR ( $P=0.66$ ), time and concentrate ( $P=0.22$ ) or time, CMR and concentrate ( $P=0.78$ ).

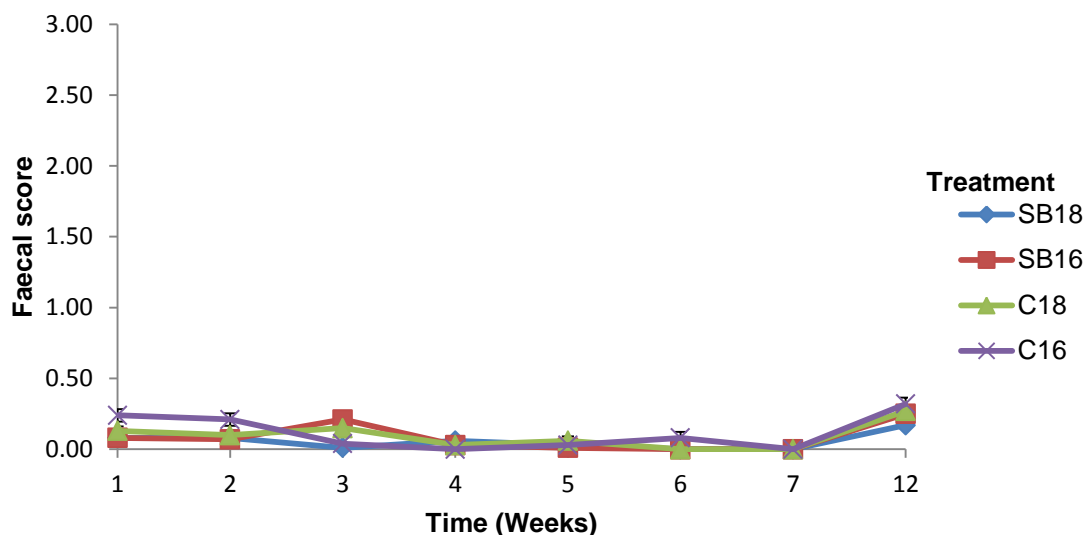
Across the duration of the trial there was no significant effect of CMR ( $P=0.7$ ), concentrate ( $P=0.56$ ) or interaction between CMR and concentrate ( $P=0.75$ ), time and CMR ( $P=0.17$ ), time and concentrate ( $P=0.1$ ) or time, CMR and concentrate ( $P=0.54$ ).

Time had a highly significant effect on mean bloom scores both pre-weaning ( $P<0.01$ ) and during the entire trial ( $P<0.01$ ).



## 4.7 Faecal consistency score

Figure 9 demonstrates the progression of mean faecal consistency scores across the trial. Although scores remained relatively low, with a mean score of 0.09 for all calves during the entire trial, scores were lowered from a mean of 0.13 in week 1 to 0 at weaning (week 7) only to increase to a mean of 0.25 by week 12.



**Figure 9: Weekly mean faecal consistency score of calves (n=48) fed calf milk replacer (CMR) with added sodium butyrate (SB) and concentrate with reduced crude protein (CP) content per treatment group.**

**SB18 (n=12) = CMR with SB and control concentrate (18% CP).**

**SB16 (n=12) = CMR with SB and concentrate with reduced CP (16% CP).**

**C18 (n=12) = Control CMR and control concentrate (18% CP).**

**C16 (n=12) = Control CMR and concentrate with reduced CP (16% CP).**

Mean faecal scores were not significantly affected pre-weaning (weeks 1-6) by CMR ( $P=0.29$ ), concentrate ( $P=0.45$ ) or by interaction between CMR and concentrate ( $P=1.0$ ), time and CMR ( $P=0.43$ ) or time and concentrate ( $P=0.69$ ). There was a strong tendency to significance of influence from interaction between time, CMR and concentrate ( $P=0.053$ ).

During the entire trial (weeks 1-12), there was no effect of CMR ( $P=0.15$ ), concentrate ( $P=0.32$ ) or by interaction between CMR and concentrate ( $P=0.88$ ), time and CMR ( $P=0.5$ ), time and concentrate ( $P=0.61$ ) or time, CMR and concentrate ( $P=0.27$ ).

Time had a highly significant effect on faecal score pre-weaning ( $P=<0.01$ ) and during weeks 1-12 ( $P=<0.01$ ).

## 4.8 Clinical respiratory disease incidence

There were 17 separate incidences of clinical BRD during the trial (see Table 4); 35% of the total population of calves were affected. Treatment group SB16 had the greatest number of cases, whilst C16 had the fewest. After chi<sup>2</sup> analysis (see appendix 9.1) there was a tendency (P=0.08) for treatment to affect clinical cases. The full list of affected calves is available in appendix 4.

**Table 4: Clinical respiratory disease incidence per treatment group.**

**SB18= CMR with SB and control concentrate (18% CP).**

**SB16 = CMR with SB and concentrate with reduced CP (16% CP).**

**C18 = Control CMR and control concentrate (18% CP).**

**C16 = Control CMR and concentrate with reduced CP (16% CP).**

	SB18	SB16	C18	C16	Total	Total population affected (%)	P value
<b>Number of clinical respiratory disease cases</b>	4	7	5	1	17	35	0.08

## 4.9 Clinical diarrhoea incidence

Table 5 shows clinical diarrhoea incidence, which remained low for all groups. Chi<sup>2</sup> analysis (see appendix 9.2) revealed no significant effect (P=0.48) of treatment on diarrhoea incidence. Appendix 5 contains a complete list of affected calves.

**Table 5: Clinical diarrhoea incidence per treatment group.**

**SB18= CMR with SB and control concentrate (18% CP).**

**SB16 = CMR with SB and concentrate with reduced CP (16% CP).**

**C18 = Control CMR and control concentrate (18% CP).**

**C16 = Control CMR and concentrate with reduced CP (16% CP).**

	SB18	SB16	C18	C16	Total	Total population affected (%)	P value
<b>Number of clinical diarrhoea cases</b>	0	1	2	2	5	10	0.48

## 4.10 Mortality

Two calves died during the trial, resulting in a mortality rate of 4% (see Table 6), but following chi<sup>2</sup> analysis (see appendix 9.3) there was no significant influence of treatment on mortality (P= 0.55). Full calf mortality is available in appendix 3.

**Table 6: Mortality of calves per treatment group.**

**SB18= CMR with SB and control concentrate (18% CP).**

**SB16 = CMR with SB and concentrate with reduced CP (16% CP).**

**C18 = Control CMR and control concentrate (18% CP).**

**C16 = Control CMR and concentrate with reduced CP (16% CP).**

	<b>SB18</b>	<b>SB16</b>	<b>C18</b>	<b>C16</b>	<b>Total</b>	<b>Total population affected (%)</b>	<b>P value</b>
<b>Calf mortality</b>	0	0	1	1	2	4	0.55

## 4.11 Performance

A parallel study conducted to investigate the effects of SB and CP level on calf performance. It concluded that SB caused a significant (P=<0.05) increase in daily live weight gain during the first week of the trial. There was no difference between start weight and feed intake during the first week, therefore the difference can be attributed to the SB supplementation. During the entire trial (weeks 1-12) calves fed SB had numerically higher live weight and daily live weight gain (see appendix 12), but the difference was not significant (P=>0.05).

There was no effect on performance of calves fed concentrates with either 16% or 18% CP. Calves fed 16% CP concentrate showed superior performance pre-weaning, with higher liveweight and DLWG during weeks 1-2, weeks 1-3 and weeks 1-6. However, calves fed 18% CP concentrate showed higher liveweight at week 12, and higher DLWG during weeks 6-12 and across weeks 1-12. There were no significant effects of treatment on other calf performance indicators.

The most cost effective diet contained SB and concentrate with 16% CP, providing the lowest cost per kilogram of liveweight gain (see appendix 14). The control treatment containing standard CMR and 18% CP was the most expensive diet per kilogram of DLWG to 12 weeks.

## CHAPTER 5: Discussion

### 5.1 Calf health

There were very few cases of calf scours, with no significant influence of treatment. There was a strong tendency of time by treatment interaction on faecal consistency score ( $P=0.053$ ), with calves fed SB and 18% CP found to have the healthiest faecal consistency and calves fed supplemented with SB to have healthier faecal scores than those fed the control CMR. Similarly Górká *et al.* (2011) found calves fed SB have improved faecal consistency, but like Kelly *et al.* (2014), results of this trial were not significant.

Because dehydration is linked to diarrhoea (Smith, 2009) it was unaffected by treatment; had diarrhoea incidence been higher it is likely that dehydration scores would have risen accordingly. All cases of diarrhoea were identified and treated with electrolytes before the calf could become truly dehydrated and illness could progress. It is possible that, had the calves been left untreated and clinical illness been allowed to develop, any potential effects of treatment may have been influential. However treatment of ill animals is required by law, and is reflective of standard industry practice (The Welfare of Farmed Animals (England) Regulations, 2007), thus any illness was treated promptly, regardless of any potential negative impact on the study.

Health score indicators of BRD, cough frequency, nasal discharge, ocular discharge and ear droop, were not significantly influenced by either treatment, supported by clinical BRD incidence analysis. Although not significant, there was a tendency ( $P=0.08$ ) for cases to be higher among calves fed SB. Similarly, although there was no significant effect of treatment on clinical diarrhoea incidence, supported by Kelly *et al.* (2014), calves supplemented with SB had numerically lower levels of clinical diarrhoea which supports findings by Górká *et al.* (2011). When hypothetical  $\chi^2$  analyses were performed on BRD and diarrhoea incidence (see appendices 9.4 and 9.5), using the same proportion of observed incidences with increased replicates there was a significant ( $P<0.05$ ) difference between treatments for both illnesses, suggesting more replicates would yield significant results.

From trial start to weaning mean faecal scores and ear droop scores decreased, and bloom score increased. This was likely due to new calves being transported, increasing stress (Pedersen *et al.*, 2009) lowering immune function (Blecha, 2001) and rendering calves more susceptible to diarrhoea (Kehoe and Heinrichs, 2005) and respiratory disease (Lorenz *et al.*, 2011). These scores improved up to weaning; this is likely due to the calves acclimatising to their environment and diet, and remaining in stable social groups. Post-weaning bloom scores were slightly depressed, while faecal and cough scores increased. It is possible that the newly dynamic social groups, combined with increased cold stress from the new environment (Stull and Reynolds, 2008) negatively impacted on calf health, as evidenced by the significant impact of time on these health criteria.

CP level had no influence on any aspect of calf health, reinforcing the findings of Nonnecke *et al.* (2000) that increased dietary protein is not influential on immunological status.

## 5.2 Calf performance

Calves fed SB showed a significant increase in growth during the first week, but not for the entire trial, suggesting that SB is only beneficial to growth for a limited time. This corresponds with findings that SB can increase pre-weaning growth rates, but have no effect post-weaning (Kelly *et al.*, 2014; Guilloteau *et al.*, 2009). However, lack of influence on rumen girth suggests that SB did not affect rumen growth; in contrast Górká *et al.* (2011) found SB increased rumen and papillae size. These trials euthanised calves in order to examine reticulo-ruminal epithelial structure; it is possible that SB increased rumen epithelial development, possibly resulting in improved growth in week 1.

The two levels of CP produced higher DLWG at differing stages of the trial; 16% CP pre-weaning and 18% CP post-weaning. This suggests that over time CP requirements increase. Hill *et al.* (2008) found highest levels of DLWG changed over time with differing levels of dietary CP, but that 18% CP was preferable pre-weaning, reducing to 15-16% CP post-weaning. However, results correspond with the assertion by Hill *et al.* (2008) that feed conversion efficiency is maximised at 16% CP.

## 5.3 Limitations

The health score system is subjective, and certain criteria are vague and only give an indication of calf health. Whilst BRD and diarrhoea were identified, the causal organisms were not. Analysis of faecal samples, for instance would allow clinical diagnosis and a greater understanding of calf health status. However, scoring was consistent, being conducted by one person throughout the trial. It may be necessary to take serum IgG samples in order to quantify immunological status of the calves and measure response to treatment (Quigley *et al.*, 2001). Health scoring was undertaken in the morning when calves were being fed. Despite being labour efficient it may have been inappropriate. Cough score may have been influenced by the increase in activity at feeding. Increased dust levels from fresh bedding, typically supplied during feeding exacerbated cough score (Bazeley and Hayton, 2007). Calves should be tested between feeds when settled. The faecal score system used is designed to assess pre-weaned calves. Although faecal consistency remains a key health indicator in adult cattle, consistency will vary; a separate reference system should be devised for weaned calves.

Although there were only 12 replicates per treatment the trial was of a similar scale to other studies on SB (Kelly *et al.*, 2014). Greater numbers were not possible with the available resources. Calf procurement was staggered due to limited space, so environmental challenge presented to the calves varied over time (AHVLA, 2013). All calves were kept in the same building, which is a poorly ventilated and potentially exacerbated any cases of BRD. Whilst having clinically ill calves may enable distinction between treatments, different batches faced different environmental challenges at different stages of the trial as clinical cases of BRD rise during winter (AHVLA, 2013) when the calf is more susceptible to pneumonia (Nonnecke *et al.*, 2009). If batches arriving contain equal numbers of calves per treatment then environmental effects on results would be mitigated.

Calves purchased from other farms were of unknown health status, and no clinical tests were undertaken to determine disease prevalence; it is likely that BRD was introduced by calves already carrying pathogens, meaning the health of neighbouring calves was disproportionately affected.

## 5.4 Validity of results

The trial had few replicates for each treatment (n=12). Whilst total calves (n=48) were comparable to similar trials (Kelly *et al.*, 2014), numbers may have insufficient to produce a statistically significant result. With the level of disease that was reported in this study 13 calves per treatment would be necessary to produce an effect of treatment on BRD (P=0.04); 43 calves per treatment would be necessary to produce an effect on diarrhoea incidence (P=0.03). Whilst this does not prove any effect of SB or CP on calf health, it suggests that there were insufficient replicates to produce a statistically significant result.

Calves arrived in batches of inconsistent size and health status with a mean age of 19 days ( $\pm 16$  days) (See appendix 1). Inconsistent batch numbers affected randomisation of treatment allocation, and the large age range would have influenced rumen development, as the first three weeks are vital for rumen development (Kehoe *et al.*, 2007). SB only influenced calf growth in the first week of the trial, so calves may have been too old for any beneficial effects of SB. The range of ages would likely have influenced disease incidence (Svensson and Liberg, 2006), and even reduced welfare (Hindhede *et al.*, 1999).

It is unlikely that weaning calves at 38 days rather than 42 days would have influenced calf health as no significant difference to calf health was detected either pre or post-weaning. Had newborn calves been studied then weaning date may have influenced results, but average calf age at weaning was 57 days.

Because 12 calves' final health scores were excluded (due to being fed alternate concentrates during the final week) total replicates were reduced by 25% for the final score. However separate analysis demonstrated no effect on significance of results following inclusion of these calves (see appendix 15).

## 5.5 Recommendations

Due to the small scale of the trial, it is recommended that the trial be repeated with more replicates to ensure results are statistically significant. All calves should be of similar health status and ages, ideally less than 5 days old, to investigate the influence of SB on early rumen development. Health scores should be accompanied by other diagnostic tools to determine calf immune status and clinically diagnose infectious disease (McGuirk, 2008).

## **CHAPTER 6: Conclusion**

The results of this study indicate that supplementing calves with sodium butyrate in calf milk replacer and reducing crude protein content in concentrates has no significant influence on calf health. There was a tendency of time and treatment interaction towards improved faecal consistency pre-weaning in calves fed sodium butyrate and 18% crude protein concentrate, and a tendency of higher incidence of respiratory disease in calves fed sodium butyrate.

Sodium butyrate fed with reduced crude protein content provided more cost efficient liveweight gain and could therefore provide financial benefits without increased risk of compromising calf health, but further research is needed to ascertain the full effects on health of sodium butyrate and reduced crude protein content.

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## Appendices

### Appendix 1: List of calf age and breeds

#### Appendix 1.1: Age and breed of calves of calves fed milk replacer with sodium butyrate and 18% crude protein concentrate

Table 7: List of calf age and breed for calves fed milk replacer with sodium butyrate and 18% crude protein concentrate. HF= Holstein Friesian; BB=Belgian Blue; SIM= Simmental; X= cross-bred.

Herd number	Calf eartag number	Breed	Date of birth	Date of Arrival	Age at arrival (days)	Age at weaning (days)	Age at trial finish (days)
UK304244	103408	HF	17/09/2014	01/10/2014	14	52	98
UK300129	403000	BBX	13/09/2014	02/10/2014	19	57	103
UK161195	307882	BBX	16/09/2014	02/10/2014	16	54	100
UK161195	107894	SIM	21/09/2014	02/10/2014	11	49	95
UK304244	703428	HF	09/10/2014	13/10/2014	4	42	88
UK300129	403028	BBX	02/10/2014	16/10/2014	14	52	98
UK161195	607892	HF	21/09/2014	16/10/2014	25	63	109
UK161195	707900	HF	24/09/2014	16/10/2014	22	60	106
UK161195	108006	BBX	28/09/2014	16/10/2014	18	56	102
UK304244	603448	HF	30/10/2014	12/11/2014	12	50	96
UK161195	108034	BBX	12/10/2014	13/11/2014	31	69	115
UK161195	108055	HF	26/10/2014	13/11/2014	17	55	101
<b>Mean age</b>					17	55	101

#### Appendix 1.2: Age and breed of calves of calves fed milk replacer with sodium butyrate and 16% crude protein concentrate

Table 8: List of calf age and breed for calves fed milk replacer with sodium butyrate and 18% crude protein concentrate. HF= Holstein Friesian; BB=Belgian Blue; SIM= Simmental; X= cross-bred.

Herd number	Calf eartag number	Breed	Date of birth	Date of Arrival	Age at arrival (days)	Age at weaning (days)	Age at trial finish (days)
UK304244	503405	HF	15/09/2014	01/10/2014	16	54	100
UK300129	602995	HF	02/09/2014	02/10/2014	30	68	114
UK161195	507863	BBX	05/09/2014	02/10/2014	27	65	111
UK161195	507884	BBX	17/09/2014	02/10/2014	15	53	99
UK300129	302999	BBX	11/09/2014	16/10/2014	35	73	119
UK161195	707886	HF	18/09/2014	16/10/2014	28	66	112
UK161195	307896	HF	21/09/2014	16/10/2014	25	63	109
UK161195	208007	BBX	28/09/2014	16/10/2014	18	56	102
UK161195	708019	BBX	03/10/2014	16/10/2014	13	51	97
UK304244	403453	HF	04/11/2014	12/11/2014	8	46	92
UK161195	608032	BBX	10/10/2014	13/11/2014	33	71	117
UK161195	508059	HF	30/10/2014	13/11/2014	13	51	97
<b>Mean age</b>					22	60	106



### Appendix 1.3: Age and breed of calves fed control milk replacer and 18% crude protein concentrate

Table 9: List of calf age and breed for calves fed control milk replacer and 18% crude protein concentrate. HF= Holstein Friesian; BB=Belgian Blue; SIM= Simmental; X= cross-bred.

Herd number	Calf eartag number	Breed	Date of birth	Date of Arrival	Age at arrival (days)	Age at weaning (days)	Age at trial finish (days)
UK304244	603399	HF	14/09/2014	01/10/2014	17	55	101
UK304244	603406	HF	17/09/2014	01/10/2014	14	52	98
UK300129	502994	BBX	31/08/2014	02/10/2014	32	70	116
UK161195	602878	SIM	14/09/2014	02/10/2014	18	56	102
UK161195	507898	BBX	22/09/2014	02/10/2014	10	48	94
UK304244	703414	HF	29/09/2014	13/10/2014	14	52	98
UK300129	203012	BBX	22/09/2014	16/10/2014	24	62	108
UK300129	103018	BBX	26/09/2014	16/10/2014	20	58	104
UK304244	303431	HF	11/10/2014	23/10/2014	12	50	96
UK161195	508038	BBX	13/10/2014	13/11/2014	30	68	114
UK161195	708061	HF	30/10/2014	13/11/2014	13	51	97
UK161195	108068	HF	03/11/2014	13/11/2014	10	48	94
<b>Mean age</b>					18	56	111

### Appendix 1.4: Age and breed of calves fed control milk replacer and 16% crude protein concentrate

Table 10: List of calf age and breed for calves fed control milk replacer and 16% crude protein concentrate. HF= Holstein Friesian; BB=Belgian Blue; SIM= Simmental; X= cross-bred.

Herd number	Calf eartag number	Breed	Date of birth	Date of Arrival	Age at arrival (days)	Age at weaning (days)	Age at trial finish (days)
UK304244	403397	HF	11/09/2014	01/10/2014	20	58	104
UK300129	302992	BBX	30/08/2014	02/10/2014	32	70	116
UK161195	307854	BBX	31/08/2014	02/10/2014	32	70	116
UK304244	103422	HF	08/10/2014	13/10/2014	5	43	89
UK161195	207888	HF	18/09/2014	16/10/2014	28	66	112
UK161195	507891	HF	19/09/2014	16/10/2014	27	65	111
UK161195	308001	BBX	25/09/2014	16/10/2014	21	59	105
UK161195	308008	BBX	28/09/2014	16/10/2014	18	56	102
UK161195	608018	BBX	02/10/2014	16/10/2014	14	52	98
UK304244	103450	HF	01/11/2014	12/11/2014	11	49	95
UK304244	303452	HF	04/11/2014	12/11/2014	8	46	92
UK161195	308029	BBX	09/10/2014	13/11/2014	34	72	118
<b>Mean age</b>					21	59	114

## Appendix 2: List of medicines administered

Table 11: List of medicines administered during the trial (7/10/2014 – 2/11/2014).

Date	Calf number	Group	Treatment, dose and route of administration	Reason
7/10/2014	603399	C18	3.5 ml Norfenicol IM and 1.25ml Metacam SC	Coughing; temperature: 39.5°C
9/10/2014	603399	C18	3.5ml Norfenicol IM	Follow up dose
11/10/2014	403000	SB18	4.2ml Norfenicol IM	Coughing; temperature: 39.7°C
13/10/2014	403000	SB18	4.2ml Norfenicol IM	Follow up dose
15/10/2014	503405	SB16	15ml Baycox oral dose	Scouring; temperature: 39.1°C
16/10/2014	503405	SB16	3.2ml Norfenicol IM	Coughing; temperature 39.3°C
18/10/2014	503405	SB16	3.2ml Norfenicol IM	Follow up dose
20/10/2014	103422	C16	15ml Baycox oral dose	Scouring
	103018	C18	7ml Norfenicol SC and 1.25 ml Metacam SC	Coughing; temperature: 39.1°C
21/10/2014	103422	C16	3ml Norodine IM and 1 sachet Life-Aid Xtra	Scouring; temperature: 39.3°C
22/10/2014	103422	C16	1 sachet Life-Aid Xtra	Scouring
	103018	C18	7ml Norfenicol SC	Follow up dose
	307896	SB16	2.5ml Combiclav IM and 1.5ml Metacam SC	Calf dull and unresponsive; very high temperature: 40.1°C
23/10/2014	307896	SB16	2.5ml Combiclav IM	Follow up dose
24/10/2014	307896	SB16	2.5ml Combiclav	Follow up dose
27/10/2014	403028	SB18	4ml Norfenicol IM and 1.5ml Metacam SC	Coughing; temperature: 39.7°C
28/10/2014	103018	C18	3ml Combiclav IM and 1.5ml Metacam SC	Difficulty breathing
29/10/2014	403028	SB18	4ml Norfenicol IM	Follow up dose
	103018	C18	3ml Combiclav IM	Follow up dose
30/10/2014	103018	C18	3ml Combiclav IM and 1 sachet Life-Aid Xtra	Follow up dose; slightly dehydrated
31/10/2014	602995	SB16	4.6ml Norfenicol IM and 2ml Metacam SC	Coughing; temperature: 40.3°C
	103018	C18	3ml Norodine IM and 15ml Baycox oral dose	Scouring
2/11/2014	602995	SB16	4.6ml Norfenicol	Follow up dose
	403000	SB18	4.6 ml Norfenicol IM and 2ml Metacam SC	Coughing; temperature: 39.8°C

(Continued overleaf)

**Table 12: List of medicines administered during the trial (4/11/2014 – 10/12/2014).**

<b>Date</b>	<b>Calf number</b>	<b>Group</b>	<b>Treatment, dose and route of administration</b>	<b>Reason</b>
4/11/2014	403000	SB18	4.6 ml Norfenicol IM	Follow up dose
5/11/2014	307854	C16	2ml Dectomax SC	Control of parasites
7/11/2014	403028	SB18	4ml Norfenicol IM	Coughing and laboured breathing; temperature: 38.7°C
8/11/2014	603399	C18	4.5ml Norfenicol IM and 1.6ml Metacam SC	Coughing; temperature: 38.6°C
9/11/2014	403028	SB18	4ml Norfenicol IM and 1.3ml Metacam SC	Follow up dose
10/11/2014	603399	C18	4.5ml Norfenicol IM	Follow up dose
11/11/2014	403028	SB18	1.6ml Draxxin SC	Coughing; difficulty breathing
	307854	C16	20ml Vecoxan oral dose and 5ml Combivit IM	Scouring; treatment of coccidial infection
12/11/2014	403028	SB18	3ml Combiclav IM	Persistent infection; broad spectrum antibiotic effect
13/11/2014	403028	SB18	3ml Combiclav IM and 2ml Colvasone IM	Follow up dose; steroidal anti-inflammatory effect
14/11/2014	403028	SB18	3ml Combiclav and 1.4ml Metacam	Follow up dose
18/11/2014	307854	SB18	1 sachet Life-Aid Xtra	Scouring
19/11/2014	307854	SB18	1 sachet Life-Aid Xtra	Scouring
20/11/2014	307854	SB18	1 sachet Life-Aid Xtra	Scouring
21/11/2014	307854	SB18	1 sachet Life-Aid Xtra	Scouring
	708061	C18	15ml Baycox oral dose	Scouring
22/11/2014	307854	SB18	1 sachet Life-Aid Xtra	Scouring
	307854	SB18	1 sachet Life-Aid Xtra	Scouring
23/11/2014	303431	C18	3.5ml Norfenicol IM and 1.6ml Metacam SC	Coughing; temperature: 39°C
	303431	C18	3.5ml Norfenicol IM	Follow up dose
25/11/2014	403453	C16	18ml Baycox oral dose	Scouring
	108055	SB18	1 tube Orbenin topically; 2.5ml Combiclav IM; 3.25ml Norfenicol and 1.75ml Metacam SC	Eye infection, high temperature: 40°C
27/11/2014	103450	SB16	3.5ml Norfenicol IM and 1.25 ml Metacam SC	Coughing; temperature: 39.5°C
28/11/2014	108055	SB18	3.5ml Norfenicol IM	Follow up dose
	403453	C16	3ml Norodine IM and 1 sachet Life-Aid Xtra	Scouring
29/11/2014	103450	SB16	3.5ml Norfenicol IM	Follow up dose
30/11/2014	608032	SB16	4.5ml Norfenicol IM and 1.75ml Metacam SC	Coughing; temperature: 39.3°C
2/12/2014	608032	SB16	4.5ml Norfenicol IM	Follow up dose
8/12/2014	508038	C18	4.7ml Norfenicol IM and 1.9ml Metacam SC	Coughing and difficulty breathing; temperature: 38.6°C
10/12/2014	508038	C18	4.7ml Norfenicol IM	Follow up dose

(Continued overleaf)

**Table 13: List of medicines administered during the trial (15/12/2014 – 30/1/2015).**

<b>Date</b>	<b>Calf number</b>	<b>Group</b>	<b>Treatment, dose and route of administration</b>	<b>Reason</b>
15/12/2014	103018	C18	12ml Norfenicol SC	Coughing
16/12/2014	507863	SB16	16ml Norfenicol SC and 3ml Metacam SC	Coughing; temperature: 38.9°C
	208007	SB16	14ml Norfenicol SC and 2.5ml Norfenicol SC	Coughing; temperature: 38.9°C
	302999	SB16	14ml Norfenicol SC and 2.5ml Norfenicol SC	Coughing; temperature: 38.8°C
17/12/2014	703414	C18	15ml Norfenicol SC and 3ml Metacam SC	Coughing; temperature: 40.2°C
19/12/2014	403453	C16	8.5ml Norfenicol SC and 2ml Metacam SC	Coughing; temperature: 39.2°C
22/12/2014	307882	SB18	19ml Norfenicol SC and 3.5ml Metacam SC	Coughing
30/1/2015	508038	C18	20ml Norfenicol and 4ml Metacam SC	Coughing; temperature: 38.9°C

### Appendix 3: Calf deaths

**Table 14: List of calf deaths**

<b>Calf number</b>	<b>Date of death</b>	<b>Group</b>	<b>Age (days)</b>	<b>Time on trial( days)</b>	<b>Cause of death</b>
307854	28/11/2014	C16	89	57	Euthanised
603399	15/12/2014	C18	92	75	Suspected pneumonia

## Appendix 4: Clinical pneumonia incidence

Table 15: List of clinical pneumonia incidence

<b>Date</b>	<b>Calf number</b>	<b>Group</b>
07/10/2014	603399	C18
13/10/2014	403000	SB18
16/10/2014	503405	SB16
20/10/2014	103018	C18
27/10/2014	403028	SB18
31/10/2014	602995	SB16
23/11/2014	303431	C18
26/11/2014	108055	SB18
27/10/2014	103450	SB16
30/10/2014	608032	SB16
08/12/2014	508038	C18
16/12/2014	507863	SB16
16/12/2014	302999	SB16
16/12/2014	208007	SB16
17/12/2014	703414	C18
19/12/2014	403453	C16
22/12/2014	307882	SB18

## Appendix 5: Clinical diarrhoea incidence

Table 16: List of clinical diarrhoea incidence

<b>Date</b>	<b>Calf number</b>	<b>Group</b>
15/10/2014	503405	SB16
31/10/2014	103018	C18
21/11/2014	708061	C18
11/11/2014	307854	C16
25/11/2014	403453	C16

## Appendix 6: List of milk replacer refusals.

Date	Calf number	Group	Refusals/treatment given
1/10/2014	603399	C18	Stomach tubed CMR at AM feed; Bottle fed at PM feed
2/10/2014	603399	C18	Bottle fed at AM feed
6/10/2014	307854	C16	Refused 100 ml at PM feed
18/10/2014	403028	SB18	Drank ½ CMR, bottle fed ½ CMR at AM feed
29/10/2014	403028	SB18	Bottle fed CMR, refused ½ at PM feed
	103018	C18	Refused ½ CMR at PM feed
30/10/2014	103018	C18	Bottle fed CMR and Life-Aid Xtra, refused ½ at AM feed; Bottle fed CMR at PM feed
31/10/2014	103018	C18	Stomach tubed CMR and Life-Aid at AM feed, Stomach tubed CMR at PM feed
1/11/2014	103018	C18	Drank ½, stomach tubed ½ at AM and PM feeds
2/11/2014	103018	C18	Refused ¼ CMR at PM feed
3/11/2014	103018	C18	Drank 2/3, stomach tubed 1/3 AM feed
9/11/2014	403028	SB18	Refused 400ml CMR at PM feed
12/11/2014	403028	SB18	Refused 500ml CMR AM feed, Refused 200ml CMR PM feed
13/11/2014	403028	SB18	Refused 100ml CMR AM feed
11/12/2014	403453	C16	Stomach tubed CMR at AM feed

**Table 17: list of milk replacer refusals**

## Appendix 7: Feed formulation and theoretical analysis

Table 18: List of component feeds in concentrates used.

Name	Stated analysis	
	CBA 16	CBA 18
Wheatfeed	25.00	29.70
Barley	19.05	16.80
Soya Hulls	13.30	13.30
Supaflow Beet Pulp	8.60	0.00
Hipro Soya	7.50	10.00
Vivergo Wheat Distillers	5.90	7.50
US Maize Distillers	5.00	6.60
SCM Maize Germ	5.00	5.00
Cane Molasses	5.00	5.00
Calcium Carbonate	2.80	3.25
Vegetable Oil	1.00	1.00
NuStart Premix	1.00	1.00
Salt	0.85	0.85
[VOLUME]	100.00	100.00

Table 19: Analyses of nutritional content of concentrates used.

Theoretical analysis - % as fed	Stated analysis		Analysis conducted 3/10/2014		Analysis conducted 22/10/2014	
	CBA 16	CBA 18	CBA 16	CBA 18	CBA 16	CBA 18
PROTEIN	16.0	18.0	16.3	18.2	16.2	17.8
OIL (Method B)	4.6	4.9	7.3	9.1	7.9	9.7
CRUDE FIBRE	10.3	9.8	9.4	9.1	8.4	9.2
CRUDE ASH	9.0	9.0	8.2	7.0	7.2	6.6
ME (MJ/kg DM)	12.6	12.6	13.22	13.02	13.49	13.03
STARCH	18.5	18.5	28.8	26.1	30.0	26.4
SUGAR	6.7	5.7	14.0	8.6	11.3	8.9
NDF	28.0	28.1	27.6	32.8	26.9	33.8

## Appendix 8: Criteria for assessing calf health scores

### Appendix 8.1: Calf dehydration score

Table 20: Calf dehydration scoring chart.

Score	1	2	3	4	5
Response	Returns to normal quickly	Returns to normal slowly (several seconds)	Skin folds	Weak body	Dead

(Source: Adapted from Kehoe and Heinrichs, 2005).

### Appendix 8.2: Calf Cough Score

Enter the pen or hutch, and squeeze the calf's trachea with some pressure while giving it a little shake. Listen for any coughs. Use the health-scoring chart to assign cough scores to each calf. For example, a calf that scores "0" does not cough, while one that scores "3" will have repeated spontaneous coughs after this procedure.

Table 21: Calf cough scoring chart.

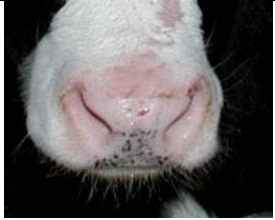



Score	0	1	2	3
Response	None	Induce single cough	Induced repeated coughs or occasional spontaneous cough	Repeated spontaneous coughs

(Source: McGuirk, 2009).



### Appendix 8.3: Nasal discharge score





Table 22: Nasal discharge scoring chart.

Score	0	1	2	3
	Normal serous discharge	Small amount of unilateral cloudy discharge	Bilateral, cloudy or excessive mucus discharge	Copious bilateral mucopurulent discharge
				

(Source: McGuirk, 2009).

### Appendix 8.4: Ocular discharge score





Table 23: Ocular discharge scoring chart.

Score	0	1	2	3
	Normal	Small amount of ocular discharge	Moderate amount of bilateral discharge	Heavy ocular discharge
				

(Source: McGuirk, 2009).

### Appendix 8.5: Ear droop score

Table 24: Ear droop scoring chart.

Score	0	1	2	3
	Normal	Ear flick or head shake	Slight unilateral droop	Head tilt or bilateral droop
				

(Source: McGuirk, 2009).

### Appendix 8.6: Calf coat bloom score





Table 25: Calf coat bloom scoring chart.

Score	1	2	3	4	5
	Dull	Slightly dull	Normal	Slight gloss	Shiny/glossy

(Source: Adapted from Linderoth, 2011).

### Appendix 8.7: Faecal consistency score

Table 26: Faecal consistency scoring chart.

Score	0	1	2	3
	Normal	Semi-formed, pasty	Loose, but stays on top of bedding	Watery, sifts through bedding
				

(Source: McGuirk, 2009).

## Appendix 9: Chi<sup>2</sup> analysis tables

### Appendix 9.1: Chi<sup>2</sup> analysis of clinical respiratory disease incidence

Table 27: Chi<sup>2</sup> analysis of clinical respiratory disease incidence

		Treatment					
	Pneumonia	SB18	SB16	C18	C16	Total	%
Observed	Yes	4	7	5	1	17	<b>0.35</b>
	No	8	5	7	11	31	<b>0.65</b>
	<b>Total</b>	12	12	12	12	48	
Expected	Yes	4.25	4.25	4.25	4.25		
	No	7.75	7.75	7.75	7.75		
O-E	Yes	-0.25	2.75	0.75	-3.25		
	No	0.25	-2.75	-0.75	3.25		
O-E <sup>2</sup>	Yes	0.06	7.56	0.56	10.56		
	No	0.06	7.56	0.56	10.56		
(O-E <sup>2</sup> )/E	Yes	0.01	1.78	0.13	2.49		
	No	0.01	0.98	0.07	1.36		
<b>Sum</b>	<b>6.83</b>						
<b>Critical Value</b>	<b>7.82</b>						
<b>P value</b>	<b>0.08</b>						

### Appendix 9.2: Chi<sup>2</sup> analysis of clinical diarrhoea incidence

Table 28: Chi<sup>2</sup> analysis of clinical diarrhoea incidence

		Treatment					
	Diarrhoea	SB18	SB16	C18	C16	Total	%
Observed	Yes	0	1	2	2	5	<b>0.10</b>
	No	12	11	10	10	43	<b>0.90</b>
	<b>Total</b>	12	12	12	12	48	
Expected	Yes	1.25	1.25	1.25	1.25		
	No	10.75	10.75	10.75	10.75		
O-E	Yes	-1.25	-0.25	0.75	0.75		
	No	1.25	0.25	-0.75	-0.75		
O-E <sup>2</sup>	Yes	1.56	0.06	0.56	0.56		
	No	1.56	0.06	0.56	0.56		
(O-E <sup>2</sup> )/E	Yes	1.25	0.05	0.45	0.45		
	No	0.15	0.01	0.05	0.05		
<b>Sum</b>	<b>2.46</b>						
<b>Critical Value</b>	<b>7.82</b>						
<b>P value</b>	<b>0.48</b>						

### Appendix 9.3: Chi<sup>2</sup> analysis of mortality

Table 29: Chi2 analysis of mortality

		Treatment					
	Diarrhoea	SB18	SB16	C18	C16	Total	%
Observed	Yes	0	0	1	1	2	<b>0.04</b>
	No	12	12	11	11	46	<b>0.96</b>
	<b>Total</b>	12	12	12	12	48	
Expected	Yes	0.5	0.5	0.5	0.5		
	No	11.5	11.5	11.5	11.5		
O-E	Yes	-0.5	-0.5	0.5	0.5		
	No	0.5	0.5	-0.5	-0.5		
O-E <sup>2</sup>	Yes	0.25	0.25	0.25	0.25		
	No	0.25	0.25	0.25	0.25		
(O-E <sup>2</sup> )/E	Yes	0.50	0.50	0.50	0.50		
	No	0.02	0.02	0.02	0.02		
<b>Sum</b>	<b>2.09</b>						
<b>Critical Value</b>	<b>7.82</b>						
<b>P value</b>	<b>0.55</b>						

### Appendix 9.4: Hypothetical Chi<sup>2</sup> analysis of clinical respiratory disease incidence

Table 30: Hypothetical Chi2 analysis of clinical respiratory disease incidence.

	Hypothetical	Treatment					
	Pneumonia	SB18	SB16	C18	C16	Total	%
Observed	Yes	4	8	5	1	18	<b>0.35</b>
	No	9	5	8	12	34	<b>0.65</b>
	<b>Total</b>	13	13	13	13	52	
Expected	Yes	4.55	4.55	4.55	4.55		
	No	8.45	8.45	8.45	8.45		
O-E	Yes	-0.55	3.45	0.45	-3.55		
	No	0.55	-3.45	-0.45	3.55		
O-E <sup>2</sup>	Yes	0.30	11.90	0.20	12.60		
	No	0.30	11.90	0.20	12.60		
(O-E <sup>2</sup> )/E	Yes	0.07	2.62	0.04	2.77		
	No	0.04	1.41	0.02	1.49		
<b>Sum</b>	<b>8.46</b>						
<b>Critical Value</b>	<b>7.82</b>						
<b>P value</b>	<b>0.04</b>						

## Appendix 9.5: Hypothetical Chi<sup>2</sup> analysis of clinical diarrhoea incidence

Table 31: Hypothetical Chi<sup>2</sup> analysis of clinical diarrhoea incidence.

	Hypothetical	Treatment					
	Diarrhoea	SB18	SB16	C18	C16	Total	%
<b>Observed</b>	<b>Yes</b>	0	3	7	7	17	<b>0.10</b>
	<b>No</b>	43	40	36	36	155	<b>0.90</b>
	<b>Total</b>	43	43	43	43	172	
<b>Expected</b>	<b>Yes</b>	4.3	4.3	4.3	4.3		
	<b>No</b>	38.7	38.7	38.7	38.7		
<b>O-E</b>	<b>Yes</b>	-4.3	-1.3	2.7	2.7		
	<b>No</b>	4.3	1.3	-2.7	-2.7		
<b>O-E<sup>2</sup></b>	<b>Yes</b>	18.49	1.69	7.29	7.29		
	<b>No</b>	18.49	1.69	7.29	7.29		
<b>(O-E<sup>2</sup>)/E</b>	<b>Yes</b>	4.30	0.39	1.70	1.70		
	<b>No</b>	0.48	0.04	0.19	0.19		
<b>Sum</b>		<b>8.98</b>					
<b>D.F.</b>		<b>3</b>					
<b>Critical Value</b>		<b>7.82</b>					
<b>P value</b>		<b>0.03</b>					

## Appendix 10: Weekly mean health scores

Appendix 10.1: Milk replacer with sodium butyrate and 18% crude protein concentrate

Dehydration score: CMR with SB and 18% CP concentrate

Table 32: Weekly dehydration score for SB18 calves. D= Dead; \*= Discarded result.

Calf eartag number	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 12
103408	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
703428	1.00	1.17	1.00	1.00	1.00	1.00	1.00	1.00
307882	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
107894	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
403000	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
108006	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
607892	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
403028	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
603448	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00*
108055	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00*
707900	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
108034	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00*

Cough frequency score: CMR with SB and 18% CP concentrate

Table 33: Weekly cough frequency score for SB18 calves. D= Dead; \*= Discarded result.

Calf eartag number	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 12
103408	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
703428	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
307882	0.00	0.00	0.67	0.67	0.67	0.00	1.00	2.00
107894	0.00	0.00	0.00	0.00	0.67	0.00	0.00	0.00
403000	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.00
108006	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
607892	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
403028	0.00	1.33	2.00	2.00	2.17	2.00	2.00	0.00
603448	0.00	0.00	0.00	0.67	0.00	0.00	0.00	2.50*
108055	0.00	0.67	0.00	0.00	0.67	0.00	0.00	0.00*
707900	0.00	0.00	0.00	0.67	0.00	0.00	0.00	1.00
108034	0.00	0.00	0.67	0.00	0.00	0.00	0.00	2.00*

### Nasal discharge score: CMR with SB and 18% CP concentrate

Table 34: Weekly nasal discharge score for SB18 calves. D= Dead; \*= Discarded result.

Calf eartag number	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 12
103408	0.00	0.00	0.00	0.67	0.00	0.00	0.00	0.00
703428	0.00	0.00	0.33	0.17	0.00	0.00	0.00	0.00
307882	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
107894	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
403000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
108006	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
607892	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
403028	0.17	0.50	0.00	0.00	0.00	0.50	0.00	1.00
603448	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00*
108055	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00*
707900	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
108034	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00*

### Ocular discharge score: CMR with SB and 18% CP concentrate

Table 35: Weekly ocular discharge score for SB18 calves. D= Dead; \*= Discarded result.

Calf eartag number	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 12
103408	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00
703428	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00
307882	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
107894	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
403000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
108006	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
607892	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
403028	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
603448	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00*
108055	0.00	1.75	0.00	0.00	0.00	0.00	0.00	0.00*
707900	0.00	0.00	0.17	0.33	0.00	0.00	0.00	0.00
108034	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00*

### Ear droop score: CMR with SB and 18% CP concentrate

Table 36: Weekly ear droop score for SB18 calves. D= Dead; \*= Discarded result.

Calf eartag number	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 12
103408	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
703428	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
307882	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
107894	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
403000	0.00	0.67	0.00	0.00	0.00	0.00	0.00	0.00
108006	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
607892	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.00
403028	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
603448	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00*
108055	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00*
707900	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

108034	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00*
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### Coat bloom score: CMR with SB and 18% CP concentrate

Table 37: Weekly coat bloom score for SB18 calves. D= Dead; \*= Discarded result.

Calf eartag number	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 12
103408	3.83	3.92	4.00	4.25	4.25	4.13	4.25	4.25
703428	3.92	3.83	3.83	4.33	4.50	4.38	4.00	4.25
307882	3.00	3.42	3.50	3.58	3.67	4.00	3.75	3.50
107894	3.33	3.50	3.58	3.67	3.83	4.00	4.00	4.00
403000	3.33	3.42	3.75	3.50	4.00	4.25	4.25	4.00
108006	3.08	3.33	3.92	3.92	3.75	3.50	3.50	3.75
607892	3.50	3.58	3.83	4.00	4.08	3.75	3.75	4.00
403028	3.25	3.50	3.92	3.83	3.92	4.25	4.25	4.00
603448	3.92	3.92	4.08	4.17	4.17	4.25	4.00	4.50*
108055	3.50	3.42	3.75	3.92	3.83	3.75	4.00	3.75*
707900	3.75	3.83	4.00	4.25	4.25	4.25	4.00	4.00
108034	3.83	3.58	3.75	3.92	3.67	3.75	3.75	3.75*

### Faecal consistency score: CMR with SB and 18% CP concentrate

Table 38: Weekly faecal consistency score for SB18 calves. D= Dead; \*= Discarded result.

Calf eartag number	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 12
103408	0.00	1.00	0.00	0.00	0.17	0.00	0.00	0.00
703428	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
307882	0.00	0.00	0.00	0.00	0.17	0.00	0.00	1.00
107894	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00
403000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
108006	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
607892	0.00	0.00	0.00	0.33	0.00	0.00	0.00	0.00
403028	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00
603448	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00*
108055	0.00	0.00	0.17	0.33	0.00	0.00	0.00	0.00*
707900	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.50
108034	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.50*



Appendix 10.2: Milk replacer with sodium butyrate and 16% crude protein concentrate

Dehydration score: CMR with SB and 16% CP concentrate

Table 39: Weekly dehydration score for SB16 calves. D= Dead; \*= Discarded result.

Calf eartag number	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 12
503405	1.00	1.00	1.25	1.00	1.00	1.00	1.00	1.00
708019	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
507884	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
307896	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
208007	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
507863	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
602995	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
707886	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
302999	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
508059	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00*
608032	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00*
403453	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00*

Cough frequency score: CMR with SB and 16% CP concentrate

Table 40: Weekly cough frequency score for SB18 calves. D= Dead; \*= Discarded result.

Calf eartag number	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 12
503405	0.00	0.67	1.33	0.00	0.00	1.00	0.00	1.00
708019	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
507884	0.00	0.00	0.00	0.83	0.00	0.00	0.00	0.00
307896	0.00	0.00	0.00	0.17	0.00	0.00	0.00	0.00
208007	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
507863	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
602995	0.00	0.00	0.67	0.00	0.67	1.00	1.00	1.50
707886	0.00	0.00	0.00	0.67	0.00	0.00	0.00	0.00
302999	0.00	0.00	0.00	0.00	0.00	0.00	2.00	1.00
508059	0.00	0.00	0.67	0.00	0.00	0.00	0.00	2.00*
608032	0.67	0.00	0.00	0.00	0.00	0.00	2.00	0.00*
403453	0.00	0.67	0.00	1.00	0.00	2.50	0.00	0.00*

### Nasal discharge score: CMR with SB and 16% CP concentrate

Table 41: Weekly nasal discharge score for SB18 calves. D= Dead; \*= Discarded result.

Calf eartag number	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 12
503405	0.00	0.00	0.17	0.00	0.00	0.00	0.00	0.00
708019	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
507884	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00
307896	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
208007	0.00	0.33	0.00	0.33	0.00	0.00	0.00	0.00
507863	0.00	0.00	0.00	0.00	0.17	0.00	0.00	0.00
602995	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
707886	0.00	0.00	0.00	0.00	0.33	0.00	0.00	0.00
302999	0.00	0.67	0.00	0.00	0.00	0.00	0.00	0.00
508059	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00*
608032	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00*
403453	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00*

### Ocular discharge score: CMR with SB and 16% CP concentrate

Table 42: : Weekly ocular discharge score for SB18 calves. D= Dead; \*= Discarded result.

Calf eartag number	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 12
503405	0.00	0.00	0.00	0.17	0.00	0.00	0.00	0.00
708019	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
507884	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
307896	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
208007	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
507863	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
602995	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00
707886	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
302999	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
508059	0.00	0.00	0.00	0.00	0.00	0.50	0.00	0.25*
608032	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00*
403453	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00*

### Ear droop score: CMR with SB and 16% CP concentrate

Table 43: Weekly ear droop score for SB18 calves. D= Dead; \*= Discarded result.

Calf eartag number	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 12
503405	0.00	0.00	0.67	0.00	0.00	0.00	0.00	0.00
708019	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
507884	0.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00
307896	1.00	1.00	1.00	0.00	0.33	0.00	0.00	0.00
208007	0.00	0.00	0.00	0.33	0.00	0.00	0.00	0.00
507863	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
602995	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
707886	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
302999	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
508059	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00*
608032	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00*
403453	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00*

### Coat bloom score: CMR with SB and 16% CP concentrate

Table 44: Weekly coat bloom score for SB18 calves. D= Dead; \*= Discarded result.

Calf eartag number	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 12
503405	3.50	3.50	3.08	3.92	3.75	4.13	4.00	4.00
708019	3.33	3.33	3.58	3.83	3.50	3.50	3.25	3.50
507884	3.83	3.75	3.67	3.92	3.92	4.00	4.25	4.00
307896	3.50	3.92	4.00	4.17	4.25	4.50	4.25	3.75
208007	3.50	3.67	3.67	3.83	3.92	3.75	3.75	4.00
507863	3.50	3.83	4.00	4.00	4.33	4.25	4.25	3.75
602995	3.92	3.83	3.83	3.83	4.00	4.25	4.25	4.00
707886	3.75	3.92	4.00	4.08	4.08	4.00	4.00	4.25
302999	3.25	3.50	3.83	4.00	3.92	4.00	3.75	3.75
508059	3.67	3.75	3.92	3.92	3.92	4.00	4.00	4.00*
608032	3.83	3.67	3.75	3.67	3.58	4.00	3.50	3.00*
403453	3.83	3.75	3.83	3.83	3.67	4.00	3.75	4.25*

### Faecal consistency score: CMR with SB and 16% CP concentrate

Table 45: Weekly faecal consistency score for SB18 calves. D= Dead; \*= Discarded result.

Calf eartag number	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 12
503405	0.33	0.00	0.67	0.00	0.00	0.00	0.00	0.00
708019	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
507884	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.50
307896	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.50
208007	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
507863	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.50
602995	0.00	0.00	0.17	0.00	0.00	0.00	0.00	0.00
707886	0.00	0.00	0.00	0.00	0.17	0.00	0.00	0.00
302999	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
508059	0.00	0.00	0.17	0.00	0.00	0.00	0.00	0.00*
608032	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00*
403453	0.67	0.50	1.50	0.33	0.00	0.00	0.00	0.50*

Appendix 10.3: Control milk replacer and 18% crude protein concentrate

Dehydration score: Control CMR and 18% CP concentrate

Table 46: Weekly dehydration score for C18 calves. D= Dead; \*= Discarded result.

Calf eartag number	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 12
603399	1.00	1.00	1.08	1.00	1.00	1.00	1.00	D
603406	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
303431	1.00	1.00	1.08	1.00	1.00	1.00	1.00	1.00
703414	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
607878	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
507898	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
502994	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
203012	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
103018	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
508038	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00*
708061	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00*
108068	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00*

Cough frequency score: Control CMR and 18% CP concentrate

Table 47: Weekly cough frequency score for C18 calves. D= Dead; \*= Discarded result.

Calf eartag number	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 12
603399	0.67	0.67	0.00	0.00	0.50	1.00	2.00	D
603406	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
303431	0.00	0.00	0.00	1.33	0.00	0.00	0.00	0.00
703414	0.00	0.00	0.00	0.67	0.00	1.25	1.00	0.00
607878	0.00	0.00	0.00	0.67	1.33	0.00	0.00	1.00
507898	0.67	0.00	0.00	0.67	0.33	0.00	0.00	0.00
502994	0.67	0.00	0.00	0.00	0.00	0.00	2.00	0.00
203012	0.00	0.00	0.00	0.00	0.67	0.00	0.00	1.00
103018	0.00	0.67	1.00	0.67	0.00	0.00	0.00	2.00
508038	0.00	0.50	1.33	0.83	0.00	0.00	0.00	2.00*
708061	0.67	0.00	0.00	0.00	1.00	0.00	2.00	0.00*
108068	0.00	0.00	0.67	0.67	1.50	0.00	0.00	2.00*

### Nasal discharge score: Control CMR and 18% CP concentrate

Table 48: Weekly nasal discharge score for C18 calves. D= Dead; \*= Discarded result.

Calf eartag number	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 12
603399	0.33	0.00	0.17	0.00	0.00	0.00	0.00	D
603406	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
303431	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
703414	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
607878	0.00	0.00	0.17	0.00	0.33	0.00	0.00	0.00
507898	0.33	0.00	0.17	0.50	0.00	0.00	0.00	0.00
502994	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
203012	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
103018	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
508038	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00*
708061	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00*
108068	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.25*

### Ocular discharge score: Control CMR and 18% CP concentrate

Table 49: Weekly ocular discharge score for C18 calves. D= Dead; \*= Discarded result.

Calf eartag number	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 12
603399	0.00	0.00	0.00	0.00	0.00	0.00	0.00	D
603406	0.00	0.17	0.00	0.00	0.17	0.00	0.00	0.00
303431	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00
703414	0.00	0.00	0.00	0.08	0.00	0.00	0.00	0.00
607878	0.33	0.50	0.00	0.08	0.33	0.00	0.00	1.00
507898	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.50
502994	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
203012	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
103018	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
508038	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00*
708061	0.00	0.00	0.00	0.00	0.33	0.00	0.50	0.00*
108068	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00*

### Ear droop score: Control CMR and 18% CP concentrate

Table 50: Weekly ear droop score for C18 calves. D= Dead; \*= Discarded result.

Calf eartag number	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 12
603399	0.67	0.67	0.00	0.00	0.00	0.00	0.00	D
603406	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
303431	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
703414	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
607878	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
507898	0.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00
502994	1.33	2.00	2.00	0.50	0.00	0.00	0.00	0.00
203012	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
103018	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
508038	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00*
708061	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00*
108068	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00*

### Coat bloom score: Control CMR and 18% CP concentrate

Table 51: Weekly coat bloom score for C18 calves. D= Dead; \*= Discarded result.

Calf eartag number	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 12
603399	3.67	3.67	4.00	4.25	3.92	4.25	4.25	D
603406	4.00	3.83	4.00	3.92	3.83	4.00	4.50	4.25
303431	4.00	4.17	4.33	4.33	4.42	4.50	4.25	3.75
703414	4.00	3.50	3.83	4.08	3.92	3.88	3.75	3.75
607878	3.00	3.08	3.33	3.75	3.83	4.00	4.00	3.75
507898	3.17	3.42	3.75	3.67	3.92	4.00	4.00	3.75
502994	3.00	3.33	3.33	3.58	3.75	4.00	4.25	3.75
203012	3.33	3.33	3.75	3.92	3.83	4.00	3.50	3.50
103018	3.00	3.00	3.58	3.75	3.58	3.50	3.75	4.00
508038	3.67	3.67	3.75	3.83	3.67	3.75	3.75	3.50*
708061	3.75	3.67	4.17	4.25	4.17	4.25	4.25	4.25*
108068	3.92	4.00	4.25	4.42	4.25	4.25	4.25	3.75*

### Faecal consistency score: Control CMR and 18% CP concentrate

Table 52: Weekly faecal consistency score for C18 calves. D= Dead; \*= Discarded result.

Calf eartag number	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 12
603399	0.00	0.33	0.50	0.00	0.00	0.00	0.50	D
603406	0.33	0.33	0.33	0.00	0.00	0.00	0.00	0.00
303431	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
703414	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
607878	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
507898	0.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00
502994	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00
203012	0.00	0.00	0.00	0.00	0.33	0.00	0.00	0.00
103018	0.00	0.33	0.50	0.33	0.00	0.00	0.00	0.50
508038	0.00	0.00	0.17	0.00	0.00	0.00	0.00	0.00*
708061	0.50	0.00	0.17	0.00	0.00	0.00	0.00	0.50*
108068	0.00	0.00	0.17	0.00	0.33	0.00	0.00	0.00*

## Appendix 10.4 Control milk replacer and 16% crude protein concentrate

### Dehydration score: Control CMR and 16% CP concentrate

Table 53: Weekly dehydration score for C16 calves. D= Dead; \*= Discarded result.

Calf eartag number	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 12
403397	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
308001	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
103422	1.33	1.33	1.00	1.00	1.00	1.00	1.00	1.00
507891	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
608018	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
207888	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
307854	1.00	1.08	1.17	1.00	1.00	1.00	1.00	D
302992	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
308008	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
303452	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00*
308029	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00*
103450	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00*

### Cough frequency score: Control CMR and 16% CP concentrate

Table 54: : Weekly cough frequency score for C16 calves. D= Dead; \*= Discarded result.

Calf eartag number	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 12
403397	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
308001	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
103422	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
507891	0.00	0.00	0.00	0.00	0.00	0.00	2.00	0.00
608018	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
207888	0.00	0.00	0.67	0.00	0.67	0.00	2.00	1.00
307854	0.00	0.00	0.00	0.00	0.33	0.00	0.00	D
302992	0.00	0.00	0.67	0.00	0.33	0.00	0.00	0.00
308008	0.00	0.67	0.00	0.00	0.00	0.00	0.00	1.00
303452	0.00	0.00	0.00	0.67	0.00	0.00	0.00	2.00*
308029	0.00	0.67	0.00	0.00	0.00	0.00	0.00	2.00*
103450	0.00	0.00	0.67	0.00	0.33	0.00	0.00	2.00*

### Nasal discharge score: Control CMR and 16% CP concentrate

Table 55: Weekly nasal discharge score for C16 calves. D= Dead; \*= Discarded result.

Calf eartag number	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 12
403397	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
308001	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
103422	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
507891	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
608018	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
207888	0.00	0.00	0.17	0.00	0.00	0.00	0.00	0.00
307854	0.00	0.00	0.00	0.83	0.00	0.00	0.00	D
302992	0.33	0.67	0.00	0.00	0.00	0.00	0.00	0.00
308008	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
303452	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00*
308029	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00*
103450	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00*

### Ocular discharge score: Control CMR and 16% CP concentrate

Table 56: Weekly ocular discharge score for C16 calves. D= Dead; \*= Discarded result.

Calf eartag number	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 12
403397	0.00	0.00	0.17	0.33	0.83	0.00	0.50	0.00
308001	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
103422	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
507891	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
608018	0.00	0.00	0.00	0.00	0.00	0.00	0.25	0.00
207888	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
307854	0.00	0.00	0.00	0.00	1.67	0.50	0.00	D
302992	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
308008	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
303452	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00*
308029	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00*
103450	0.00	0.00	0.00	0.00	0.00	0.50	0.00	0.00*

### Ear droop score: Control CMR and 16% CP concentrate

Table 57: Weekly ear droop score for C16 calves. D= Dead; \*= Discarded result.

Calf eartag number	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 12
403397	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
308001	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
103422	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00
507891	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
608018	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
207888	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
307854	2.00	1.33	1.67	1.17	1.00	1.00	1.00	D
302992	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
308008	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
303452	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00*
308029	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00*



103450	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00*
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### Coat bloom score: Control CMR and 16% CP concentrate

Table 58: Weekly coat bloom score for C16 calves. D= Dead; \*= Discarded result.

Calf eartag number	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 12
403397	4.00	3.50	3.83	4.08	3.83	4.00	4.25	4.50
308001	3.17	3.58	3.75	4.00	3.58	3.50	3.25	3.00
103422	3.92	3.92	4.08	4.25	4.25	4.25	4.25	1.25
507891	3.58	3.83	3.92	3.92	3.92	4.00	3.75	4.00
608018	3.50	3.50	3.58	3.75	3.83	3.75	3.50	3.75
207888	3.50	3.58	3.83	4.17	4.00	4.00	4.00	3.75
307854	3.17	3.58	3.67	3.58	3.58	4.00	3.75	D
302992	3.33	3.33	3.67	3.83	4.00	4.50	4.50	3.75
308008	3.17	3.33	3.67	3.92	3.83	3.75	3.75	3.75
303452	4.17	4.17	4.17	4.33	4.33	4.25	4.25	4.50*
308029	3.67	3.58	3.75	3.75	3.75	3.75	3.75	3.50*
103450	3.75	3.83	3.67	4.25	4.33	4.25	4.25	3.50*

### Faecal consistency score: Control CMR and 16% CP concentrate

Table 59: Weekly faecal consistency score for C16 calves. D= Dead; \*= Discarded result.

Calf eartag number	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 12
403397	0.67	0.50	0.00	0.00	0.00	0.00	0.00	0.50
308001	0.00	0.17	0.00	0.00	0.00	0.00	0.00	1.00
103422	1.00	0.83	0.17	0.00	0.00	0.00	0.00	1.00
507891	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
608018	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
207888	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
307854	0.67	0.00	0.00	0.00	0.33	1.00	0.50	D
302992	0.00	0.67	0.00	0.00	0.00	0.00	0.00	0.50
308008	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
303452	0.50	0.00	0.33	0.00	0.00	0.00	0.00	0.00*
308029	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00*
103450	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.50*

## Appendix 11: Mean feed intake

Table 60: Mean feed intake per treatment group.

SB18= CMR with SB and control concentrate (18% CP).

SB16 = CMR with SB and concentrate with reduced CP (16% CP).

C18 = Control CMR and control concentrate (18% CP).

C16 = Control CMR and concentrate with reduced CP (16% CP).

Treatment group	Mean milk replacer intake (kg/calf)	Mean concentrate intake (kg/calf)		
		Pre-weaning (weeks 1-6)	Post-weaning (weeks 6-12)	Entire trial (weeks 1-12)
SB18	21.9	30.55	159.90	190.45
SB16	21.9	28.55	155.80	184.35
C18	21.9	26.27	160.00	186.27
C16	21.9	28.93	154.60	183.53

## Appendix 12: Mean daily live weight gain

Table 61: Mean daily live weight gain (DLWG) per treatment group.

SB18= CMR with SB and control concentrate (18% CP).

SB16 = CMR with SB and concentrate with reduced CP (16% CP).

C18 = Control CMR and control concentrate (18% CP).

C16 = Control CMR and concentrate with reduced CP (16% CP).

Treatment group	Mean DLWG (kg/calf)		
	Pre-weaning (weeks 1-6)	Post-weaning (weeks 6-12)	Entire trial (weeks 1-12)
SB18	0.79	1.17	0.98
SB16	0.77	1.15	0.96
C18	0.70	1.19	0.95
C16	0.78	1.07	0.93

## Appendix 13: Cost of feed

Table 62: Cost of milk replacer and concentrate

Cost of milk replacer (£/t)		Cost of concentrate (£/t)	
Control	1,625	18% CP	304
Sodium butyrate	1,660	16% CP	298

## Appendix 14: Cost per kilogram of daily live weight gain

Table 63: Cost (£/kg) of mean daily live weight gain (DLWG).

SB18= CMR with SB and control concentrate (18% CP).

SB16 = CMR with SB and concentrate with reduced CP (16% CP).

C18 = Control CMR and control concentrate (18% CP).

C16 = Control CMR and concentrate with reduced CP (16% CP).

Treatment group	Pre-weaning (weeks 1-6)		Post-weaning (weeks 6-12)		Entire trial (weeks 1-12)	
	Mean daily feed cost (£/calf)	Mean DLWG cost (£/kg)	Mean daily feed cost (£/calf)	Mean DLWG cost (£/kg)	Mean daily feed cost (£/calf)	Mean DLWG cost (£/kg)
<b>SB18</b>	1.20	1.53	1.06	0.90	1.12	1.14
<b>SB16</b>	1.18	0.90	1.01	0.88	1.09	1.14
<b>C18</b>	1.15	0.80	1.06	0.89	1.10	1.16
<b>C16</b>	1.16	0.91	1.00	0.93	1.07	1.15

## Appendix 15: P values from analysis including discarded results during weeks 1-12

Table 64: P values from analysis including discarded results during weeks 1-12.

	<b>CMR</b>	<b>Concentrate</b>	<b>CMR* Concentrate</b>	<b>Time</b>	<b>Time *CMR</b>	<b>Time* Concentrate</b>	<b>Time *CMR* Concentrate</b>
<b>Dehydration P value</b>	0.37	0.28	0.38	0.18	0.63	0.57	0.21
<b>Cough frequency P value</b>	0.75	0.18	0.36	<0.01	0.65	0.66	0.62
<b>Nasal discharge P value</b>	0.56	0.44	0.29	0.28	0.37	0.45	0.27
<b>Ocular discharge P value</b>	0.19	0.83	0.62	0.33	0.17	1.00	0.42
<b>Ear droop P value</b>	0.32	0.60	0.95	0.03	0.62	0.63	0.29
<b>Coat bloom P value</b>	0.7	0.64	0.86	<0.01	0.19	0.18	0.80
<b>Faecal consistency P value</b>	0.12	0.32	0.96	<0.01	0.70	0.84	0.26