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Factors influencing the development of mucosal immunity in hand-reared calves

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Abstract

The potential for rearing bull calves from dairy cows in order to supply the bull beef market is hampered by high rates of death and disease. The development of the gut-associated immune system of calves in the first three weeks of life was investigated through the influence on the health and immune responses of calves of two different forms of additives to calf milk replacer. Addition of a commercial nucleotide to calf milk replacer appeared to have no effect. A second experiment using nucleotides was designed to determine whether amount of solid feed intake could influence the effects of nucleotides on health of calves and again there was no significant effect of amount of solid feed on health of the calves. During the experiments on fatty acids and nucleotides it was noted that the appearance of the Peyer's patches in the intestinal tract varied considerably from calf to calf. A correlation between Peyer's patch numbers and incidence of diarrhoea showed that calves with a greater number of Peyer's patches in the small intestine had a lower incidence of diarrhoea.

Executive summary

- The potential for rearing bull calves from dairy cows in order to supply the bull beef market is hampered by high rates of death and disease.

- In the first three weeks of life mortality rates for hand-reared calves ranges between 2 and 15% of calves reared and the prevalence of disease averages about four to five times the mortality rate.

- The high costs of rearing calves (especially when coupled with costs incurred for treatments of diseases and losses through deaths) makes calf rearing an economically risky enterprise.

- The majority of diseases in calves in Australia are enteric diseases affecting the gut and causing severe diarrhoea or scours.

- The development of the gut-associated immune system of calves in the first three weeks of life was investigated through the influence on the health and immune responses of calves of two different forms of additives to calf milk replacer.

- In particular, observations and analyses were made on the Peyer's patches of the small intestine. These specific and important immune structures are critical for the calf against pathogens.

- The first experiment investigated the effects of the addition of 5% sunflower seed oil (poly unsaturated fatty acids) and 5% palm fat (saturated fatty acids) on health status of bull calves and the gut associated lymphoid tissue (specifically Peyer's patches) that is important in protecting young calves against disease entry through the gut.

- The second and third experiments investigated the effects of a commercial preparation of nucleotides on health status and immunity of bull calves.

- Unlike early work where replacing all the fat in calf milk replacer with unsaturated fats caused increased mortalities, the addition here of 5% unsaturated fatty acids did not cause increased mortality or morbidity in two or three week old calves.

- Addition of a commercial nucleotide to calf milk replacer appeared in the first experiment to be of benefit at 2 weeks of age but at 3 weeks it appeared to be associated with an increased rate of diarrhoea and a decreased weight gain. Because solid feed intake increases between 2 and 3 weeks it was thought that increased availability of substrates from the solid feed and availability of nucleotides for microbial development could have caused an increase in microbial proliferation to the detriment of calf health.

- A second experiment using nucleotides was designed to determine whether amount of solid feed intake could influence the effects of nucleotides on health of calves. The responses of calves to nucleotides differed from those in the first

nucleotide experiment and no advantage of addition of nucleotides to calf milk replacer was seen at two weeks of age. There was no significant effect of amount of solid feed on health of the calves.

- During the experiments on fatty acids and nucleotides it was noted that the appearance of the Peyer's patches in the intestinal tract varied considerably from calf to calf.

- A correlation between Peyer's patch numbers and incidence of diarrhoea showed that calves with a greater number of Peyer's patches in the small intestine had a lower incidence of diarrhoea.

- This important finding could be exploited further if a genetic marker for Peyer's patches could be found so that genetic selection on the basis of Peyer's patch number could be made. There is evidence from others to suggest that the numbers of Peyer's patches in other species of animals is predetermined by the genetic make-up but the function can be modified to some degree after birth.

- If a genetic marker could be found it would benefit not only the dairy bull beef industry but also the beef industry as the prevalence of diarrhoea in beef breed calves reared by their dams is about 3% and this could increase with an increase in early weaning practices.

- As a spin-off from the third experiment in which the effects of an interaction between nucleotides and solid feed was investigated, the effects on methanogens and selected other microorganisms in the developing rumen were also assessed. Both nucleotides and amount of solid feed eaten in young calves have the potential to increase rate of development of fermentation reactions in the rumen and the numbers or types being established.

- In calves given the nucleotide supplement methanogens were reduced compared to calves without the nucleotide supplement. This suggests that there is a potential to influence the early establishment of methanogens in calves and that this, in view of interactions between gut microorganisms and the immune function in the gut, could have potential for influencing the lifetime concentrations of methanogens in the rumen.

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1 Background

One of the greatest problems facing veterinarians in dairy practices is control of disease in calves. Dairy bull calves have the potential to be an economically important source of meat animals but they face the greatest challenges to survival of all livestock: they are hand-reared with milk-replacer from a few hours or days after birth and frequently have little access to colostrum because it is reserved for heifer calves or sold for human consumption. However, even with an adequate intake of colostrum, death and disease in calves is high. Mortality rates in hand-reared calves average about 6 – 7% with a usual range of 2 to 15 % (Moran 2002, Sutherland pers. comm.). The numbers of calves that need treatment for disease is about 4 – 5 times those dying (Tomlinson *et al.* 2002). The greatest losses occur within the first 1.5 to 5 weeks of age. In contrast, in beef calves reared by their mothers, mortality rates during 1.5 to 5 weeks of age are generally lower, averaging around 3%. Infections of the respiratory and digestive tracts account for more than 75% of deaths in hand-reared calves. Diarrhoea and invasion by pathogens from the intestinal tract is a major cause of disease. On welfare grounds the high level of ill health in calves needs to be reduced. On economic grounds, high mortality rates, expensive treatments and reduced growth rates contribute to poor returns to calf rearers. A reduction in disease and mortality in calves would encourage the rearing of a greater number than the estimated 175,000 calves at present reared per annum and would reduce the wastage from slaughter of about 700,000 bobby calves (Moran (2005) MLA consultancy report).

The mucosal immune system of the digestive tract (or gut-associated lymphoid tissue, GALT) is the first line of defence against a major entry point for pathogens of young animals. Rates of development of GALT can be influenced by genetic make up - breed variations in immune cell receptors have been recorded in cattle. Naturally occurring factors in milk and diet can also enhance rates of development.

Although there is much anecdotal evidence and some data on overall health advantages of diet composition or dietary additives in young calves, there is little documented scientific work (unlike for young of other livestock) on the effects of specific dietary components on the development of the important GALT defence mechanisms. Milk replacer does not contain many of the essential growth factors and other compounds present in colostrum and transition milk. These are important for rapid development of the GALT.

Based on work in other species that show an advantageous effect on GALT development, the effects of the following diet components were investigated: unsaturated fatty acids (which, although being dietary essential components, are paradoxically low in cows' milk) and nucleotides (that are unable to be synthesised fast enough for the high requirements for cell multiplication and maturation of the growing digestive tract).

Ingestion of good quality colostrum with high levels of antibodies (passive transfer of immunity) in the first 24 hours of life is very important for preventing death and disease in neonatal calves. However, even with high levels of passive immunity obtained from colostrum, up to 30% of calves can still succumb to disease (Wells *et al.* 1996). The antibodies acquired by passive immunity have little effect on preventing pathogenic microorganisms entering the body and their specificity against individual pathogens restricts their usefulness. If the antibodies are not active against an invading pathogen, then disease will result.

The **gut-associated immune system** develops within the gastro-intestinal tract and associated lymphatic organs. It has innate mechanisms that recognise more broadly the various pathogens that are ingested and it can rapidly mobilise cellular and antibody defence mechanisms to overcome the effects of the pathogens and prevent disease. Indicative of the importance of mucosal immunity is that more than 70% of all immunoglobulin secretory cells in the body are present in the intestine (Field *et al.* 1999). The most important immune structures or organs within the intestinal tract walls are the Peyer's patches. These structures are involved in innate and acquired immunity. In calves of a few weeks of age innate immunity is the more important system and is naturally present from birth and is the first line of defence against pathogens. Its relative strength or activity is thought to be determined by genetic constitution and is not dependent for its functioning on prior exposure to pathogenic microorganisms or antigens. However, it provides a link between the innate and acquired or active immune systems.

The defensive cells and secretory immunoglobulins of the mucosal immune system reduce proliferation of pathogenic microorganisms within the gut and thus reduce the incidence of disease and severity of scouring (Brandtzaeg 1996; Gil and Rueda 2002).

Cells of the immune system have specialised receptors known as **toll-like receptors** (TLRs) on the cell membrane surfaces which have an innate ability to recognise pathogenic microorganisms (Werling and Jungi 2003, Eicher *et al.* 2004, Menzies and Ingham 2006). The receptors hold a unique place in forging links between the innate functioning of immune cells and the acquired antibody secreting cells. There is also the potential for using the level of expression of the TLR genes for selection of calves with greater genetic resistance to disease (Werling and Jungi 2003).

The ten to thirteen TLRs that have been identified in mammalian cell membranes are pathogen recognition proteins or receptors and have the ability to be stimulated by specific pathogens. Toll-like receptor 2 (TLR2) recognises for instance gram positive bacteria and TLR4, gram negative bacteria. When a TLR on the surface of a cell of the innate immune system is activated by an appropriate ligand it initiates a series of reactions including stimulation of the release of cytokines that can influence the activity of e.g. lymphocytes of the active immune system. The TLRs are an important and essential link between the innate and active immune systems and in neonatal calves development of the active immune system has not reached the level seen in adults. An ability to manipulate or up regulate TLR expression and therefore the subsequent function of the receptors may help to improve development of immunity in young calves.

Activation of TLRs causes stimulation of genes ('downstream' genes) that in turn produce signalling factors important in immune function (Figure 1). MyD88 provides an important link between innate and active immunity since all TLRs, except TLR3, signal through it to produce inflammatory cytokines. Activation of NF-κB leads to induction and expression of inflammatory cytokines such as TNFα, IL-6, IL-12, which are essential in inhibiting bacterial activity. The up-regulation of these signalling molecules and transcription factor NF-κB in the TLR4 signalling pathway in the calf's tissue is in agreement with the findings of several studies that showed that unsaturated fat inhibits NF-κB activation induced by TLR4, but not its downstream signalling components (Miles and Calder, 1998; Calder, 2003; Yaqoob, 2003; Lee *et al.,* 2003; Lee *et al.,* 2004; Calder, 2006).

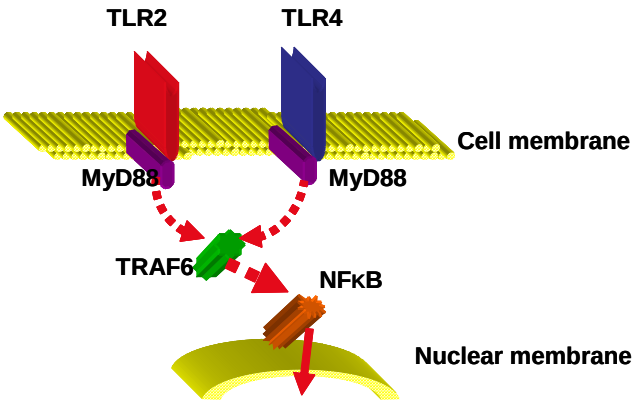


Figure 1. Simplified schematic diagram showing relationship between TLR2, TLR4 and signalling molecules, MyD88 - myeloid differentiation factor 88, TRAF6 - tumor necrosis factor-associated factor 6 and NFκB nuclear factor kappa B.

An active and rapidly maturing gut-associated immune system is vital for protecting calves from the many and varied pathogenic organisms that can contribute to producing disease. Developmental maturation of gut-associated immunity in other animals has been shown to be stimulated by a number of **dietary factors** e.g. unsaturated fatty acids, oligosaccharides, nucleotides/nucleosides and polyamines (Brandtzaeg 1996; Gil and Rueda 2002). However, there is very little specific information about rate of development of gut-associated immunity in hand-reared calves or on how its functional development or maturation could be enhanced (Yasuda *et al.* 2002, Norrman *et al.* 2003, Werling and Jungi 2003, White *et al.* 2003).

Unlike other species, calves have a relatively low requirement for **essential unsaturated fatty acids**; increasing the intake of unsaturated fatty acids causes increased incidence of ill-thrift (Roy 1980). It is suggested that the natural micro-flora of the pre-ruminant gut produce sufficient polyunsaturated fatty acids to satisfy the calf's requirements (Sklan et al. 1971; 1972). Polyunsaturated fatty acids through their products, the eicosanoids, have important roles in modulating and maintaining immune system functions. However, a balance between the fatty acids is important as excesses or deficiencies of individual fatty acids can cause major changes in immune system function. The proportions of dietary fatty acids can also influence the composition of all cell membranes including those of the cells of the immune system and those cells lining the intestinal tract. The properties of the cells can therefore be affected. The type of fat (from cow's milk, vegetable oils or hydrogenated fats) will therefore influence the protective functioning of the intestinal tract innate and active immune systems.

Calves reared by their mothers obtain milk with a composition considerably different from the **milk-replacers** used to hand-rear calves. A contributing factor to differences in the mortality rates between bull and heifer calves is that a greater proportion of heifer calves are given transition milk that is unsuitable for human consumption. The differences between transition and later milk are most marked in the composition of the non-nutritional factors e.g. nucleotides/nucleosides needed for synthesis of DNA and RNA in rapidly dividing cells (Schlemme *et al.* 2000). **Nucleotides/nucleosides**, normally present in high concentrations in milk for at least two to three weeks after calving, have been shown in other species to stimulate the rate of development of gut-associated mucosal immunity and to reduce the incidence of disease (Grimble and Westwood 2000, Theisinger *et al.* 2002). A dietary supply is therefore essential because synthesis rate of the compounds by intestinal cells is slower than demand (Theisinger *et al.* 2002).

Dried milk powders used to formulate most calf milk-replacers are generally produced from milk with low levels of nucleotides/nucleosides and calves can therefore have low intakes of the essential compounds that are necessary for the optimum development of the mucosal immune system.

2 Project objectives

The project was designed to:-

(1) determine whether specific additives or changes to the composition of calf milk replacer can improve the activity of GALT, and

(2) obtain basic information about the growth and development of GALT of Friesian and Jersey calves within the first month after birth when calves are at their most vulnerable.

3 Methodology

The project consisted of a series of experiments in which various modifications were made to the composition of calf milk replacer (CMR) and the effects on health and immune status assessed. In addition development of immunity in a few Jersey calves was assessed for comparison with the Friesian-Holstein calves that were used in all experiments.

Experiment 1. The first experiment carried out in two parts was to determine the effect of adding saturated or unsaturated fatty acids to CMR on health and immunity. The first part examined the effects of the modified CMR for three weeks from 2 – 5 days of age and was followed by an experiment that examined the effects of feeding the diets for a shorter period, from 5 days to two weeks of age.

Experiment 2. This examined the effect on health and immunity of adding a commercial nucleotide preparation to calves from 3 to 5 days of age to 2 or 3 weeks of age.

Experiment 3. This experiment examined whether there could be an interaction between nucleotides added to CMR and amount of solid feed eaten by calves, on their health and immunity.

Experiment 4. The histomorphology of the Peyer's patches of Jersey calves from 5 days to was measured.

Experiment 5. The distribution, numbers and or sizes of Peyer's patches from calves in experiments 2 to 3 were determined and correlated with severity of diarrhoea.

Additional experimental data. Using rumen fluid samples collected by stomach tube from calves in experiment 3, molecular biological techniques were used to identify key microorganisms. The methods, results and discussion of each of the experiments will be described separately to be followed by a general discussion of the results and significance.

4 Five separate experiments.

Experiment 1 – Effect of saturated or unsaturated fats on immunity of calves.

Methods

The experiment was carried out in two parts. In **Part 1** Friesian bull calves were kept for three weeks and were obtained from three different dairy farms. In **Part 2** calves kept for two weeks all came from a single farm.

Bull calves were obtained from commercial dairy farms at 2 – 4 days of age and transported to the Bundoora campus of La Trobe University. For the first day after arrival the calves were fed an electrolyte solution. They were weighed within two days of arrival and divided into three treatment groups. The treatment groups in both part 1 and 2 were,

1. Controls fed commercial calf milk replacer (CMR),
2. Saturated fat group fed CMR plus palm oil added at a rate of 5% of the dry matter to the CMR and,
3. Unsaturated fat group fed CMR plus sunflower seed oil added at a rate of 5% of the dry matter to the CMR.

Calves were fed twice a day at a rate of approximately 5% of their body weight at each feed. Water was available at all times and straw or calf pellets or mash was also available. Calves were housed in an 'Ecoshelter' (Redpath, Bendigo East, Victoria) containing six pens (2.5 x 3 m), three calves per pen. Bedding was a mixture of sawdust and wood shavings.

The health status of calves was recorded each day so that the incidence of disease could be assessed. Feeding behaviour, rectal temperature (scored 0 for 38.5 – 39.4^{o}C; 1 for 38.5 – 39.9 ^{o}C; 2 for 39.5-39.9^{o}C; 4 for >40.0^{o}C), presence or absence of eye or nasal discharge or coughing (scored 0 for no observations, 1 for presence) and faecal consistency (evidence of diarrhoea, 0 for normal; 1 for thin; 2 for watery) were used to assess health status.

In **Part 1**, blood samples were collected at 5 days, 12 – 14 days and 20 – 21 days and in **Part 2** at 5 and 12-14 days, in order to measure serum immunoglobulin G (IgG) concentrations by an ELISA method (Bethyl Laboratories). Calves were weighed at weekly intervals.

At either 14 or 21 days of age calves were given 5-bromo-2'- deoxyuridine (BrdU) by intravenous injection. The BrdU was dissolved in 20 ml 0.9% saline solution and given at a dose rate of 10 mg/kg body weight. Calves were killed one hour later with an overdose of barbiturate and samples of the intestinal tract collected. Collection of tissues was usually completed within 20 minutes of the calf dying.

Samples were collected from the jejunal and ileal Peyer's patches (JPP & IPP) and from mesenteric lymph nodes lying close to the jejunum and ileum (JLN & ILN) for histological examination and for measurement of mRNA expression of TLR 2 and TLR 4. Tissues were also collected and analysed from a region of the jejunum where Peyer's patches were not visible.

Results

Body weights

There was no significant difference in body weights or body weight gains among the treatments (Table 1.1) in either Part 1 (3 weeks old calves) or Part 2 (2 week old calves).

Table 1.1 Body weights and body weight gains of calves fed for 3 weeks (Part 1) or 2 weeks (Part 2) on calf milk replacer (Control) or on calf milk replacer with the addition of either 5% palm oil (Saturated fat) or 5% sunflower seed oil (Unsaturated fat). Means ± SD, numbers in brackets are numbers of calves.

	Age	Body weight (kg)		
		Control	Saturated fat	Unsaturated fat
Part 1 (7) 3 weeks old	Initial 3-5 d	45.7 ± 3.4	45.9 ± 2.3	41.9 ± 4.8
	Final 21 d	52.0 ± 5.1	51.1 ± 2.3	50.0 ± 5.0
	Change over 15-16 days	6.2 ± 2.2	5.1 ± 3.5	8.1 ± 3.5
Part 2 (8) 2 weeks old	Initial 7 d	44.6 ± 2.9	46.7 ± 4.3	43.3 ± 3.3
	Final 14 d	46.3 ± 3.0	47.8 ± 4.3	44.7 ± 4.6
	Change over 7 days	1.8 ± 1.5	1.5 ± 1.8	1.3 ± 2.7

IgG concentrations

There was no difference in the final concentrations of serum IgG of the Part 1 calves kept until 21 days of age (Table 1.2) or in Part 2 calves kept for 2 weeks. However, the samples taken at week 1 in Part 1 showed a significant lower concentration in the Saturated fat Group with evidence of failure of passive transfer of maternal antibodies in two of the calves. As has been observed previously, IgG concentrations in such animals (in contrast to those with adequate IgG concentrations) increase rather than decrease with time.

Table 1.2 Serum immunoglobulin G (IgG) concentrations of calves fed for 3 weeks (Part 1) or 2 weeks (Part 2) on calf milk replacer (Control) or on calf milk replacer with the addition of either 5% palm oil (Saturated fat) or 5% sunflower seed oil (Unsaturated fat). Means ± SD, numbers in brackets are numbers of calves.

	Age	Serum IgG mg/ml		
		Control	Saturated fat	Unsaturated fat
Part 1 (7) 3 weeks old	7 d	17.6 ± 5.3	9.7 ± 3.9 *	18.5 ± 15.4
	14 d	13.3 ± 3.9	10.1 ± 3.9	13.0 ± 5.1
	20 d	14.3 ± 4.4	10.2 ± 3.8	11.7 ± 6.9
	Change	- 3.2 ± 5.3	+ 0.4 ± 5.0	- 6.7 ± 9.7
Part 2 (8) 2 weeks old	5 d	17.44 ± 4.49	12.46 ± 2.18	16.14 ± 3.67
	10 d	17.51 ± 3.81	15.25 ± 3.65	18.18 ± 4.53
	13-14 d	12.20 ± 2.95	10.43 ± 1.75	13.05 ± 3.00
	Change	-5	-3	-2

* P<0.05

Health parameters

Except for the behaviour of the calves in the group given Saturated fat for three weeks (Part 1) there were no treatment differences in the health status of the three week (Table 3) or two week old calves (Table 1.3). The calves in the Saturated fat group kept for three weeks (Part 1) appeared more listless than those in the other two treatment groups however, this group of calves also showed a significantly lower concentration of serum IgG (Table 2) and this could have contributed to their more listless behaviour in the first two weeks of age.

Table 1.3 Cumulative health parameters of calves fed for 3 weeks (Part 1) or 2 weeks (Part 2) on calf milk replacer (Control) or calf milk replacer with addition of either 5% palm oil (Saturated fat) or 5% sunflower seed oil (Unsaturated fat). Means ± SD, numbers in brackets are numbers of calves.

		Score		
	Health parameter	Control (7)	Saturated fat (7)	Unsaturated fat (7)
Part 1	Temperature score	1.2 ± 0.7	3.4 ± 2.9	2.7 ± 2.6
	Behaviour	0.4 ± 0.5	1.7 ± 1.2*	0.2 ± 0.4
	Presence of cough	0	0.5 ± 0.7	0.2 ± 0.4
	Nasal discharge	0	0	0
	Eye discharge	0.1 ± 0.3	0.5 ± 0.7	0.4 ± 0.5
	Faecal consistency	4.2 ± 3.5	4.7 ± 3.0	6.5 ± 4.3
	Average health score	1.0 ± 0.7	1.8 ± 1.2	1.7 ± 1.0
Part 2	Temperature score	0.12 ± 0.16	0.12 ± 0.16	0.11 ± 0.11
	Behaviour	0	0.13 ± 0.35	0
	Presence of cough	0	0	0
	Nasal discharge	0	0	0
	Eye discharge	0.58 ± 0.28	0.52 ± 0.26	0.48 ± 0.2
	Faecal consistency	0.20 ± 0.14	0.16 ± 0.15	0.22 ± 0.29
	Average health score	0.13 ± 0.19	0.15 ± 0.19	0.15 ± 0.22

* P<0.05

For definition of scores see methods section.

Histomorphology of intestinal tract and immuno-histology

Part 1 – three week old calves

There were no significant differences between treatments for heights, areas and circumferences of villi near the JPP or IPP or between lengths, areas and circumferences of the follicles of the Peyer's patches (Table 1.4). Consistent with reported differences between the morphology of the JPP and IPP, the villi were significantly larger ($P < 0.05$) and the follicles significantly smaller ($P < 0.05$) in the jejunum than the ileum ($P<0.05$).

Table 1.4 Measurements on villi and follicles of Peyer's patches in jejunum and ileum of three week old calves fed milk replacer (Control) or milk replacer with the addition of either 5% palm oil (Saturated fat) or 5% sunflower seed oil (Unsaturated fat). Means ± SD, n=7

	Control	Saturated	Unsaturated
		Jejunal Peyer's patches	
Villus Height (µm)	508 ± 91	460 ± 92	499 ± 98
Villus Area (x10^3 µm^2)	75.7 ± 23.1	63.5 ± 16.8	70.9 ± 20.2
Villus Circumference (µm)	1.33 ± 0.21	1.19 ± 0.19	1.29 ± 0.2
Follicular Height (µm)	235 ± 34	213 ±42	221 ± 37
Follicular Area (x 10^3 µm^2)	92.2 ± 34.3	72.9 ± 30.4	80.7 ± 24.6
Follicular Circumference (mm)	1.16 ± 0.20	1.02 ± 0.23	1.14 ± 0.16
		Ileal Peyer's patches	
Villus Height (µm)	337 ± 46	291 ± 46	315 ± 82
Villus Area (x10^3 µm^2)	39.5 ± 9.4	38.2 ± 9.3	41.9 ± 14.3
Villus Circumference (µm)	0.96 ± 0.09	0.89 ± 0.11	0.92 ± 0.18
Follicular Height (µm)	1030 ± 412	867 ± 209	1009 ± 208
Follicular Area (x 10^3 µm^2)	357.3 ± 198.8	311.7 ± 90.9	340.2 ± 126.4
Follicular circumference (mm)	2.62 ± 0.91	2.35 ± 0.48	2.54 ± 0.54

The proliferation of cells measured by counting numbers of cells incorporating bromodeoxyuridine (BrdU) was at a significantly higher rate in the dome area of the jejunum of calves given the Saturated fat supplement compared with those in the Control group (Table 1.4). The dome area of the Peyer's patches contains M-cells, mainly, but not exclusively, located towards the luminal surface of the dome. M-cells are important in the entry of pathogenic and non-pathogenic organisms from the lumen of the gut into tissues and transmission or presentation of antigens to B-lymphocytes. In the more central part of the dome, B-lymphocytes rather than T-lymphocytes are located and it is thought that interactions between M-cells and B-

lymphocytes in the dome are important for the development of active immunity. From the data it is not possible to state categorically that it is the B-lymphocytes rather than M-cells that were proliferating in the dome area but from the location of the BrdU stained cells it is most likely that it was B-lymphocyte proliferation in the domes that was significantly greater in the calves supplemented with Saturated fat compared with Controls. An endpoint histological observation of this nature does not allow specific conclusions to be made regarding the impact of the fats on immune function: an increased rate of proliferation could be interpreted as increased active immunity because the B-lymphocytes were actively dividing and therefore it showed a response or an ability to respond to pathogens or antigens entering the tissue: on the other hand, the increased rate of proliferation could indicate decreased immunity or disease resistance in the calves given Saturated fat and as a result B-lymphocytes were required in greater numbers to provided protection for the calves.

No significant differences were observed in rates of proliferation of cells in other areas of the JPP or IPP (Tables 1.5). No differences among treatments were observed in numbers of B- and T-lymphocytes in the JPP or IPP of the three week old calves. However, in the jejunum, B-lymphocyte numbers tended to be higher (except in the domes) in the calves supplemented with Saturated fat. The lower numbers in the domes is not inconsistent with the increased rate of cell proliferation observed in the domes: B-lymphocytes produced in the domes (or follicles) can enter the blood circulation or move to other areas of the Peyer's patches such as follicle associated epithelium on the luminal surfaces. From the data it would appear that the type of fat added to CMR for three weeks after birth can influence the immune system of young calves but it is not possible from the data to determine a direction, beneficial or deleterious, of the change.

Table 1.5 Numbers of proliferating cells (BrdU labelled), B-lymphocytes and T-lymphocytes in the JPP and IPP of three week old calves (Part 1) fed milk replacer (Control)or milk replacer with the addition of either 5% palm oil (Saturated fat) or 5% sunflower seed oil (Unsaturated fat). Means ± SD, n=7

		no. of cells/ mm	
	Control	**Saturated**	**Unsaturated**
	Jejunal Peyer's patches		
BrdU labelled cells			
Follicle	33.2 ± 7.1	33.0 ± 5.9	31.3 ± 7.0
Interfollicular area	15.5 ± 2.4	14.1.± 1.2	13.7 ± 1.9
Dome	11.4 ± 1.1[a]	15.7 ± 4.4[b]	12.1 ± 1.2[ab]
B-lymhocytes			
Follicle	30.9 ± 8.2	34.5 ± 7.8	26.0 ± 4.2
Interfollicular area	9.7 ± 4.2	10.3 ± 2.2	8.9 ± 3.1
Dome	22.2 ± 6.1	21.8 ± 6.3	29.6 ± 9.7
Follicle associated epithelium	33.9 ± 3.1	42.8 ± 11.9	33.8 ± 9.6
T-lymphocytes			
Follicle	14.7 ± 4.2	14.8 ± 5.0	13.3 ± 5.6
Interfollicular area	73.2 ± 16.2	68.1 ± 17.2	65.4 ± 13.5
Interepithelial lymphocytes	42.5 ± 6.13	43.8 ± 14.5	41.9 ± 8.5
	Ileal Peyer's patches		
BrdU labelled cells			
Follicle	19.9 ± 2.5	19.7 ± 4.7	20.1 ± 4.8
Interfollicular area	17.1 ± 3.4	14.9 ± 4.9	14.1 ± 2.4
Dome	12.4 ± 3.0	12.7 ± 3.5	13.3 ± 3.6
B-lymhocytes			
Follicle	17.5 ± 6.4	21.8 ± 4.7	21.5 ± 5.7
Interfollicular area	15.2 ± 4.5	15.0 ± 5.2	16.8 ± 4.8
Dome	20.2 ± 5.0	18.9 ± 4.9	17.9 ± 4.4
Follicle associated epithelium	21.6 ± 5.6	29.4 ± 5.9	27.8 ± 6.9
T-lymphocytes			
Follicle	3.8 ± 1.1	4.8 ± 2.0	4.1 ± 1.4
Interfollicular area	49.8 ± 4.8	50.7 ± 10.0	39.8 ± 8.3
Interepithelial lymphocytes	52.7 ± 11.1	41.2 ± 9.4	41.2 ± 12.8

Superscript letters a, b in the same row indicate significant differences, P<0.05

In the JLN there was a significant decrease in B-lymphocyte numbers in the follicles in Saturated and Unsaturated fat supplemented calves compared with the Controls (Table 1.6). Again it is difficult to attribute a possible functional effect of the difference. The mesenteric lymph nodes are usually considered part of the mucosal immune system however the lymphocytes that are produced and maturing there have greater roles in systemic immunity than do cells produced in the Peyer's patches. Although a decrease in B-lymphocytes may indicate a decrease in ability of the calves to rapidly respond to infection by mounting an antibody response, a decrease in B-lymphocytes could also indicate that the calves had a greater overall level of immunity or disease resistance and therefore there are fewer B-lymphocytes present. There seemed to be a trend for cells in the follicles of JLN from calves supplemented with Saturated fat to have a higher rate of cell division than those from calves in the other two groups and it is in the follicles that more B-lymphocytes than

T-lymphocytes are found and divide. A significantly lower number of B-lymphocytes in the follicles of the calves fed Saturated fat compared with Controls, coupled with a trend for these calves to have had a higher rate of proliferation of follicular cells may indicate an increased need for B-lymphocytes because of a prior or ongoing infection but it is not really valid to draw such conclusions from the type of data available. No other significant differences in B- and T-lymphocyte numbers among treatments were observed in JLN or ILN, nor in the rates of proliferation of cells in the lymph nodes.

Part 2 – two week old calves

The area of the jejunum follicles was smaller than that of IPP and interfollicular areas were larger. There were no significant differences between treatments for follicular (where B cells are found) and interfollicular areas (where T cells are found) in jejunal tissues however, follicular height was significantly greater in Unsaturated calves than Saturated calves. Villi heights and circumferences were slightly smaller in the jejunum than ileum but jejunal villus area was slightly larger than that of the ileum. There were no significant differences between treatments.

Problems were encountered with histochemical staining of JPP and IPP and only small numbers (Table 1.6) of samples could be examined and conclusions regarding the effects of different fats on cell proliferation could not be made.

Table 1.6 Numbers of proliferating cells (BrdU labelled), B-lymphocytes and T-lymphocytes in the JLN and ILN of three week old calves (Part 1) fed milk replacer (Control)or milk replacer supplemented with the addition of either 5% palm oil (Saturated fat) or 5% sunflower seed oil (Unsaturated fat). Means ± SD, n=7

		no. cells/ mm	
	Control	**Unsaturated**	**Saturated**
		Jejunal lymph nodes	
BrdU labelled cells			
Follicles	33.1 ± 5.5	32.2 ± 7.4	36.9 ± 10.5
Paracortex	8.4 ± 1.4	10.1 ± 2.5	9.1 ± 1.5
B-lymphocytes			
Follicles	48.5 ± 9.1[a]	35.4 ± 6.0[b]	37.7 ± 3.9[b]
Paracortex	10.3 ± 2.4	10.5 ± 2.2	11.5 ± 2.5
T-lymphocytes			
Follicles	15.8 ± 2.7	20.6 ± 6.4	18.5 ± 5.3
Paracortex	45.7 ± 25.3	41.2 ± 16.2	51.5 ± 18.5
		Ileal lymph nodes	
BrdU labelled cells			
Follicles	33.4 ± 6.7	31.4 ± 5.8	34.8 ± 5.7
Paracortex	9.3 ± 1.3	8.5 ± 1.4	8.3 ± 0.9
B-lymphocytes			
Follicles	34.0 ± 71	38.7 ± 6.5	34.4 ± 5.0
Paracortex	12.5 ± 2.2	12.9 ± 2.3	11.2 ± 2.3
T-lymphocytes			
Follicles	22.9 ± 8.8	17.1 ± 6.5	15.1 ± 4.1
Paracortex	42.7 ± 10.8	38.9 ± 16.7	32.0 ± 9.9

Superscript letters a, b in the same row indicate significant differences, $P<0.05$

Table 1.7 Measurements on villi and follicles of Peyer's patches in jejunum and ileum of two week old calves fed milk replacer (Control) or milk replacer with the addition of either 5% palm oil (Saturated fat) or 5% sunflower seed oil (Unsaturated fat). Means ± SE, n=8 except Sat n=7

	Control	Saturated	Unsaturated
		Jejunal Peyer's patches	
Follicular Area (x10^3 µm^2)	98.2 ± 9.7	76.8 ± 12.4	101.5 ± 9.6
Follicular Height (µm)	296.7 ± 18.8^{ab}	240.9 ± 21.2^{a}	315.8 ± 17.3^{b}
Interfollicular Area (x10^3 µm^2)	67.2 ± 8.0	47.5 ± 8.4	50.9 ± 5.5
Villus Area (x10^3 µm^2)	148.3 ± 3.4	155.6 ± 14.6	136.4 ± 13.4
Villus Height (µm)	731.7 ± 21.5	770.0 ± 52.6	729.7 ± 44.5
Villus Circumference (µm)	2021.4 ± 45.7	2097.1 ± 130.1	1947.5 ± 110.8
		Ileal Peyer's patches	
Follicular Area (x10^3 µm^2)	254.1 ± 24.5	214.4 ± 24.7	254.3 ± 38.7
Follicular Height (µm)	651.7 ± 30.8	588.5 ± 30.3	690.3 ± 75.5
Interfollicular Area (x10^3 µm^2)	28.4 ± 1.8	36.4 ± 5.0	35.7 ± 2.5
Villus Area (x10^3 µm^2)	125.5 ± 12.8	135.1 ± 10.1	130.3 ± 14.6
Villus Height (µm)	595.3 ± 30.5	604.6 ± 18.2	603.0 ± 33.4
Villus Circumference (µm)	1866.6 ± 123.7	1857.0 ± 98.0	1883.1 ± 114.3

Table 1.8 Numbers of proliferating cells (BrdU labelled), B-lymphocytes and T-lymphocytes in the jejunal Peyer's patches of two week old calves (Part 2) fed milk replacer (Control)or milk replacer with tha addition of either 5% palm oil (Saturated fat) or 5% sunflower seed oil (Unsaturated fat). Means ± SD, number in brackets = n.

		no. of cells/ mm	
	Control	**Saturated**	**Unsaturated**
BrdU labelled cells			
Follicle	121.5 ± 6.5 (7)	119.1 ± 8.7 (8)	115.1 ± 8.0 (8)
Interfollicular area	24.2 ± 2.6 (7)	23.5 ± 4.0 (8)	24.4 ± 2.5 (8)
Dome	25.0 ± 3.0 (7)	20.8 ± 1.1 (7)	20.9 ± 2.7 (7)
B-lymhocytes			
Follicle	123.3 ± 6.5 (3)	91.4 ± 8.8 (4)	116.0 ± 12.3 (5)
Interfollicular area	18.0 ± 6.2 (3)	15.4 ± 2.4 (4)	26.2 ± 4.7 (5)
Dome	59.3 ± 19.5 (3)	33.1 ± 6.6 (4)	63.0 ± 13.9 (5)
Follicle associated epithelium	24.9 ± 8.9 (3)	24.7 ± 12.6 (4)	92.2 ± 4.7 (5)
T-lymphocytes			
Follicle	1.4 ± 0.3 (6)	8.1 ± 7.0 (5)	2.4 ± 0.8 (4)
Interfollicular area	77.3 ± 12.3 (6)	124.1 ± 13.4 (5)	131.6 ± 18.5 (4)
Dome	20.3 ± 1.7 (6)	28.0 ± 5.2 (5)	30.2 ± 3.8 (4)
Interepithelial lymphocytes	105.2 ± 11.9 (6)	125.5 ± 20.8 (5)	122.4 ± 9.7 (4)

Toll-like receptor measurements three and two week old calves

The mRNA expression in the tissues from calves given saturated or unsaturated fats was calculated by the $2^{-(\Delta\Delta Ct)}$ method (Livak and Schmittgen 2001) as fold difference from that in the Control calves.

At 3 weeks of age the 2 to 2.5 fold increase in TLR2 mRNA expression in JPP and IPP of calves fed Unsaturated fats was not significantly different from that of Controls or that of calves fed Saturated fats. At 2 weeks of age the almost two-fold increase in mRNA expression of TLR2 in IPP and JLN of calves given Saturated palm oil (Figure 2) were not significantly different from Controls or that of calves fed Unsaturated, sunflower seed oil. The only significant differences in relative expression of TLR mRNA was for that of TLR2 at 2 weeks in the areas of jejunum without Peyer's patches where expression was reduced in calves given Saturated and Unsaturated fats compared with the Control calves.

Expressions of downstream signalling genes relative to control

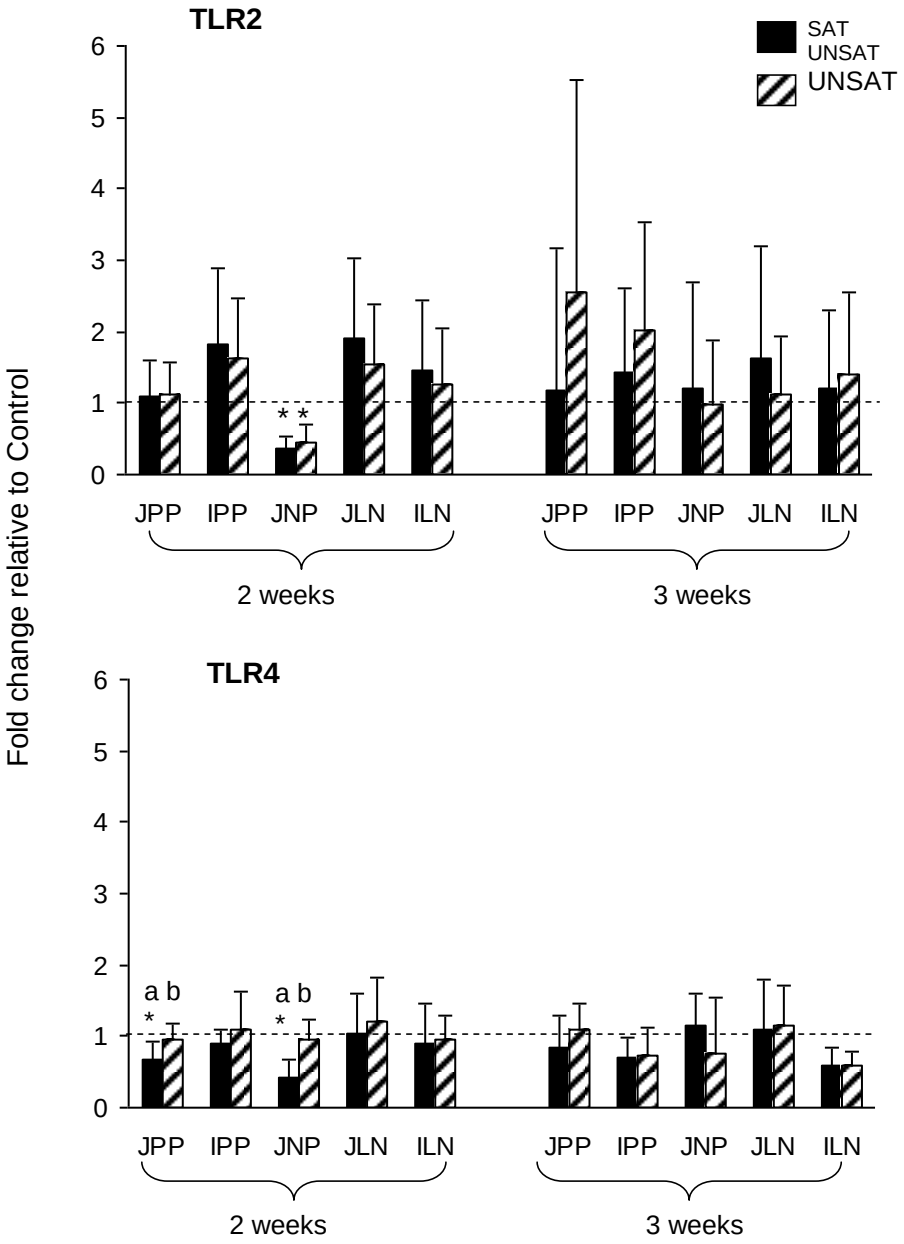
Quantitative real time PCR (qPCR) of mRNA expression was measured for three genes that are stimulated by TLR2 or TLR4 when these are stimulated by bacterial activity and expressed relative to the level in Control calves. These were measured as it was thought that, though significant differences in expression of TLR2 and TLR4 among the treatments were not observed, there may be significant effects of the treatments on the genes stimulated by those receptors.

At two weeks (Part 2) the mRNA expression of MyD88 (Myeloid differentiation factor 88) in JLN relative to Controls (calculated as $2^{-\Delta\Delta Ct}$) was significantly ($P < 0.05$) lower (2.64 ± 1.38) in calves on the Saturated fat treatment compared with those on the Unsaturated treatment (4.46 ± 1.94). The approximately four fold difference between those on the Unsaturated fat treatment relative to Controls was also significantly

different (Figure 1.1). Similar significant differences ($P < 0.05$) were seen in the ILN mRNA expression of MyD88 at two weeks of age between the Saturated (2.24 ± 0.86) and Unsaturated fat calves (4.46 ± 1.51) and between Unsaturated treatment fat calves relative to Control calves.

At three weeks of age (Part 1) the only significant differences for MyD88 expression was seen in areas of the jejunum without Peyer's patches (Figure 3) between Saturated fat and Control calves in IPP (1.56 ± 0.76) and Unsaturated fat treatment relative to Controls (2.13 ± 0.37) calves.

Figure 1.1. mRNA expression of Toll-like receptor 2 (TLR2) and TLR4 in jejunal Peyer's patches (JPP), ileal Peyer's patches (IPP), jejunal wall without Peyer's patches (JNP), jejunal lymph node (JLN), and ileal lymph node (ILN) of calves at 2 and 3 weeks of age given calf milk replacer with addition of either 5% added palm oil (Saturated) or Sunflower seed oil (Unsaturated).

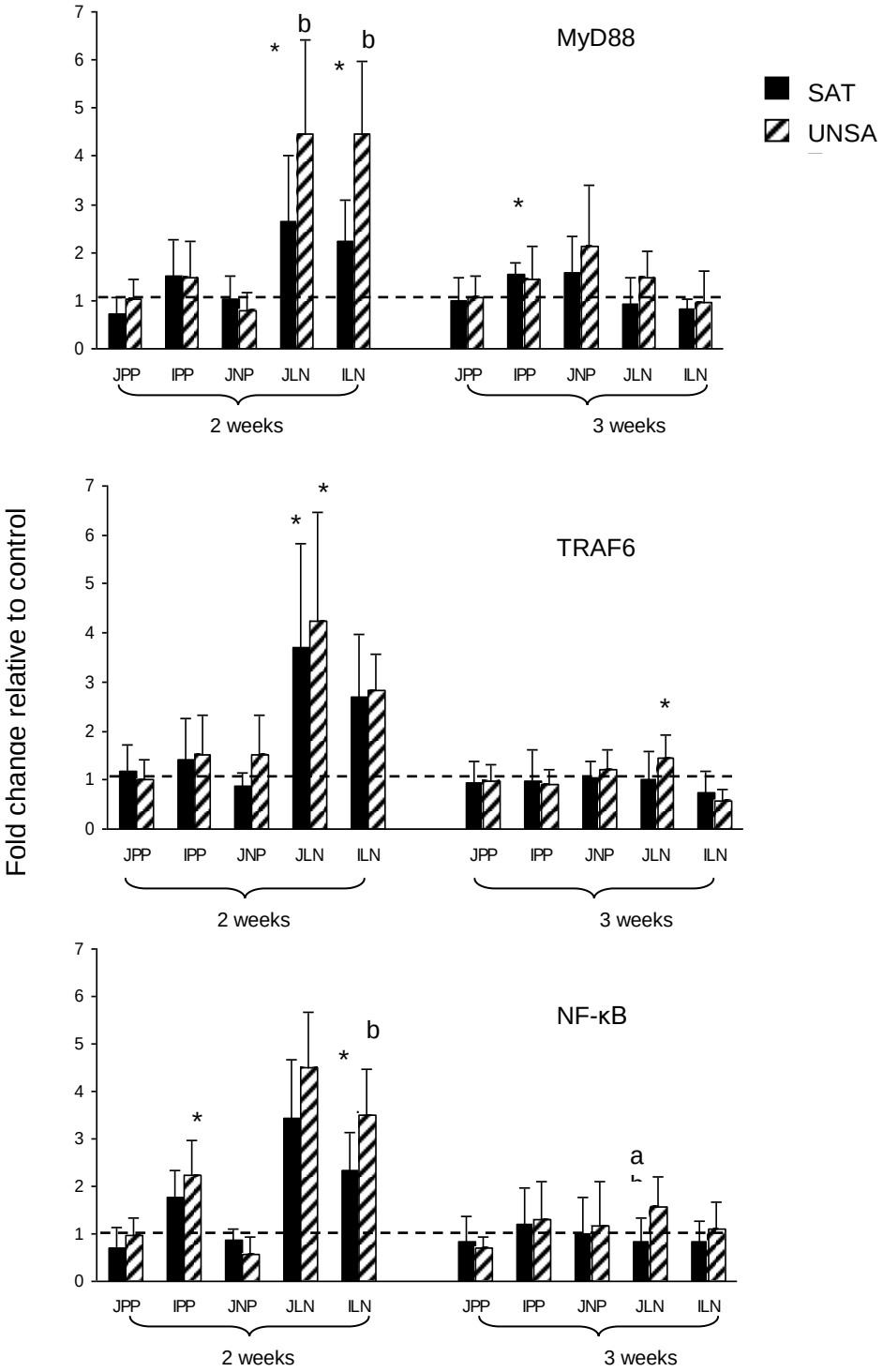


Fold differences from Control calves given calf milk replacer without added oil (Control) calculated from $2^{-(\Delta\Delta Ct)}$ values. Dotted line indicates level of mRNA in Control calves. Means ± SD, N = 8; a, b different letters for the same tissue and gene indicate a significant difference between Saturated and Unsaturated.
* significantly different from Control, $P < 0.05$.

For TRAF6 (Tumour necrosis factor - receptor associated factor 6), there was a significantly higher fold difference ($2^{-\Delta\Delta Ct}$) in calves on Saturated (3.69 ± 2.21) and Unsaturated fats (1.44 ± 2.21) relative to Control in JLN at two weeks of age and at three weeks in the same tissue between Unsaturated fat treatment calves relative to Controls (Figure 1). Between Saturated and Unsaturated fat treatments there were no significant differences in mRNA expression of TRAF6 in any of the tissues.

At two weeks of age, mRNA expression of NF-κB relative to Controls ($2^{-\Delta\Delta Ct}$) was significantly ($P < 0.05$) higher in IPP of Saturated (1.76 ± 1.21) and Unsaturated fat (2.24 ± 1.3) calves. For both the JLN and ILN NF-κB expression was significantly greater in Saturated fat calves (3.45 ± 0.83 and 2.35 ± 0.85 and Unsaturated fat calves (2.35 ± 0.85 and 3.49 ± 1.09) relative to Controls (Figure 1.2). In areas of jejunum without Peyer's patches, mRNA expression of NF-κB in Saturated fat and Unsaturated fat calves was lower relative to Controls but only in the Unsaturated fat calves was the difference significant (0.57 ± 1.17). Between the Saturated and Unsaturated fat treatments significant differences were only seen in the ILN at two weeks of age and in JLN at three weeks of age. Expression in Saturated fat calves was lower in ILN (2.35 ± 0.77) and JLN (0.85 ± 0.43) than in Unsaturated fat calves (4.49 ± 0.38 and 1.09 ± 0.98) (Figure 1.2).

Figure 1.2. mRNA expression of MyD88, TRAF6 and NF-κB in tissues of 2 and 3 week old calves given milk replacer with added saturated fat (SAT) or unsaturated fat (UNSAT).



Fold differences relative to control calves given milk replacer without additives (Control) were calculated from $2^{(-\Delta\Delta Ct)}$ values. Dotted lines indicate level of mRNA in Control calves. Means ± SD, N = 8, JPP – jejunal Peyer's patches; IPP – ileal Peyer's patches; JNP – area of jejunum without Peyer's patches; JLN – jejunal lymph node; ILN – ileal lymph node.
* means of mRNA fold expression significantly ($P < 0.05$) different from Control,
a,b: different letters indicate significant ($P < 0.0\ 5$) difference between saturated and unsaturated values in tissue type.

Experiment 2 – Effect of nucleotides on immunity of calves

Methods

Thirty-two Friesian calves were obtained from the DPI Ellinbank research herd. Calves were kept in pens, three calves per pen in an 'Ecoshelter' (Redpath, Bendigo East, Victoria) containing six pens (2.5 x 3 m). Bedding was a mixture of sawdust and wood shavings. Calves were fed calf milk replacer (CMR) at 10% of their body weight divided into two feeds per day. Water, hay and calf meal were available at all times. The calves were divided into two groups of 16 calves each, a Control group was fed CMR and the Nucleotide group was fed CMR to which was added nucleotides in the form of the proprietary product, *Ascogen* (Chemoforma Ltd, Switzerland), 1 g per feed from five days of age.

Calves were weighed at 5, 10, 13/14 and 19/20 days of age, blood samples were taken for leukocyte counts and serum IgG measurements on the same days as calves were weighed. The temperature and health status of calves was recorded each day so that the incidence of disease could be assessed.

At two weeks and three weeks of age, eight calves per group were killed one hour after intravenous administration of 5-bromo-2'-deoxyuridine (BrdU) (for measurement of cell proliferation). The BrdU was dissolved in 0.9% saline solution and given at a dose rate of 10 mg/kg body weight. Calves were killed with an overdose of barbiturate and samples of the intestinal tract collected. Collection of tissues was usually completed within 20 minutes of the death of the calf.

Samples were collected from the JPP and IPP and from JLN and ILN for histological examination and for measurement of TLR2 and TLR 4 mRNA expression. Tissues were also collected from a region of the jejunum where Peyer's patches were not visible, for mRNA expression of TLR2 and TLR4.

Results

Body weights

Calves fed nucleotides had higher relative weight gains to two weeks of age but from two to three weeks their weight gains were lower than that of Controls (Table 2.1). There were no significant differences in initial or final body weights, body weight gains or relative weight gains (Table 2.2) between the treatments.

Table 2.1 Live weights (kg) and weight gains (g/d) of calves (kg) reared on calf milk replacer with (Nucleotide) or without (Control) added nucleotides. Mean ± SD.

Weight	N	Control	Nucleotide
Live - Initial			
Calves killed at 2 weeks	8	44.3 ± 4.3	44.9 ± 3.5
Calves killed at 3 weeks	8	43.7 ± 2.7	44.8 ± 3.4
All calves	16	44.0 ± 3.5	44.8 ± 3.3
Live – at 2 weeks			
Calves killed at 2 weeks	8	45.2 ± 4.6	46.5 ± 4.0
Calves killed at 3 weeks	8	45.4 ± 3.0[1]	47.3 ± 3.2
All calves	16	45.3 ± 3.8	46.9 ± 3.5
Live – at 3 weeks	8	49.5 ± 2.9	50.2 ± 3.5
Gain from 5d to 2 weeks			
Calves killed at 2 weeks	8	104.6 ± 177.3	206.5 ± 202.9
Calves killed at 3 weeks	8	222.0 ± 113.0[1]	279.2 ± 135.5
All calves	16	159.5 ± 157.7[2]	242.8 ± 170.8
Gain from 5d to 3 weeks	8	383.8 ± 94.2	366.8 ± 96.0

1, n=7; 2, n=15

Table 2.2 Relative weight gain (as a % of initial weight) of calves reared on calf milk replacer with (Nucleotide) or without (Control) added nucleotides. Mean ± SD.

Weight gain Treatment	N	Control	Nucleotide
At 2 weeks of age (gain/d as % of initial weight)			
Calves killed at 2 weeks	8	0.24 ± 0.4	0.46 ± 0.47
Calves killed at 3 weeks	8	0.58 ± 0.31[1]	0.6 ± 0.32
All calves	16	0.37 ± 0.36[2]	0.55 ± 0.40
At 3 weeks of age (gain/d as % of initial weight)	8	0.88 ± 0.22	0.82 ± 0.24
From 2 to 3 weeks of age (gain/d as % of initial weight)	8	1.37 ± 0.27[1]	1.12 ± 0.55
From 2 to 3 weeks of age (gain/d as % of weight at 2 weeks)	8	1.33 ± 0.29[2]	1.06 ± 0.54

1, n=7; 2, n=15

There were no differences in serum 1gG concentrations Table 2.3 between groups

Table 2.3 Serum IgG concentration (mg/ml) at 5, 10 and 14, 18 and 19-22 days of age in Friesian bull calves hand-reared from 5 days to 3 weeks of age fed CMR only (C3W: N= 8) or CMR plus 2g/day of *Ascogen* (N3W: N = 8), expressed as means ± SEM.

	Serum IgG Concentration (mg/ml)					
Treatment Group (N = 8)	**5 Days**	**10 Days**	**14 Days**	**18 Days**	**19-22 Days**	**Change from 5 to 13 or 14 days**
Control (C2W)	16.37 ± 7.53	9.28 ± 3.01	10.08 ± 4.12			-6.28 ± 3.44
Nucleotides (N2W)	16.41 ± 4.81	12.44 ± 3.90	12.82 ± 3.74			-3.50 ± 1.32
Control (C3W)	24.86 ± 5.45	15.78 ± 2.14	13.97 ± 1.98	13.92 ± 1.69	14.67 ± 2.28	-10.89 ± 4.30
Nucleotides (N3W)	18.35 ± 2.45	14.70 ± 1.62	13.53 ± 1.56	12.62 ± 1.31	13.62 ± 1.57	-4.82 ± 1.57

Serum IgG concentrations and leukocyte counts

At the start of the feeding experiment (5 days of age) the serum IgG concentrations of Control calves reared to two weeks of age were 16.37 ± 7.53 mg/ml (n = 8) and in the Nucleotide treatment group concentrations were similar, 16.41 ± 4.81 mg/ml (n = 8). At two and three weeks of age there were no significant differences between treatments (Table 2.3)

Although the calves were all obtained from the same herd (DPI Ellinbank Research herd) and over the same season and were randomly assigned to treatment times (two or three weeks) as they arrived, the calves kept until three weeks of age started the experiment with higher concentrations than those kept for two weeks. Again no significant differences were seen between treatments at 2 or 3 weeks of age.

Health parameters

The total number of days with diarrhoea per calf was lower in the Nucleotide group (Table 2.4) as was the total number of days on which diarrhoea was observed as a percentage of the total days in the experiment. For each measure, differences were not statistically significant. Nucleotide calves reared for three weeks (Table 2.5) had proportionately fewer days with diarrhoea in their first and second weeks than the Control calves but in the third week the Control calves had proportionately fewer days with diarrhoea than the Nucleotide fed calves.

The higher incidence of diarrhoea in Nucleotide calves during week 3 could account for their lower rate of growth during that period compared to the Controls (Table 2.2).

Rectal temperatures were measured each day and average daily temperature for each calf was calculated. There was no difference between treatments in rectal temperatures of the calves but the temperatures of the Nucleotide treated calves were lower than in the Control calves in the first two weeks but tended to be higher in the third week.

T**able 2.4.** Evidence of diarrhoea in calves reared on calf milk replacer with (Nucleotide) or without (Control) added nucleotides. Mean ± SD.

Diarrhoea observed Treatment	n	Control	Nucleotide
5d to 2 weeks			
Number of days per calf	8	2.9 ± 2.5	1.8 ± 2.4
% of total days	8	30.8 ± 26.5	18.0 ± 25.4
5d to 3 weeks	8		
Number of days per calf	8	3.4 ± 1.9	2.5 ± 2.4
% of total days	8	21.0 ± 11.9	15.5 ± 14.8
All calves, % days	16	25.9 ± 20.5	17.1 ± 20.2

Table 2.5 Evidence of diarrhoea (days diarrhoea recorded as % of total days) in calves reared to three weeks on calf milk replacer with (Nucleotide) or without (Control) added nucleotides. Calculated using total calf days per treatment group.

	% days with diarrhoea		
Treatment	1st week	2nd week	3rd week
Control	54.2	51.8	8.2
Nucleotide	29.2	33.9	20.4

There was no significant difference between treatment for abnormal faecal consistency but the incidence of watery faeces was significantly lower ($p<0.05$) in 2week old calves supplemented with nucleotides (1.4 ± 1.4%) than nucleotide treated calves (12.4 ± 3.9%).

There were no significant differences in total leukocyte numbers in whole blood between the control and nucleotide treated calves or in differential counts except that nucleotide calves at 10 days had significantly higher ($p<0.05$) percent eosinophils.

Table 2.6 Histomorphology

	Control 2 week	Nucleotide 2 week	Control 3 week	Nucleotide 3 week
Jejunum				
Follicular area **($x10^3$ μm^2)**	90.6 ± 14.3[a]	125.5± 14.6[ab]	135.5 ± 15.1[ab]	153.5 ± 15.2[b]
Follicular height **(µm)**	424.7 ± 25.7[a]	499.5 ± 26.6[ab]	520.1 ± 25.5[ab]	545.3 ± 25.9[b]
Interfollicular area **($x10^3$ μm^2)**	85.7 ± 7.5	107.0 ± 17.8	91.7 ± 11.3	90.4 ± 9.5
Villus area **($x10^3$ μm^2)**	110.4 ± 6.6[a]	123.3 ± 5.8[ab]	142.3 ± 2.3[b]	135.5 ± 8.5[ab]
Villus circumference (µm)	1730.9 ± 93.2[a]	1880.3 ± 48.2[a]	2100.6 ± 42.8[b]	2053.3 ± 103.7[ab]
Villus height **($x10^3$ μm^2)**	661.3 ± 36.8[a]	702.0 ± 24.9[ab]	806.7 ± 15.6[b]	760.4 ± 46.4[ab]
Ileum				
Follicular area **($x10^3$ μm^2)**	163.7 ± 33.4	214.5 ± 34.0	260.2 ± 74.2	228.3 ± 36.5
Follicular height **(µm)**	628.8 ± 87.0	712.9 ± 57.8	782.6 ± 161.6	757.9 ± 88.7
Interfollicular area **($x10^3$ μm^2)**	34.2 ± 3.8	34.7 ± 4.2	31.5 ± 3.3	33.2 ± 4.2
Villus area **($x10^3$ μm^2)**	120.7 ± 1.8	105.6 ± 8.0	123.8 ± 5.8	126.2 ± 10.2
Villus circumference (µm)	1840.2 ± 56.0	1707.2 ± 79.1	1888.0 ± 100.4	1869.0 ± 87.8
Villus height **($x10^3$ μm^2)**	697.8 ± 18.7	650.7 ± 28.4	648.4 ± 34.0	654.8 ± 29.9

	Control 2 weeks	Nucleotide 2 weeks	Control 3 weeks	Nucleotide 3 weeks
BrdU labelled cells (cells/mm)	(6)	(8)	(7)	(8)
Domes	24.0 ± 3.4	26. ± 2.4	18.4 ± 3.0	18.7 ± 1.4
Follicles	44.8 ± 11.0[a]	86.1 ± 4.4[b]	82.8 ± 11.9[b]	88.4 ± 4.9[b]
Interfollicular areas	25.5 ± 1.7[a]	23.0 ± 2.0[ab]	17.1 ± 1.5[b]	19.7 ± 1.2[ab]
B-lymphocytes/mm	(6)	(7)	(7)	(5)
Domes	44.8 ± 3.9	47.9 ± 6.5	54.5 ± 13.1	65.3 ± 7.1
Follicles	44.1 ± 11.4[a]	120.6 ± 13.5[b +]	111.2 ± 22.3[b]	117.2 ± 9.2[b]
Interfollicular areas	9.4 ± 1.8	10.5 ± 2.5[+]	10.2 ± 1.7	13.8 ± 2.7
FAE	45.8 ± 14.2	42.2 ± 9.9	63.8 ± 13.8	114.7 ± 19.7
T-lymphocytes/mm	(6)	(8)	(7)	(7)
Domes	22.5 ± 1.9	20.1 ± 2.2	22.3 ± 4.7	22.8 ± 4.9
Follicles	13.3 ± 5.5	5.6 ± 1.0	8.2 ± 2.4	4.7 ± 0.8
Interfollicular areas	91.0 ± 19.5	122.3 ± 7.8	117.7 ± 8.4	11.5 ± 6.9
IELs	72.0 ± 9.9	80.6 ± 12.1	87.3 ± 6.4	85.7 ± 6.5

	Control 2 weeks	Nucleotide 2 weeks	Control 3 weeks	Nucleotide 3 weeks
BrdU labelled cells (cells/mm)	(6)	(8)	(8)	(8)
Domes	20.9 ± 3.9	18.2 ± 1.6	16.8 ± 1.2	13.6 ± 1.4
Follicles	56.5 ± 15.7	91.5 ± 15.7	81.1 ± 1.0	75.1 ± 11.1
Interfollicular areas	22.2 ± 3.4	25.8 ± 3.9	20.4 ± 3.0	31.6 ± 8.7
B-lymphocytes/mm	(6)	(8)	(7)	(7)
Domes	46.1 ± 6.7	61.0 ± 6.4	60.8 ± 7.4	47.4 ± 5.1
Follicles	83.2 ± 26.8	145.1 ± 9.2	111.0 ± 20.9	134. ± 15.3
Interfollicular areas	25.7 ± 4.1^{+}	27.0 ± 3.7	33.4 ± 3.3	49.7 ± 10.2
FAE	31.9 ± 8.3	39.0 ± 10.5	45.4 ± 10.0	40.8 ± 7.3
T-lymphocytes/mm	(6)	(8)	(7)	(7)
Domes	19.9 ± 6.9	16.1 ± 1.6	17.2 ± 1.2	23.1 ± 4.0
Follicles	15.0 ± 6.9	1.5 ± 0.3	5.0 ± 2.9	3.2 ± 1.7
Interfollicular areas	62.6 ± 11.4	83.4 ± 8.4	73.4 ± 10.3	60.6 ± 8.9
IELs	66.4 ± 10.8	60.4 ± 5.9	65.4 ± 4.9	56.7 ± 5.0

Different letters within rows represent significant differences, $p < 0.05$
+ n = 5

Table 2.8 Follicular and villus morphology of JPP of calves fed commercial calf milk replacer (CMR) without additives for 2 weeks; (C2W: N = 6), calves fed CMR with *Ascogen* added at 2g/day for 2 weeks; (N2W: N = 8) and calves fed CMR without additives for 3 weeks; (C3W: N = 8) and fed with CMR with *Ascogen* added at 2g/day for 3 weeks (N3W: N = 8), expressed as means ± SEM

Treatment Group	Follicular Area ($x10^3$ µm^2)	Follicular Height (µm)	Interfollicular Area ($x10^3$ µm^2)	Villus Area ($x\ 10^3$ µm^2)	Villus Height (µm)	Villus Circumference (µm)
C2W	100.0 ± 11.9	456.8 ± 15.3	63.8 ± 5.7	116.4 ± 3.1^{ac}	685.6 ± 14.3	1816.9 ± 32.2^{ac}
N2W	119.1 ± 17.6	484.2 ± 34.4	84.5 ± 5.7	122.9 ± 5.9^{c}	706.5 ± 23.9	1909.6 ± 39.8^{c}
C3W	137.2 ± 14.0	526.0 ± 21.0	88.8 ± 12.2	142.5 ± 2.4^{bc}	807.2 ± 15.7	2100.9 ± 42.9^{bc}
N3W	152.7 ± 15.4	545.5 ± 25.7	90.2 ± 9.6	135.8 ± 8.4^{c}	759.9 ± 46.7	2053.2 ± 103.7^{c}

Different letters indicate significant differences, $p < 0.05$

Table 2.9 Follicular and villus morphology of IPP of calves fed commercial calf milk replacer (CMR) without additives for 2 weeks; (C2W: N = 6), calves fed CMR with *Ascogen* added at 2g/day for 2 weeks; (N2W: N = 8) and calves fed CMR without additives for 3 weeks; (C3W: N = 7) and fed with CMR with *Ascogen* added at 2g/day for 3 weeks (N3W: N = 8), expressed as means ± SEM

Treatment Group	Follicular Area ($x10^3$ µm^2)	Follicular Height (µm)	Interfollicular Area ($x10^3$ µm^2)	Villus Area ($x\ 10^3$ µm^2)	Villus Height (µm)	Villus Circumference (µm)
C2W	144.0 ± 33.6	613.2 ± 88.6	30.6 ± 1.9	118.3 ± 2.7	667.1 ± 16.2	1810.1 ± 28.0
N2W	212.0 ± 34.8	706.4 ± 60.6	32.1 ± 4.0	108.6 ± 6.1	663.5 ± 24.7	1750.8 ± 60.3
C3W	228.1 ± 77.3	704.7 ± 163.6	30.7 ± 3.7	125.7 ± 6.3	654.5 ± 38.6	1901.6 ± 114.9
N3W	234.0 ± 37.4	764.9 ± 89.9	30.7 ± 3.3	130.0 ± 9.2	661.9 ± 25.5	1896.0 ± 76.8

Health Data

Figure 2.1. Mean daily rectal temperatures (˚C) of Friesian bull calves hand-reared from 5 to 14 days of age with commercial calf milk replacer (CMR) only (C2W: N = 8) or CMR with *Asocogen* added at 2/g day (N2W: N = 8) and calves reared from 5 to 21 days of age with commercial calf milk replacer (CMR) only (C3W: N = 8) or CMR with *Asocogen* added at 2/g day (N3W: N = 8). Error bars represent standard error

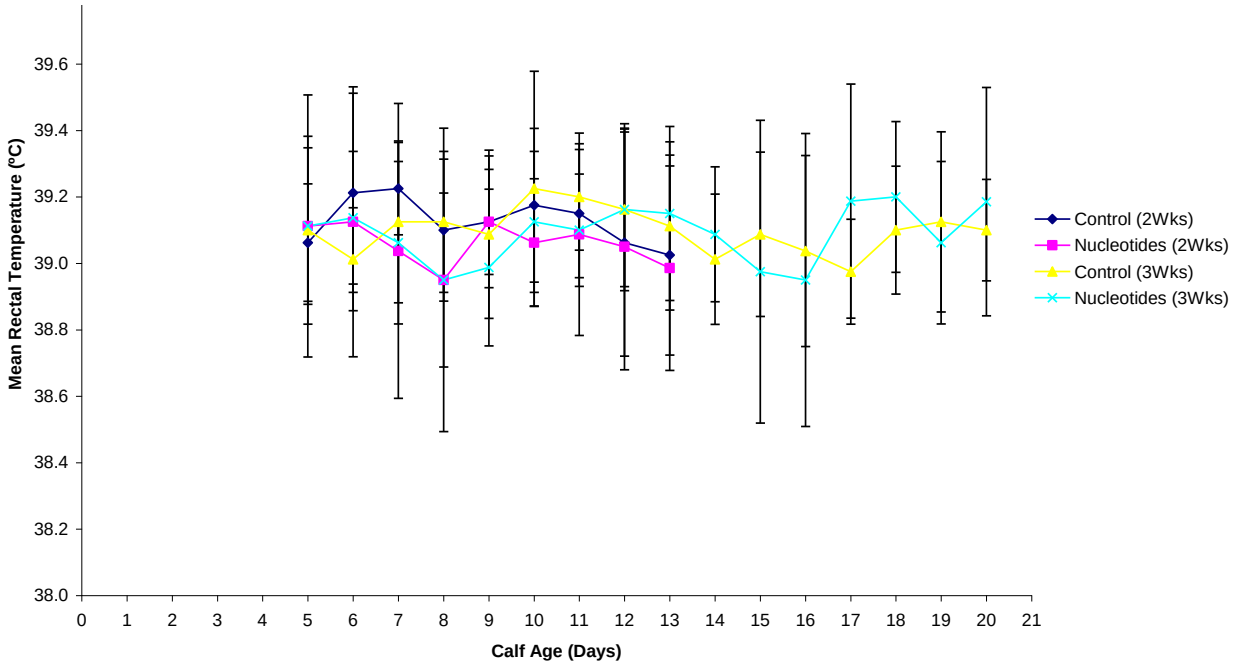


Figure 2.2. Mean rectal temperatures (°C) of Nucleotide and Control calves recorded daily after the morning feed, Mean ± SD.

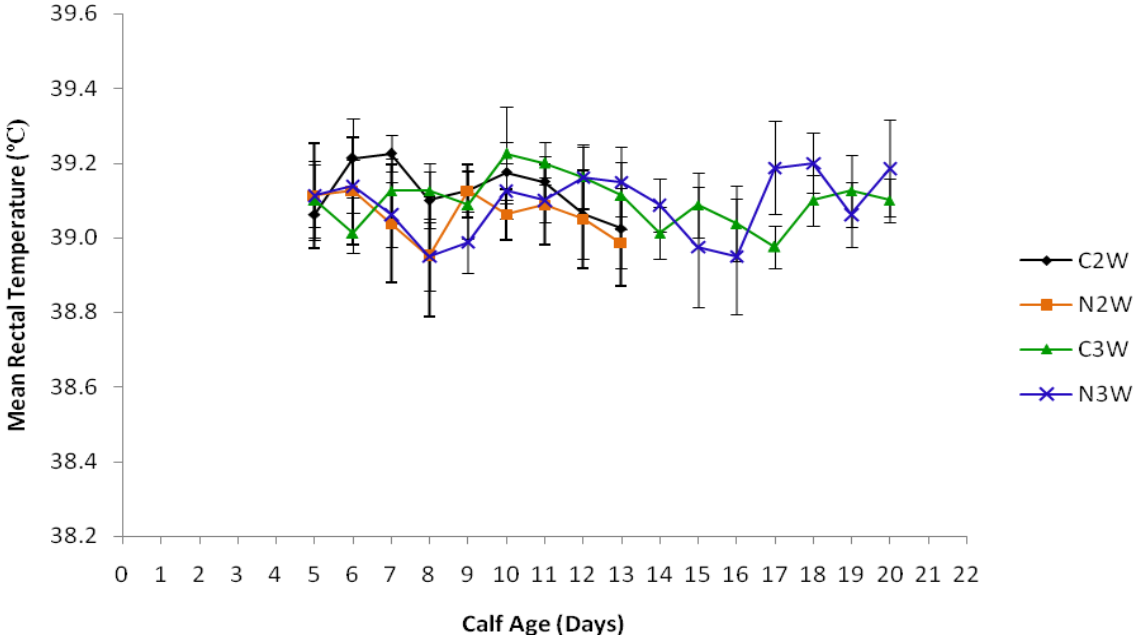


Figure 2.3 The incidence of loose, thin or watery and overall abnormal faeces (as a mean percentage of days on treatment ± SEM) in Friesian bull calves from 5 to 14 days of age with commercial calf milk replacer (CMR) only (C2W: N = 8) or CMR with *Asocogen* added at 2/g day (N2W: N = 8) and calves reared from 5 to 21 days of age with commercial calf milk replacer (CMR) only (C3W: N = 8) or CMR with *Asocogen* added at 2/g day (N3W: N = 8)

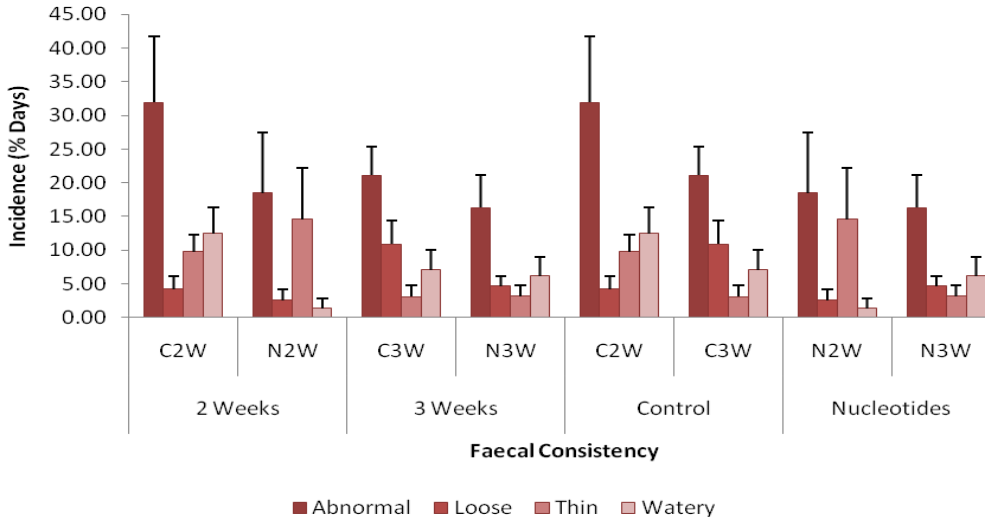


Table 2.10 Feeding behaviour (as a mean percentage of days on treatment ± SEM) in Friesian bull calves from 5 to 14 days of age with commercial calf milk replacer (CMR) only (C2W: N = 8) or CMR with *Asocogen* added at 2/g day (N2W: N = 8) and calves reared from 5 to 21 days of age with commercial calf milk replacer (CMR) only (C3W: N = 8) or CMR with *Asocogen* added at 2/g day (N3W: N = 8)

	Treatment Group			
Feeding Behaviour	**C2W**	**N2W**	**C3W**	**N3W**
Keen	93.06 ± 3.60	95.49 ± 3.23	97.66 ± 1.64	100.00 ± 0.00
Needs Encouragement	2.78 ± 1.82	2.95 ± 1.94	1.56 ± 1.56	0.00 ± 0.00
Refuses to Feed	2.78 ± 1.82	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Since submitting the previous report we have re-examined the data on diarrhoea in the calves and at two weeks it appears that the severity was reduced in the nucleotide treated calves compared with Controls. There were fewer days with very watery diarrhoea in calves given nucleotides from 5 to 14 days of age but in the third week of life there was no significant difference between treatment groups (Fig 2.1).

Figure 2.4. Faecal consistency of calves on the control and nucleotide treatments (n=8).
a,b indicate a significant difference between control and nucleotide treatment at 2 weeks (P<0.05).

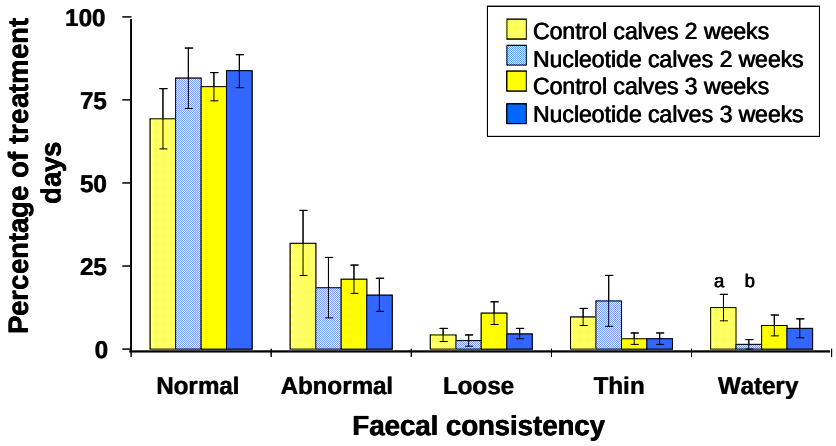


Table 2.11 Total numbers of white blood cells at 5, 10 and 13-14 days of age in Friesian bull calves hand-reared from 5 days to 2 weeks of age fed CMR only (C2W: N= 8) or CMR plus 2g/day of *Ascogen* (N2W: N = 8), expressed as means ± SEM

	Age (Days)		
	5	**10**	**13-14**
C2W	$78.7 \times 10^5 \pm 8.7 \times 10^5$	$100.3 \times 10^5 \pm 12.5 \times 10^5$	$75.7 \times 10^5 \pm 12.6 \times 10^5$
N2W	$59.1 \times 10^5 \pm 6.9 \times 10^5$	$86.0 \times 10^5 \pm 15.3 \times 10^5$	$84.8 \times 10^5 \pm 14.2 \times 10^5$

Table 2.12 Differential white blood cell ratios at 5, 10 and 13-14 days of age in Friesian bull calves hand-reared from 5 days to 2 weeks of age fed CMR only (C2W: N= 8) or CMR plus 2g/day of *Ascogen* (N2W: N = 8), expressed as means ± SEM

		Lymphocytes	Monocytes	Neutrophils	Basophils	Eosinophils	N:L Ratio
5 Days	C2W	71.38 ± 6.02	4.63 ± 0.87	23.63 ± 5.50	0.00 ± 0.00	0.38 ± 0.26	0.41 ± 0.13
	N2W	72.50 ± 5.25	6.88 ± 1.14	14.50 ± 2.53	0.13 ± 0.13	0.38 ± 0.26	0.22 ± 0.05
10 Days	C2W	69.43 ± 3.61	6.00 ± 1.40	24.57 ± 4.11	0.00 ± 0.00	0.00 ± 0.00	0.38 ± 0.08
	N2W	64.75 ± 4.88	5.25 ± 1.41	29.88 ± 5.60	0.00 ± 0.00	0.38 ± 0.18	0.53 ± 0.14
13 – 14 Days	C2W	81.38 ± 4.39	3.75 ± 1.16	14.50 ± 4.20	0.13 ± 0.13	0.25 ± 0.16	0.21 ± 0.07
	N2W	70.00 ± 4.83	5.75 ± 1.13	24.25 ± 5.00	0.00 ± 0.00	0.00 ± 0.00	0.40 ± 0.12

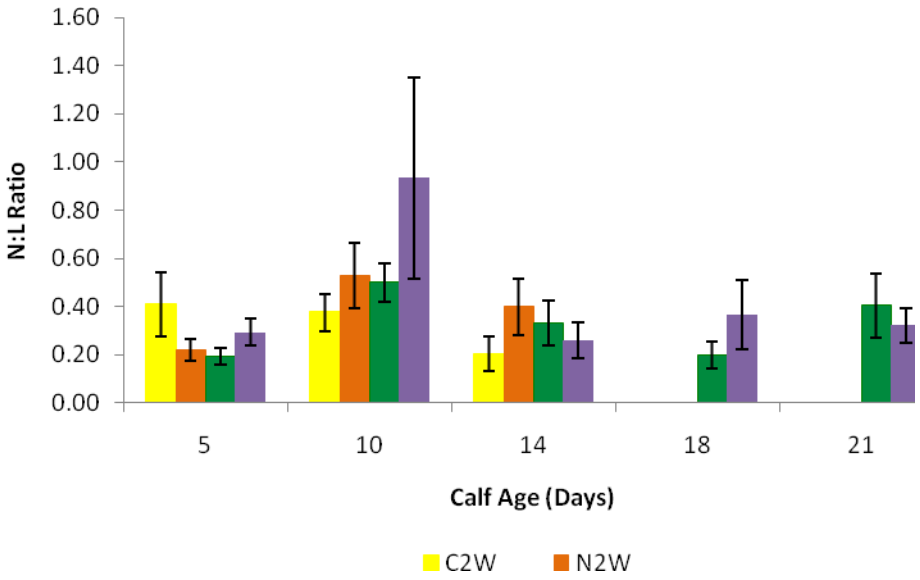
Table 2.13 Total numbers of white blood cells at 5, 10, 14, 18 and 19-22 days of age in Friesian bull calves hand-reared from 5 days to 3 weeks of age fed CMR only (C3W: N= 8) or CMR plus 2g/day of *Ascogen* (N3W: N = 8), expressed as means ± SEM

	Age (Days)				
	5	**10**	**14**	**18**	**19-22**
C3W	$63.4 \times 10^5 \pm 7.3 \times 10^5$	$65.1 \times 10^5 \pm 7.8 \times 10^5$	$73.4 \times 10^5 \pm 9.8 \times 10^5$	$83.8 \times 10^5 \pm 8.9 \times 10^5$	$101.9 \times 10^5 \pm 11.0 \times 10^5$
N3W	$72.3 \times 10^5 \pm 12.6 \times 10^5$	$79.5 \times 10^5 \pm 8.7 \times 10^5$	$94.6 \times 10^5 \pm 11.1 \times 10^5$	$85.8 \times 10^5 \pm 7.1 \times 10^5$	$89.5 \times 10^5 \pm 6.0 \times 10^5$

Table 2.14 Differential white blood cell ratios at 5, 10, 14, 18 and 19-22 days of age in Friesian bull calves hand-reared from 5 days to 3 weeks of age fed CMR only (C3W: N= 8) or CMR plus 2g/day of *Ascogen* (N3W: N = 8), expressed as means ± SEM

		Lymphocytes	**Monocytes**	**Neutrophils**	**Basophils**	**Eosinophils**	**N:L Ratio**
5 Days	C3W	77.63 ± 3.14	7.50 ± 1.34	14.38 ± 2.32	0.13 ± 0.13	0.38 ± 0.26	0.19 ± 0.03
	N3W	70.38 ± 3.28	9.88 ± 1.72	19.50 ± 2.71	0.00 ± 0.00	0.25 ± 0.16	0.29 ± 0.06
10 Days	C3W	63.13 ± 3.30	5.50 ± 1.80	30.38 ± 3.17	0.00 ± 0.00	0.00 ± 0.00	0.50 ± 0.08
	N3W	59.00 ± 7.29	4.75 ± 1.36	36.25 ± 8.05	0.00 ± 0.00	0.00 ± 0.00	0.94 ± 0.42
14 Days	C3W	73.00 ± 4.43	5.38 ± 1.45	21.63 ± 4.90	0.00 ± 0.00	0.00 ± 0.00	0.33 ± 0.09
	N3W	75.13 ± 3.08	6.13 ± 2.39	18.25 ± 4.11	0.00 ± 0.00	0.50 ± 0.27	0.26 ± 0.07
18 Days	C3W	80.13 ± 4.27	5.38 ± 1.76	14.25 ± 3.48	0.13 ± 0.13	0.13 ± 0.13	0.20 ± 0.06
	N3W	73.38 ± 5.82	4.50 ± 1.51	21.25 ± 6.14	0.13 ± 0.13	0.75 ± 0.31	0.37 ± 0.14
20 - 22 Days	C3W	70.25 ± 5.04	4.38 ± 1.36	24.00 ± 5.10	0.13 ± 0.13	0.13 ± 0.13	0.40 ± 0.14
	N3W	71.13 ± 4.24	7.63 ± 1.21	20.88 ± 4.13	0.13 ± 0.13	0.25 ± 0.16	0.32 ± 0.07

Figure 2.5 Ratio of neutrophils to lymphocytes at 5, 10, 14, 18 and 21 days in Friesian bull hand-reared from 5 to 14 days of age with commercial calf milk replacer (CMR) only (C2W: N = 8) or CMR with *Asocogen* added at 2/g day (N2W: N = 8) and calves reared from 5 to 21 days of age with commercial calf milk replacer (CMR) only (C3W: N = 8) or CMR with *Asocogen* added at 2/g day (N3W: N = 8)



Total Leukocyte numbers

There were no significant differences between treatments or times samples were taken in Control or Nucleotide fed calves (Table 2.15).

Table 2.15 Total leukocyte numbers in Control calves and calves fed Nucleotides

Days of age	Total leukocyte numbers (x 10^6 cells/ml whole blood) 5	10	13 - 14	18	20 - 22
Two week Control	7.32 ± 0.93	8.50 ± 1.22	7.29 ± 1.13		
Nucleotide	5.91 ±0.69	8.60 ± 1.53	8.48 ± 1.42		
Three week Control	6.34 ± 0.73	6.51 ± 1.25	7.34 ± 0.98	8.38 ± 0.89	10.19 ± 1.10
Nucleotide	7.23 ± 1.25	7.95 ± 0.87	9.46 ± 1.11	8.58 ± 0.71	8.95 ± 0.60

Morphology

Table 2.16 Follicular and villus morphology of JPP of calves fed commercial calf milk replacer (CMR) without additives for 2 weeks; (C2W: N = 6), calves fed CMR with *Ascogen* added at 2g/day for 2 weeks; (N2W: N = 8) and calves fed CMR without additives for 3 weeks; (C3W: N = 8) and fed with CMR with *Ascogen* added at 2g/day for 3 weeks (N3W: N = 8), expressed as means ± SEM

Treatment Group	Follicular Area ($x10^3$ µm^2)	Follicular Height (µm)	Interfollicular Area ($x10^3$ µm^2)	Villus Area ($x\ 10^3$ µm^2)	Villus Height (µm)	Villus Circumference (µm)
C2W	100.0 ± 11.9	456.8 ± 15.3	63.8 ± 5.7	116.4 ± 3.1^{ac}	685.6 ± 14.3	1816.9 ± 32.2^{ac}
N2W	119.1 ± 17.6	484.2 ± 34.4	84.5 ± 5.7	122.9 ± 5.9^{c}	706.5 ± 23.9	1909.6 ± 39.8^{c}
C3W	137.2 ± 14.0	526.0 ± 21.0	88.8 ± 12.2	142.5 ± 2.4^{bc}	807.2 ± 15.7	2100.9 ± 42.9^{bc}
N3W	152.7 ± 15.4	545.5 ± 25.7	90.2 ± 9.6	135.8 ± 8.4^{c}	759.9 ± 46.7	2053.2 ± 103.7^{c}

Different letters indicate significant differences, p < 0.05

Table 2.17 Follicular and villus morphology of IPP of calves fed commercial calf milk replacer (CMR) without additives for 2 weeks; (C2W: N = 6), calves fed CMR with *Ascogen* added at 2g/day for 2 weeks; (N2W: N = 8) and calves fed CMR without additives for 3 weeks; (C3W: N = 7) and fed with CMR with *Ascogen* added at 2g/day for 3 weeks (N3W: N = 8), expressed as means ± SEM

Treatment Group	Follicular Area ($x10^3$ µm^2)	Follicular Height (µm)	Interfollicular Area ($x10^3$ µm^2)	Villus Area ($x\ 10^3$ µm^2)	Villus Height (µm)	Villus Circumference (µm)
C2W	144.0 ± 33.6	613.2 ± 88.6	30.6 ± 1.9	118.3 ± 2.7	667.1 ± 16.2	1810.1 ± 28.0
N2W	212.0 ± 34.8	706.4 ± 60.6	32.1 ± 4.0	108.6 ± 6.1	663.5 ± 24.7	1750.8 ± 60.3
C3W	228.1 ± 77.3	704.7 ± 163.6	30.7 ± 3.7	125.7 ± 6.3	654.5 ± 38.6	1901.6 ± 114.9
N3W	234.0 ± 37.4	764.9 ± 89.9	30.7 ± 3.3	130.0 ± 9.2	661.9 ± 25.5	1896.0 ± 76.8

Preliminary conclusions

Although there was no significant difference between treatment groups, the body weight data, evidence of diarrhoea and temperatures show there to be a trend for nucleotides to have a beneficial effect on the calves at least in the first two weeks. There is no specific and identifiable cause for the lower rates of gain in the calves given nucleotides than the Control calves between 2 and 3 weeks of age (Table 2.1) but a decrease in incidence of diarrhoea in the Control calves in that period could have induced compensatory growth and improved their rate of gain (Figure 2.3).

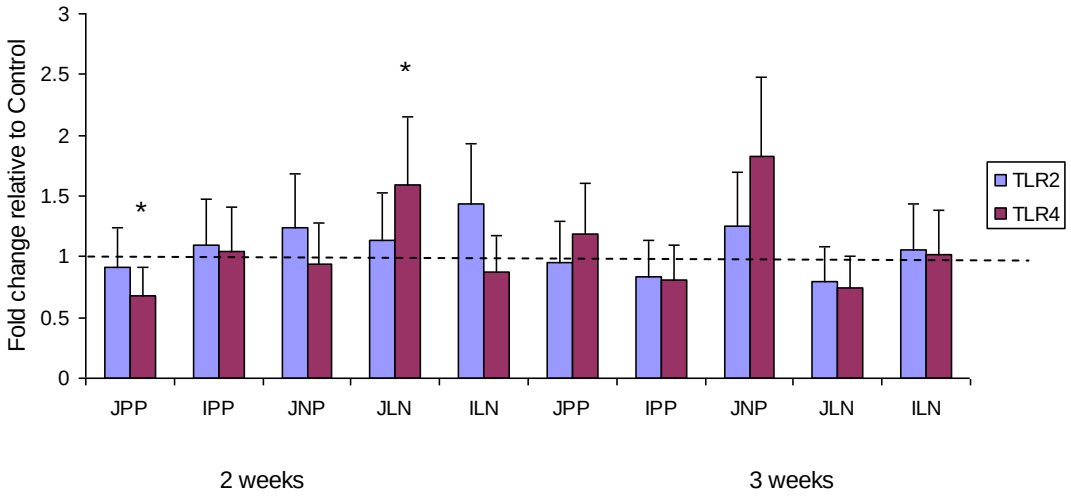
mRNA expression of TLR2 and TLR4 in intestinal and lymph node tissues

In intestinal tissues the only significant difference in mRNA expression between Control and Nucleotide treatments was a lower fold expression for TLR4 ($P < 0.05$) at 2 weeks of age in jejunal Peyer's patches.

There was a significant ($P < 0.05$) fold increase in TLR4 mRNA expression in JLN of two week old Nucleotide treated calves relative to the Control calves (Figure 2.6), while no differences ($P > 0.05$) were observed for mRNA of TLR2 and TLR4 expressions in JLN nor ILN at three weeks.

Expressions of TLR2 and TLR4 did not follow a particular trend as each tissue was affected differently by the addition of Nucleotide to calf milk replacer. Nucleotide supplementation lowered the expression of TLR4 mRNA in JPP at two weeks of age (suggesting a beneficial effect on immune function) but the increase in its expression at three weeks of age suggests that this beneficial effect was no longer present.. At three weeks of age there was a trend for weight gains to be lower and temperatures and incidence of diarrhoea to be higher in the Nucleotide calves than the Controls whereas at two weeks of age the trend was reversed. The trends suggest that, unlike the Control calves at three weeks of age or the two week old Nucleotide calves, the three week Nucleotide calves were more susceptible to infections or that some other factor contributed to a reduction in their well-being between two and three weeks of age.

Figure 2.6 mRNA expression of TLR2 and TLR4 in jejunal Peyer's patches (JPP), ilealPeyer's patches (IPP), jejunal wall without Peyer's patches (JNP), jejunal lymph node (JLN), and ileal lymph node (ILN) of calves given calf milk replacer with Nucleotides.



Fold differences from Control calves given calf milk replacer without added Nucleotides calculated from $2^{-(\Delta\Delta Ct)}$ values.
Dotted line indicates level of mRNA in Control calves. Means ± SD, N = 8;
* means mRNA fold expression is significantly different from Control, $P < 0.05$

Preliminary conclusions

There is still no clear reason or cause of the lower weight gains in the Nucleotide treated calves between two and three weeks of age however it is possible that there was an interaction between feed intake and nucleotide ingestion. We noticed, subjectively, that rumen development of some of the calves was larger than in previous experiments but we made no record of this because it was not a part of the protocol. However, it may be that it is of importance.

Even when solid feed in the form of calf mash or pellets is made available to calves from soon after birth they eat little in the first two weeks of age but after that the volume eaten begins to increase. Rumen growth and development is stimulated by

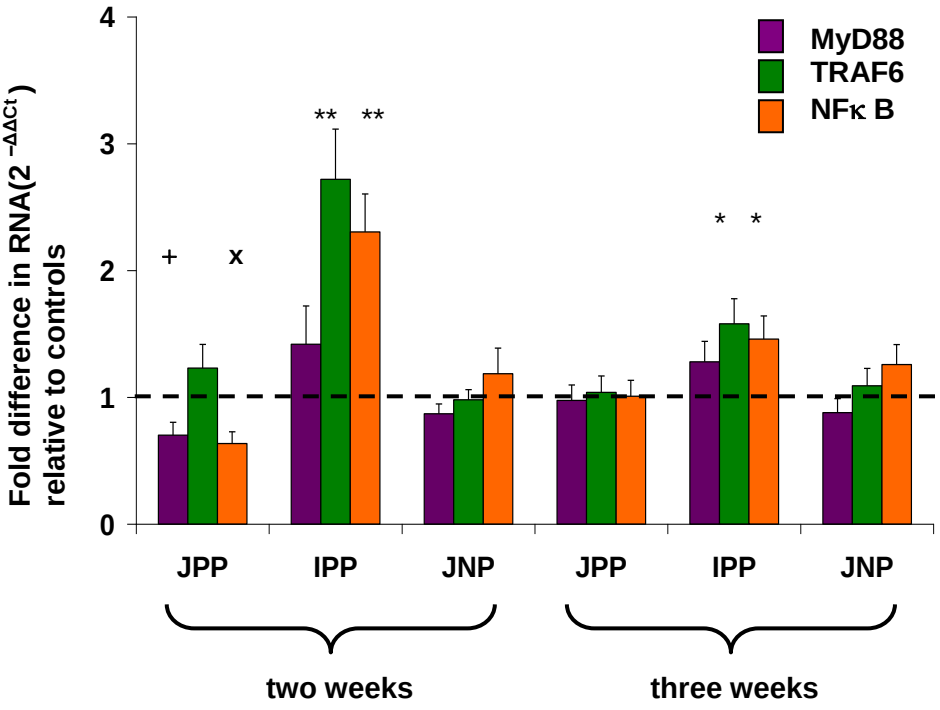
the provision of solid feed and rumen microflora increase and fermentation rate increases. With an increase in rumen development it is possible that nucleotides could be used by rumen microflora before reaching the small intestine where they could be used by the calves' tissues.

A supply of preformed nucleotides would allow the rumen microflora to multiple more rapidly than would normally occur. This in turn could have increased the rate of fermentation in the rumen and result in the production of higher concentrations of products such as ammonia and volatile fatty acids. The liver of young calves has not fully developed the capacity to metabolise such products of fermentation and it is possible that concentrations of these products reached levels in the blood which could have had deleterious effects.

The design of the experiment and data on body weights and health parameters were reported in Milestone report 3. In Milestone 4 the plasma IgG concentrations, leukocyte counts and gene expression of TLR2 and TLR4 in the two and three week old calves were reported. mRNA expression of the downstream signalling genes (Fig 2.7) that can be induced by action of pathogens on TLRs has been completed. In JLN and ILN there were no significant differences in expression of myeloid differentiation factor 88 (MyD88), tumor necrosis factor 6 (TRAF6) and nuclear factor kappa B (NFκB) of nucleotide fed calves compared with controls. However, there were significant differences in mRNA expression in the Peyer's patches of the jejunum and ileum at both two and three weeks of age (Fig 2.6). In JPP the mRNA expression of MyD88 and NFκB was significantly lower than in Controls. This reflects the decrease in mRNA expression in TLR4 in JPP reported in Milestone 4 and perhaps the lower incidence of watery diarrhoea at two weeks of age (Fig 2.3).

In IPP, mRNA expression of TRAF6 and NFκB at two and three weeks of age was significantly higher relative to the Controls. However, there was no increase in mRNA expression of TLR2 or TLR4. This suggests that the increase in gene expression of the two downstream genes could have been induced through other receptors or pathways.

Figure 2.7. mRNA expression of MyD88, TRAF6 and NFκB in JPP, IPP and non-Peyer's patch tissue of jejunum (JNP) in calves at two and three weeks of age given nucleotides.



Data expressed as mean fold difference ($2^{-\Delta\Delta Ct}$) ± SEM relative to control calves (dotted line), Significant fold difference: +, p=0.069; x, p=0.036; **, p=0.012; *, p < 0.05. n = 8,

The results from Experiment 2 suggest that nucleotides could influence immunity of young calves. However, it is still possible that there had been an interaction between dietary nucleotides, increased solid feed intake and the development of the rumen with consequent proliferation of the microorganisms.

In order to determine whether the possible beneficial effects of nucleotides seen at two weeks of age and the possible deleterious effects at three weeks were due to an interaction between nucleotides and calf meal intake Experiment 2 was repeated with modifications.

Experiment 3 – Effects of supplementary nucleotides and solid feed restriction.

Forty-two calves were obtained from the DPI Ellinbank dairy herd and divided into four treatment groups, 10 calves per group.

1. **Group C** (control) was CMR and had *ad lib.* access to calf meal.
2. **Group CR** (restricted control) was fed CMR and was given calf meal at 60% of the intake of Group C.
3. **Group N** was fed similar to Group C except that from 5 days of age 1g nucleotides (Ascogen, Chemaforma, Switzerland) were added to CMR at each feed (total 2g per day).

4. **Group NR** was fed similar to group CR except that 2g nucleotides were added to the CMR as for Group N.

Calves were fed CMR twice a day at 10% of the body weight per day. Hay and water was available at all times and calf meal provided from 5 days. Calves were weighed at 5 days, 13-14 and 20 days of age and blood samples collected on the same days. At three weeks of age the calves were given an intravenous injection of bromo deoxyuridine (to measure cell proliferation in histological samples) and killed one hour later. Samples from the intestinal tract and mesenteric lymph nodes were collected. In addition samples of rumen fluid were collected in order to determine whether the nucleotides had an effect on the rumen function and microorganisms.

The feeding experiment was completed at the beginning of November and only preliminary collation of data on weight gains and calf health has been carried out.

Serum IgG Concentrations

Table 3.1 Serum IgG concentration (mg/ml) at 5, 14 and 18-21 days of age in Friesian bull calves hand-reared with unrestricted access to calf meal fed commercial calf milk replacer (CMR**)** only, (CU: N = 10), or CMR with *Asocogen* added at 2/g day with (NU: N = 10) and calves with restricted access to calf meal fed CMR only (CR: N = 10) or CMR plus 2g/day of *Ascogen* (NR: N = 10). Expressed as means ± SEM

	Serum IgG Concentration (mg/ml)		
Treatment Group (N = 10)	**5 Days**	**14 Days**	**18-21 Days**
CU	10.76 ± 2.20	8.23 ± 1.99	7.84 ± 1.74
NU	7.07 ± 2.62	5.87 ± 2.59	6.05 ± 2.38
CR	10.89 ± 1.81	9.09 ± 1.36	8.26 ± 1.21
NR	10.82 ± 3.26	9.44 ± 3.22	10.08 ± 3.12

Table 3.2 Serum IgG concentration (mg/ml) at 5, 14 and 18-21 days of age in Friesian bull calves hand-reared commercial calf milk replacer (CMR) only, (CO: N = 20), or CMR with *Asocogen* added at 2/g day with (NUCL: N = 20). Expressed as means ± SEM

	Serum IgG Concentration (mg/ml)		
Treatment Group (N = 20)	**5 Days**	**14 Days**	**18-21 Days**
Control	10.82 ± 1.38	8.66 ± 1.18	8.05 ± 1.03
Nucleotides	8.95 ± 2.08	7.65 ± 2.05	8.06 ± 1.97

Preliminary calculation of weight data (Table 3.3) showed that percentage weight gains of calves were slightly higher (but not significantly) in the calves given nucleotides compared to controls between start of the experiment and 2 weeks of age and between 2 weeks and 3 weeks of age. This response is different from the previous experiment with nucleotides (Milestone 3 report) where weight gains of nucleotide fed calves were lower between weeks 2 and 3 than between the start of the experiment and 2 weeks of age.

Table 3.3. Weight gains of calves as a percentage of initial weight or weight at weeks of age. Mean ± SD, n = 10.

	Weight gain to 2 weeks (% initial wt)	Weight gain to 3 weeks (% initial wt)	Weight gain between 2 and 3 weeks (% initial wt)	Weight gain between 2 and 3 weeks (% weight at 2 weeks)
Control	0.727 ± 0.519	0.957 ± 0.333	1.331 ± 0.432	1.262 ± 0.426
Control restricted	0.767 ± 0.371	0.874 ± 0.318	1.018 ± 0.495	0.957 ± 0.466
Nucleotide	0.824 ± 0.746	1.044 ± 0.505	1.372 ± 0.414	1.281 ± 0.375
Nucleotide restricted	0.749 ± 0.550	0.895 ± 0.424	1.108 ± 0.778	1.053 ± 0.680

There was no significant effect of treatment on days with diarrhoea or severity of diarrhoea (Table 3.4)

Figure 3.1 Change in liveweight (kg) from 5 to 20 days of age in Friesian bull calves hand-reared with unrestricted access to calf meal fed commercial calf milk replacer (CMR) only, (CU: N = 10), or CMR with *Asocogen* added at 2/g day with (NU: N = 10) and calves with restricted access to calf meal fed CMR only (CR: N = 10) or CMR plus 2g/day of *Ascogen* (NR: N = 10). Expressed as mean ± SEM

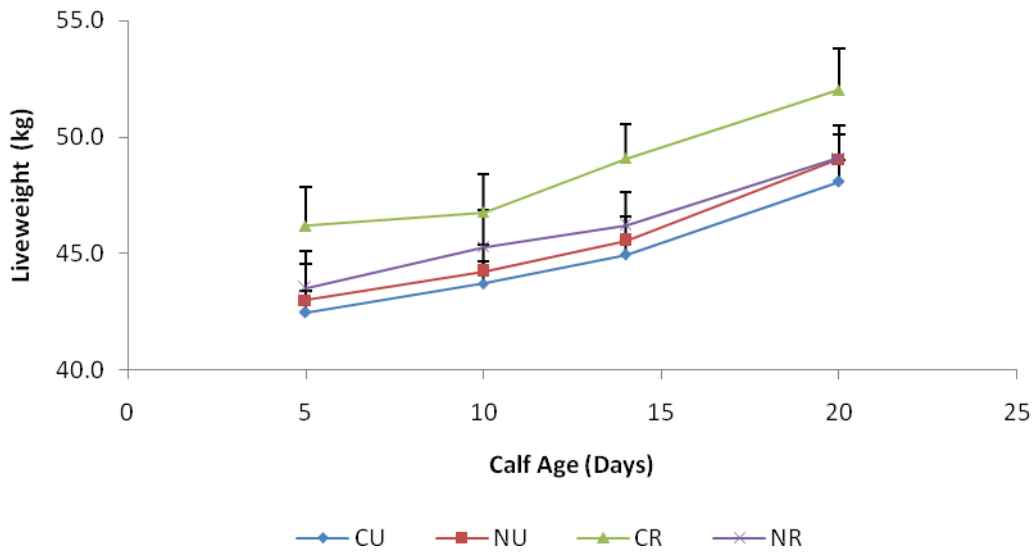


Figure 3.2 Liveweight gain (kg ± SEM) as a percentage of initial weight (5 days of age) throughout the study period in Friesian bull calves hand-reared with unrestricted access to calf meal fed commercial calf milk replacer (CMR) only, (CU: N = 10), or CMR with *Asocogen* added at 2/g day with (NU: N = 10) and calves with restricted access to calf meal fed CMR only (CR: N = 10) or CMR plus 2g/day of *Ascogen* (NR: N = 10)

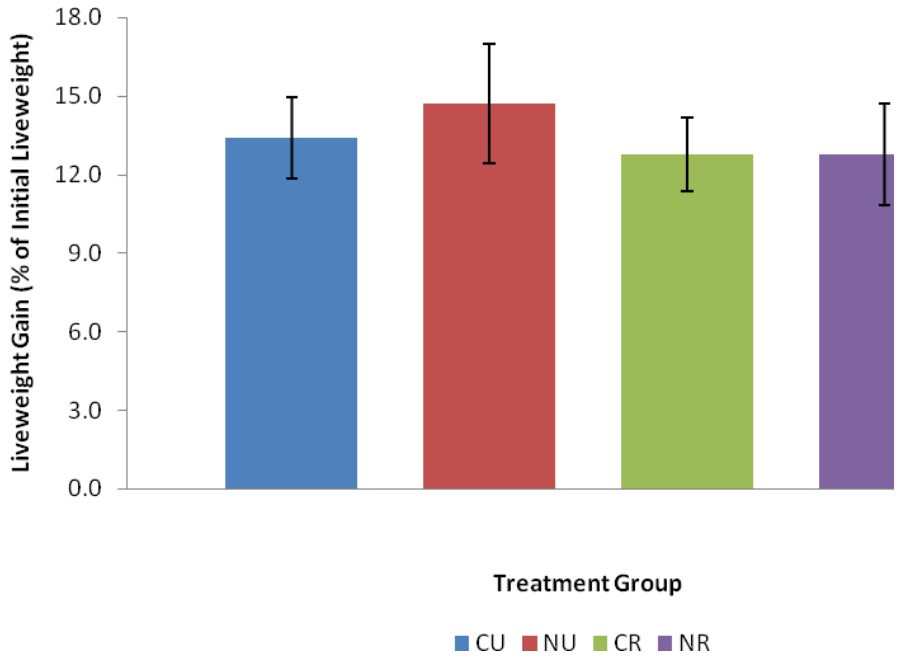


Figure 3.3 Liveweight gain (kg) as a percentage of initial weight (5 days of age) at 2 and 3 weeks of age in Friesian bull calves hand-reared with unrestricted access to calf meal fed commercial calf milk replacer (CMR) only, (CU: N = 10), or CMR with *Asocogen* added at 2/g day with (NU: N = 10) and calves with restricted access to calf meal fed CMR only (CR: N = 10) or CMR plus 2g/day of *Ascogen* (NR: N = 10)

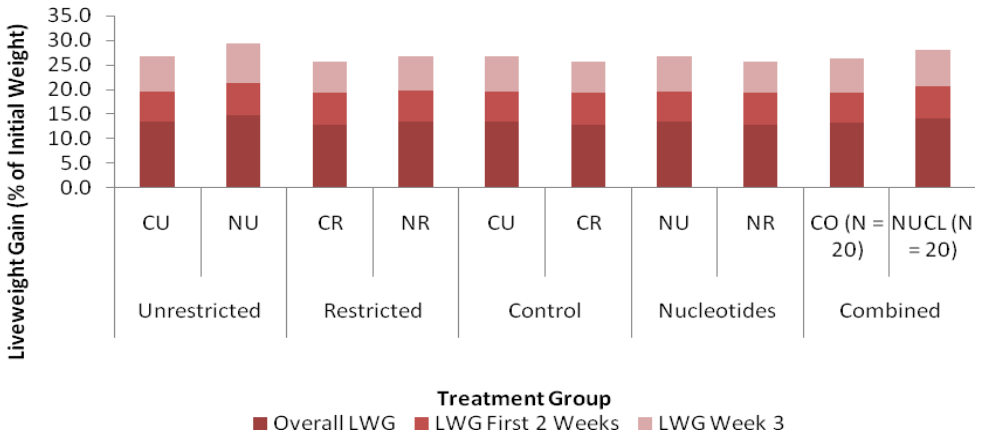


Table 3.4. Faecal consistency (days as a percentage of total days on experiment). Mean ± SD, N = 10.

	Normal faeces (% total d)	Loose faeces (% total d)	Thin faeces (% total d)	Watery faeces (% total d)
Control	81.7 ± 24.4	9.7 ± 10.1	6.6 ± 14.7	2.0 ± 6.3
Control restricted	78.8 ± 23.0	12.4 ± 14.6	7.6 ± 10.3	1.3 ± 2.7
Nucleotide	82.6 ± 12.9	9.9 ± 9.7	4.9 ± 3.8	2.5 ± 4.4
Nucleotide restricted	80.6 ±18.4	13.1 ± 13.2	6.3 ± 7.2	0.6 ±.0

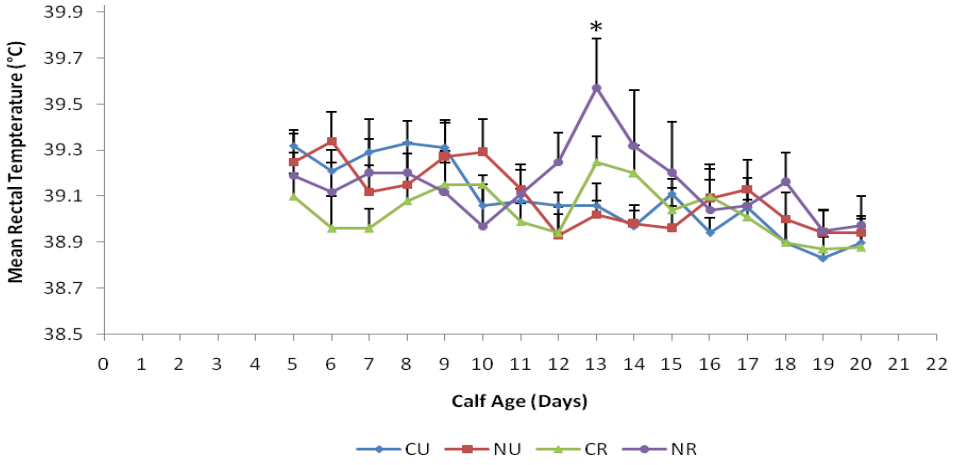
Differential leukocyte counts and analysis of serum IgG concentrations were carried out by a fourth year Agricultural Science student as part of her final year project and were checked again. Her results for the neutrophil:lymphocyte ratios showed that nucleotide supplemented calves had lower ratios (nucleotide 0.33; nucleotide restricted 0.29) than control (0.45) and control restricted calves (0.28) indicating a lower rate of infection. Preliminary analysis of serum IgG concentrations showed that the decrease in concentration from 5 days to 3 weeks did not follow a consistent pattern. The decrease in concentration in Controls was -7.32 ± 5.88 mg/ml and in control restricted calves -6.96 ± 4.93, nucleotide supplemented, -4.35 ± 2.56 and nucleotide restricted calves, -8.76 ± 4.97 mg/ml.

No differences between treatments were seen in the empty weights of the rumen. Total weight of the rumen of Controls was 1.03 kg ± 0.17, control restricted 1.11 ± 0.15, nucleotide group 1.08 ± 0.23 and nucleotide restricted 1.05 ± 0.15.

Nucleotides and Calf Meal (2009)

Health data

Figure 3.4 Mean daily rectal temperatures (˚C) of Friesian bull calves hand-reared from 5 to 21 days of age with unrestricted access to calf meal fed commercial calf milk replacer (CMR) only, (CU: N = 10), or CMR with *Asocogen* added at 2/g day with (NU: N = 10) and calves with restricted access to calf meal fed CMR only (CR: N = 10) or CMR plus 2g/day of *Ascogen* (NR: N = 10). Error bars represent standard error



*Indicates at 13 days of age, rectal temperatures were significantly higher, ($p < 0.05$), in NU calves compared to CR, or NR calves

Figure 3.5 Mean daily rectal temperatures (˚C) of Friesian bull calves hand-reared from 5 to 21 days of age with commercial calf milk replacer (CMR) only, (CO: N = 20), or CMR with *Asocogen* added at 2/g day with (NUCL: N = 20). Error bars represent standard error

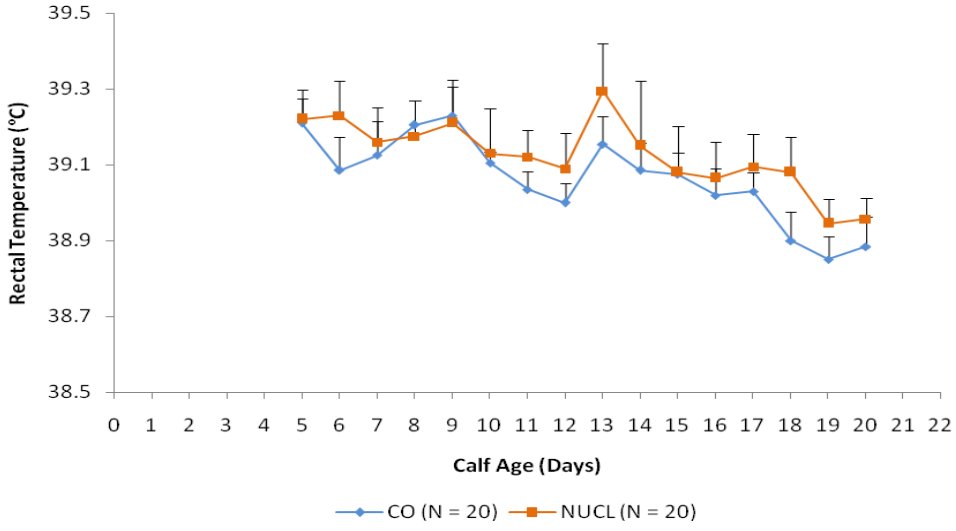
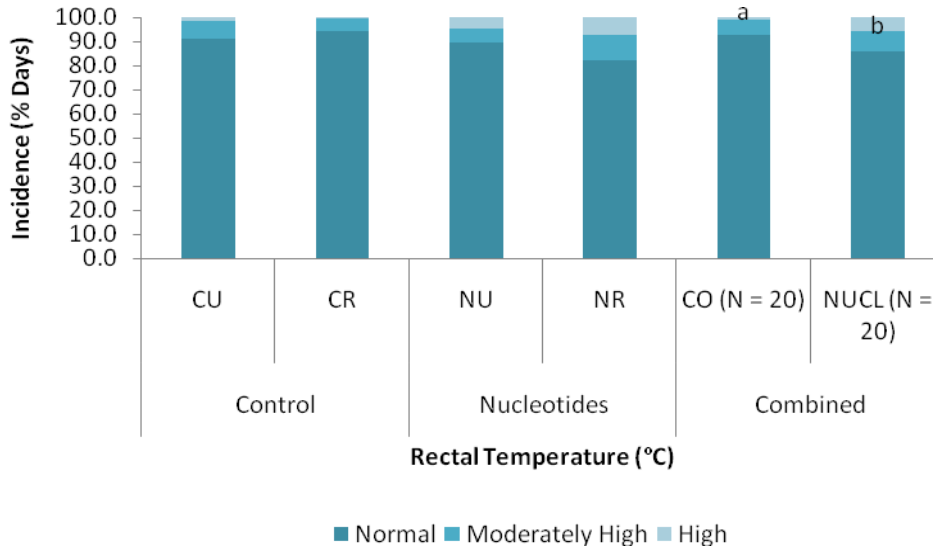


Figure 3.6 The incidence of normal (39.5˚C), moderately high (39.6 – 39.9˚C) or high (≥40.0˚C), expressed as a percentage of study days in Friesian bull calves hand-reared from 5 to 21 days of age with unrestricted access to calf meal fed commercial calf milk replacer (CMR) only, (CU: N = 10), or CMR with *Asocogen* added at 2/g day with (NU: N = 10) and calves with restricted access to calf meal fed CMR only (CR: N = 10) or CMR plus 2g/day of *Ascogen* (NR: N = 10)



Different letters indicate significant differences between groups in the incidence of high temperatures; ($p < 0.05$)

Figure 3.7 The incidence of overall, dry, watery or wet and thick eye discharge, expressed as a percentage of study days in Friesian bull calves hand-reared from 5 to 21 days of age with unrestricted access to calf meal fed commercial calf milk replacer (CMR) only, (CU: N = 10), or CMR with *Asocogen* added at 2/g day with (NU: N = 10) and calves with restricted access to calf meal fed CMR only (CR: N = 10) or CMR plus 2g/day of *Ascogen* (NR: N = 10)

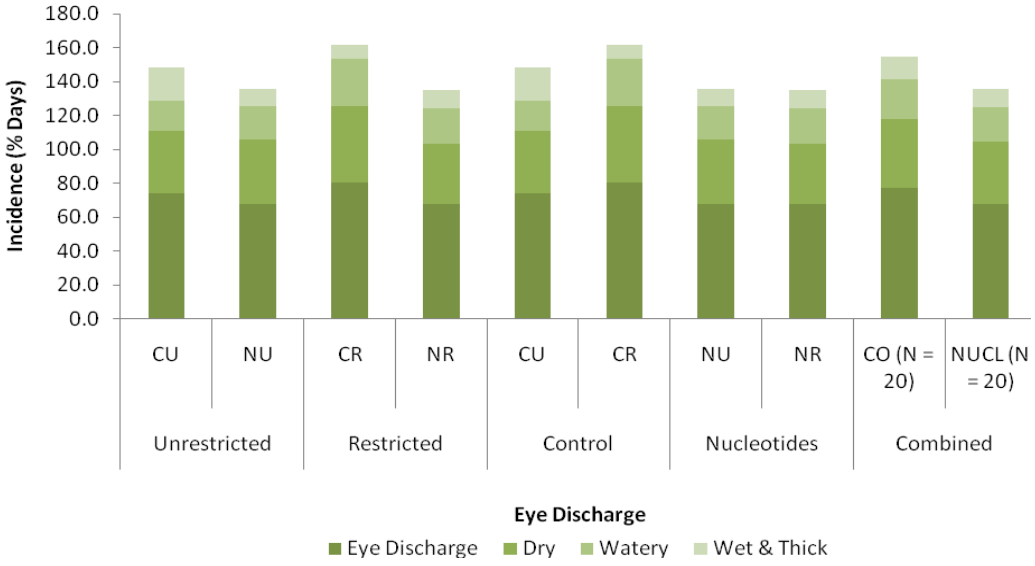


Figure 3.8 The incidence of none or serous nasal discharge, expressed as a percentage of study days (± SEM) in Friesian bull calves hand-reared from 5 to 21 days of age with unrestricted access to calf meal fed commercial calf milk replacer (CMR) only, (CU: N = 10), or CMR with *Asocogen* added at 2/g day with (NU: N = 10) and calves with restricted access to calf meal fed CMR only (CR: N = 10) or CMR plus 2g/day of *Ascogen* (NR: N = 10)

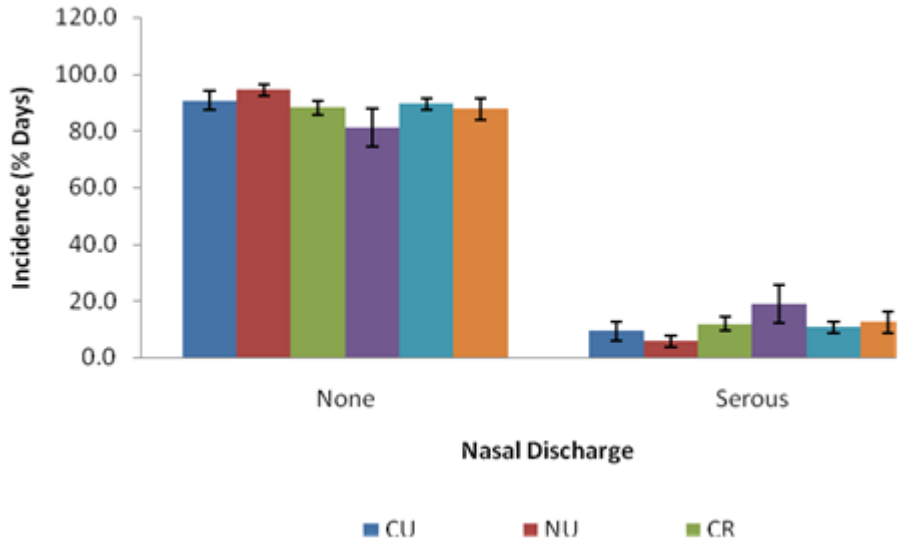
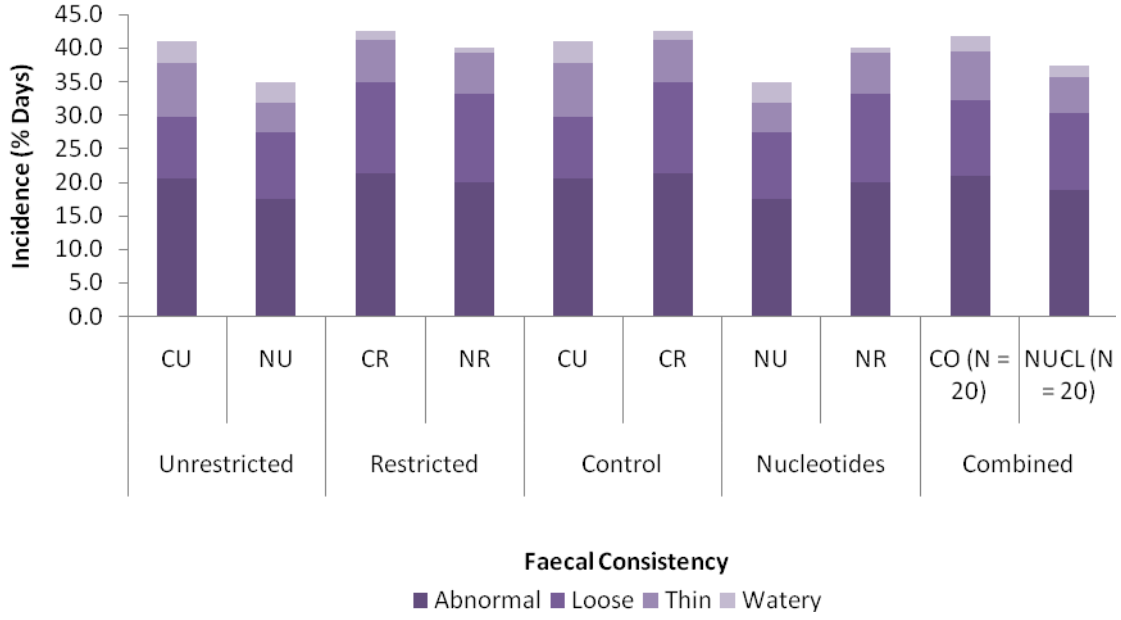


Figure 3.9 The incidence of total abnormal, loose, thin or watery faeces, expressed as a percentage of study days in Friesian bull calves hand-reared from 5 to 21 days of age with unrestricted access to calf meal fed commercial calf milk replacer (CMR) only, (CU: N = 10), or CMR with *Asocogen* added at 2/g day with (NU: N = 10) and calves with restricted access to calf meal fed CMR only (CR: N = 10) or CMR plus 2g/day of *Ascogen* (NR: N = 10)



White cell counts

Table 3.5 Total numbers of white blood cells at 5, 14 and 18-21 days of age in Friesian bull calves hand-reared with unrestricted access to calf meal fed commercial calf milk replacer (CMR) only, (CU: N = 10), or CMR with *Asocogen* added at 2/g day with (NU: N = 10) and calves with restricted access to calf meal fed CMR only (CR: N = 10) or CMR plus 2g/day of *Ascogen* (NR: N = 10). Expressed as means ± SEM

	Age (Days)		
	5	**14**	**19-21**
CU	$56 \times 10^5 \pm 6.2 \times 10^5$	$68 \times 10^5 \pm 9.0 \times 10^5$	$80 \times 10^5 \pm 6.6 \times 10^5$
NU	$59 \times 10^5 \pm 7.0 \times 10^5$	$84 \times 10^5 \pm 8.2 \times 10^5$	$69 \times 10^5 \pm 5.7 \times 10^5$
CR	$60 \times 10^5 \pm 7.5 \times 10^5$	$80 \times 10^5 \pm 9.4 \times 10^5$	$82 \times 10^5 \pm 9.6 \times 10^5$
NR	$60 \times 10^5 \pm 8.4 \times 10^5$	$78 \times 10^5 \pm 10 \times 10^5$	$79 \times 10^5 \pm 6.9 \times 10^5$

Table 3.6 Differential white blood cell ratios at 5, 14 and 18-21 days of age in Friesian bull calves hand-reared with unrestricted access to calf meal fed commercial calf milk replacer (CMR) only, (CU: N = 10), or CMR with *Asocogen* added at 2/g day with (NU: N = 10) and calves with restricted access to calf meal fed CMR only (CR: N = 10) or CMR plus 2g/day of *Ascogen* (NR: N = 10). Expressed as means ± SEM

		Lymphocytes	**Monocytes**	**Neutrophils**	**Basophils**	**Eosinophils**	**N:L Ratio**
5 Days	CU	51.2 ± 4.7	13.0 ± 3.5	35.1 ± 5.9	0.4 ± 0.2	0.3 ± 0.2	0.8 ± 0.2
	NU	55.4 ± 6.5	12.0 ± 2.1	32.6 ± 6.8	0.1 ± 0.1	0.2 ± 0.2	1.0 ± 0.5
	CR	64.0 ± 4.1	8.7 ± 2.1	27.1 ± 4.5	0.1 ± 0.1	0.1 ± 0.1	0.5 ± 0.1
	NR	57.6 ± 4.3	16.1 ± 3.3	25.6 ± 4.3	0.6 ± 0.4	0.2 ± 0.2	0.5 ± 0.1
14 Days	CU	63.5 ± 7.2	9.7 ± 3.1	26.1 ± 7.0	0.4 ± 0.3	0.3 ± 0.2	0.7 ± 0.3
	NU	58.1 ± 7.3	11.0 ± 2.4	30.6 ± 6.7	0.1 ± 0.1	0.2 ± 0.2	0.8 ± 0.3
	CR	60.9 ± 3.5	13.3 ± 2.7	24.2 ± 3.8	1.1 ± 0.5	0.4 ± 0.2	0.4 ± 0.1
	NR	55.6 ± 5.9	11.1 ± 2.5	32.4 ± 5.6	0.3 ± 0.2	0.6 ± 0.4	0.7 ± 0.2
19-21 Days	CU	55.7 ± 6.4	10.3 ± 2.1	32.6 ± 6.2	0.3 ± 0.2	1.1 ± 0.6	0.9 ± 0.3
	NU	59.0 ± 5.5	19.4 ± 4.7	20.6 ± 4.5	0.3 ± 0.2	0.7 ± 0.4	0.4 ± 0.1
	CR	64.2 ± 6.4	12.3 ± 2.0	21.6 ± 5.8	0.8 ± 0.7	1.1 ± 0.4	0.6 ± 0.4
	NR	62.4 ± 6.0	14.9 ± 3.1	21.8 ± 6.8	0.7 ± 0.4	0.2 ± 0.2	0.5 ± 0.2

Table 3.7 Total white blood cells at 5, 14 and 18-21 days of age in Friesian bull calves hand-reared commercial calf milk replacer (CMR) only, (CO: N = 20), or CMR with *Asocogen* added at 2/g day with (NUCL: N = 20). Expressed as means ± SEM

	Age (Days)		
	5	**14**	**19-21**
Control	$58 \times 10^5 \pm 4.7 \times 10^5$	$74 \times 10^5 \pm 6.5 \times 10^5$	$81 \times 10^5 \pm 5.6 \times 10^5$
Nucleotides	$59 \times 10^5 \pm 5.3 \times 10^3$	$81 \times 10^5 \pm 6.3 \times 10^5$	$74 \times 10^5 \pm 4.5 \times 10^5$

Table 3.8 Differential white blood cell ratios at 5, 14 and 18-21 days of age in Friesian bull calves hand-reared commercial calf milk replacer (CMR) only, (CO: N = 20), or CMR with *Asocogen* added at 2/g day with (NUCL: N = 20). Expressed as means ± SEM

		Lymphocytes	**Monocytes**	**Neutrophils**	**Basophils**	**Eosinophils**	**N:L Ratio**
5 Days	Control	57.3 ± 3.4	11.0 ± 2.1	31.3 ± 3.8	0.3 ± 0.1	0.2 ± 0.1	0.7 ± 0.1
	Nucleotides	56.5 ± 3.8	14.1 ± 2.0	29.1 ± 4.0	0.3 ± 0.2	0.2 ± 0.1	0.8 ± 0.3
14 Days	Control	62.3 ± 4.1	11.4 ± 2.1	25.2 ± 4.0	0.7 ± 0.3	0.4 ± 0.2	0.5 ± 0.1
	Nucleotides	56.8 ± 4.6	11.1 ± 1.7	31.5 ± 4.2	0.2 ± 0.1	0.4 ± 0.2	0.8 ± 0.2
19-21 Days	Control	59.7 ± 4.5	11.3 ± 1.4	27.4 ± 4.3	0.5 ± 0.3	1.1 ± 0.3	0.8 ± 0.2
	Nucleotides	60.7 ± 4.0	17.2 ± 2.8	21.2 ± 4.0	0.5 ± 0.2	0.4 ± 0.2	0.5 ± 0.1

Table 3.9 Lymph follicle and villus dimensions

	Control U	Nucleotide U	Control R	Nucleotide R
Jejunum				
Follicular area **($x10^3$ µm^2)**	126.2 ± 13.1	137.7± 9.3	137.0 ± 7.9	141.2 ± 9.5
Follicular height **(µm)**	492.6 ± 29.4	518.1 ± 18.4	515.0 ± 17.3	538.4 ± 20.8
Interfollicular area **($x10^3$ µm^2)**	143.7 ± 11.9	161.9 ± 20.1	170.9 ± 16.8	150.4 ± 16.1
Villus area **($x10^3$ µm^2)**	134.0 ± 8.2	143.0 ± 9.0	148.7 ± 11.1	138.8 ± 7.7
Villus circumference (µm)	2008.3 ± 74.3	1943.4 ± 52.1	2052.4 ± 70.4	19.48.0 ± 62.9
Villus height **($x10^3$ µm^2)**	756.0 ± 33.2	722.0 ± 24.2	761.2 ± 27.4	750.0 ± 26.3
Ileum				
Follicular area **($x10^3$ µm^2)**	163.7 ± 33.4	214.5 ± 34.0	260.2 ± 74.2	228.3 ± 36.5
Follicular height **(µm)**	628.8 ± 87.0	712.9 ± 57.8	782.6 ± 161.6	757.9 ± 88.7
Interfollicular area **($x10^3$ µm^2)**	34.2 ± 3.8	34.7 ± 4.2	31.5 ± 3.3	33.2 ± 4.2
Villus area **($x10^3$ µm^2)**	120.7 ± 1.8	105.6 ± 8.0	123.8 ± 5.8	126.2 ± 10.2
Villus circumference (µm)	1840.2 ± 56.0	1707.2 ± 79.1	1888.0 ± 100.4	1869.0 ± 87.8
Villus height **($x10^3$ µm^2)**	697.8 ± 18.7	650.7 ± 28.4	648.4 ± 34.0	654.8 ± 29.9

Experiment 4 – Jersey Gross Morphology

Total JPPs were 79.7 ± 7.9, 72.4 ± 5.1 and 67.0 ± 10.7 in the calves reared for 1, 2 or 3 weeks respectively (mean ± SEM).

Table 4.1 Numbers of Jejunal Peyer's Patches (JPPs) in different length categories in 1, 2 or 3 week old hand-reared Jersey bull calves reared with commercial CMR at 10% of body weight from 2-5 days of age (means ± SEM)

Rearing Group	<0.5cm	0.5-3.0cm	3.5-6.0cm	6.5-9.0cm	9.5-12cm	>12.5cm
1 Week (N = 3)	33.0 ± 7.5	25.0 ± 7.4	14.0 ± 1.7	6.7 ± 2.3	0.7 ± 0.3	0.3 ± 0.3
2 Weeks (N = 5)	29.4 ± 4.4	22.8 ± 2.1	14.2 ± 2.7	4.2 ± 1.0	1.0 ± 1.0	0.8 ± 0.6
3 Weeks (N = 3)	28.7 ± 6.7	19.7 ± 3.3	13.3 ± 1.7	4.7 ± 1.3	0.3 ± 0.3	0.3 ± 0.3

Table 4.2 Total length (cm) of the small intestine, lengths (cm) of the jejunum, ileum, ileal Peyer's Patch involution and of all jejunal Peyer's Patches and distance (cm) between the last jejunal Peyer's Patch and the beginning of the ileal Peyer's Patch in 1, 2 or 3 week old hand-reared Jersey bull calves reared with commercial CMR at 10% of body weight from 2-5 days of age (means ± SEM)

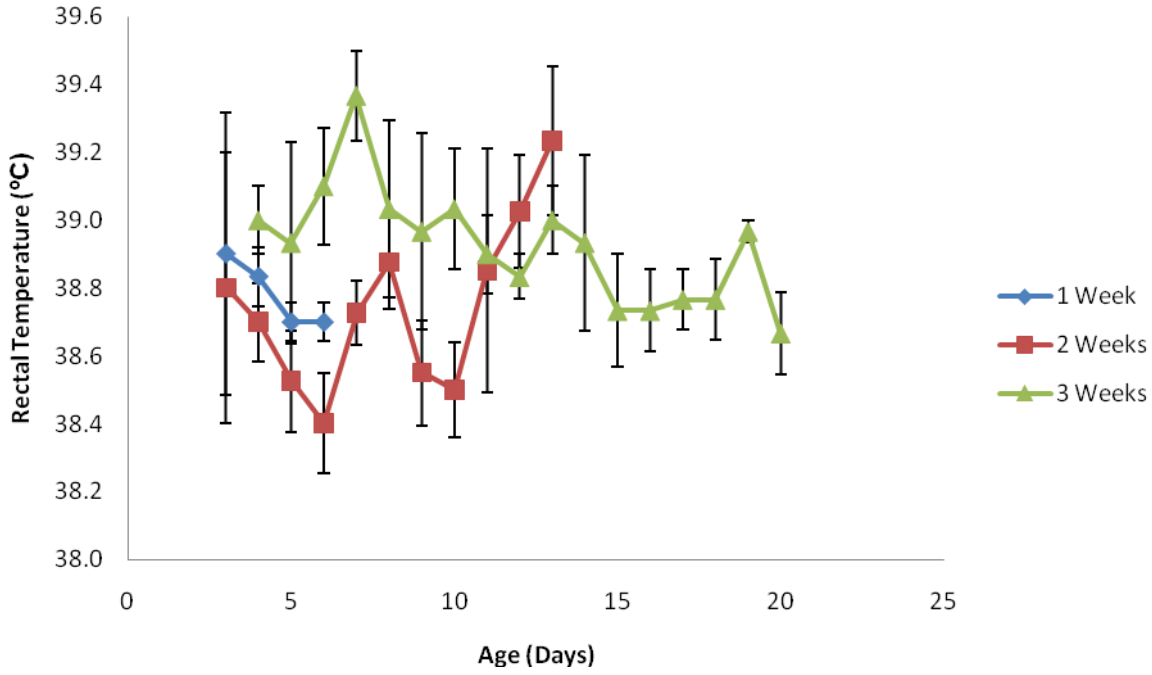
	Measure (cm)		
	1 Week (N = 3)	**2 Weeks (N = 5)**	**3 Weeks (N = 3)**
Total Length (jejunum + ileum)	1394.3 ± 30.9	1629.0 ± 19.9	1625.0 ± 175.0
Jejunal Length	1210.6 ± 22.1	1387.3 ± 32.9	1405.1 ± 132.2
Ileal Length	150.4 ± 17.0	192.2 ± 2.1	181.8 ± 26.7
Distance (last JPP to IPP)	33.3 ± 3.9	49.5 ± 15.7	38.1 ± 20.4
Length (Ileal Tapering)	36.0 ± 8.8	42.9 ± 2.1	48.7 ± 17.3
Sum of JPP Lengths	167.7 ± 6.5	157.4 ± 11.8	139.6 ± 8.2

Table 4.3 The percentage of total jejunal and ileal length that is ileum, the percentage of ileum that is ileal tapering, the percentage of jejunal and ileal length that is Peyer's patch and normal tissue and the total jejunal and ileal length as a percentage of final liveweight of 1, 2 or 3 week old hand-reared Jersey bull calves reared with commercial CMR at 10% of body weight from 2-5 days of age (means ± SEM)

	1 Week (N = 3)	**2 Weeks (N = 5)**	**3 Weeks (N = 3)**
Ileum (% total length)	10.8 ± 1.1	11.8 ± 0.2	11.1 ± 0.6
Ileal tapering (% Ileum)	25.6 ± 5.0	22.4 ± 1.3	25.0 ± 6.4
Peyer's patch tissue (% total length)	22.8 ± 0.6	21.5 ± 0.6	20.0 ± 1.0
Normal tissue (% total length)	77.2 ± 0.6	78.5 ± 0.6	80.1 ± 1.0
Total length (% liveweight)	5.3 ± 0.4	4.5 ± 1.1	4.9 ± 0.5

Jersey Calves Health Data 2007

Figure 4.1 Mean daily rectal temperatures (˚C) of Jersey bull calves hand-reared from 2-5 days of age with commercial calf milk replacer (CMR) fed at 10% of body weight to 7 days (N = 3), 14 days (N = 4) or 21 days (N = 3). Error bars represent standard error



Although rectal temperatures fluctuated throughout the study period, no calves presented with temperatures above 39.5˚C throughout the study period. (A rectal temperature of 38.5 – 38.5˚C considered normal).

Figure 4.2 The incidence of eye discharge (percentage of study days ± SEM) in Jersey bull calves reared with commercial CMR at 10% of body weight for 1 Week (N = 3), 2 Weeks (N = 4) or 3 Weeks (N = 21)

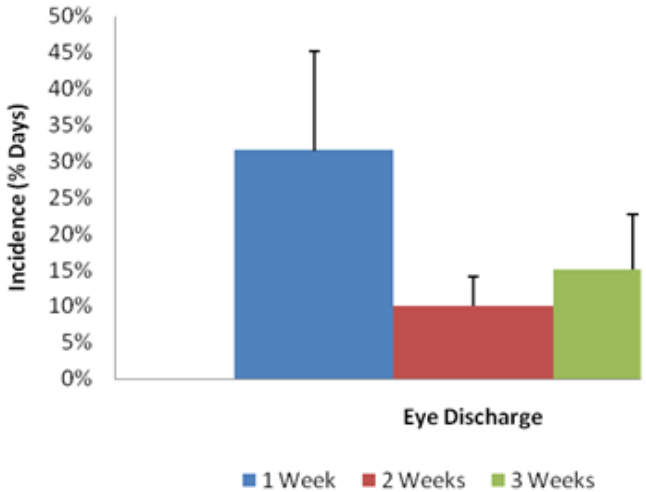
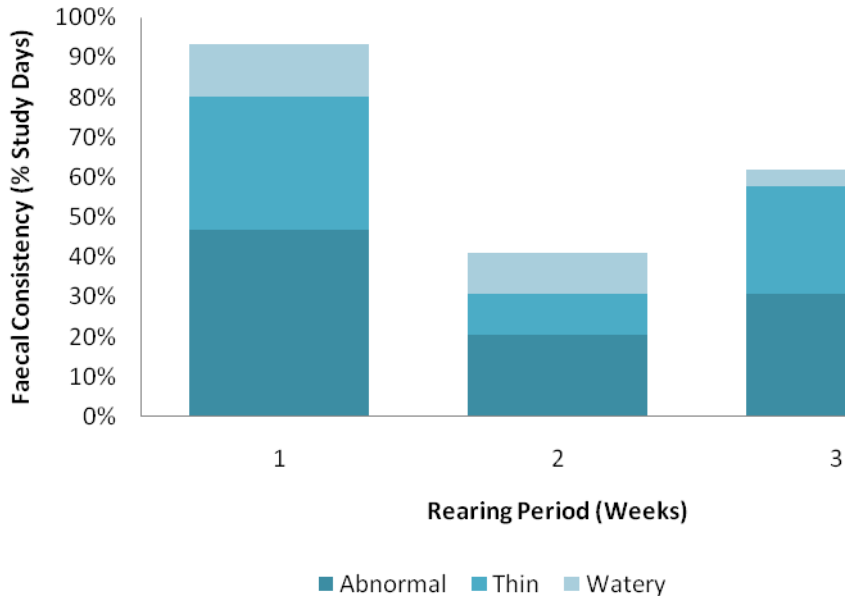
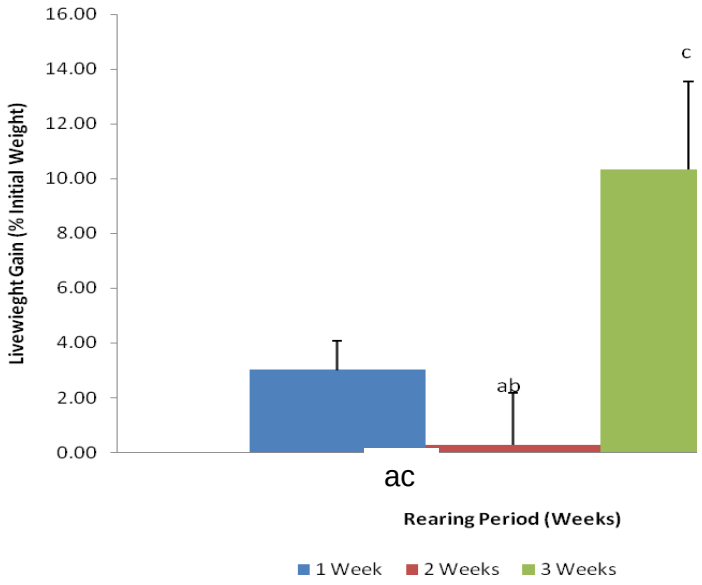


Figure 4.3 The incidence of total abnormal, thin or watery faeces (mean percentage of study days) in Jersey bull calves reared from 2-5 days old to 1, 2 or 3 weeks of age on commercial CMR at 10% of body weight



Jersey calves in this study did not present with coughing, nasal discharge or abnormal feeding, or general behaviour throughout the study period. There were no significant differences in the incidence of eye discharge or abnormal faecal consistency between calves reared for 1, 2 or 3 weeks.

Figure 4.4 Mean liveweight gain as a percentage of initial weight (± SEM) of Jersey bull calves reared from 2-5 days of age to 1 (N = 3), 2 (N = 4) or 3 (N = 3) weeks of age on commercial CMR at 10% of body weight

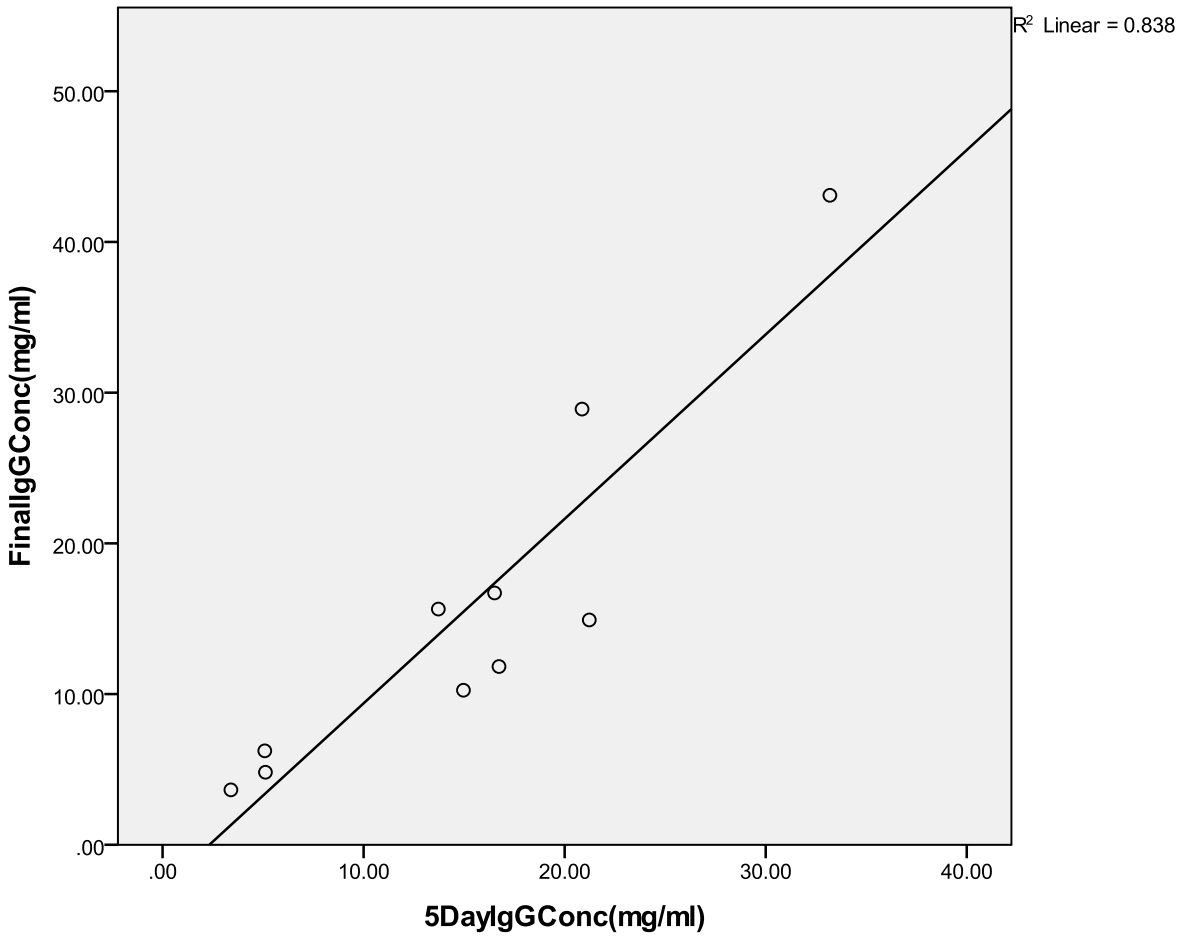


4.1 Serum IgG

Table 4.4 Serum IgG concentration (mg/ml) at 5, 7, 10, 14, 18 and 21 days of age in Jersey bull calves hand-reared for 1, 2 or 3 weeks with commercial calf milk replacer (CMR) fed at 10% of body weight. Data expressed as means ± SEM

	Serum IgG Concentration (mg/ml)					
Rearing Group	**5 Days**	**7 Days**	**10 Days**	**14 Days**	**18 Days**	**21 Days**
1 Week (N = 3)	**10.4 ± 5.3**	**13.3 ± 7.8**				
2 Weeks (N = 4)	**21.2 ± 4.3**		**14.0 ± 5.8**	**22.6 ± 6.8**		
3 Weeks (N = 3)	**11.7 ± 4.2**		**7.9 ± 3.3**	**8.2 ± 2.8**	**8.1 ± 2.4**	**8.6 ± 2.5**

Figure 4.5 Correlation between serum IgG concentration (mg/ml) at 5 days of age and final IgG concentration on day of slaughter in Jersey bull calves (N = 10) hand-reared with commercial CMR at 10% of body weight for 1, 2 or 3 weeks



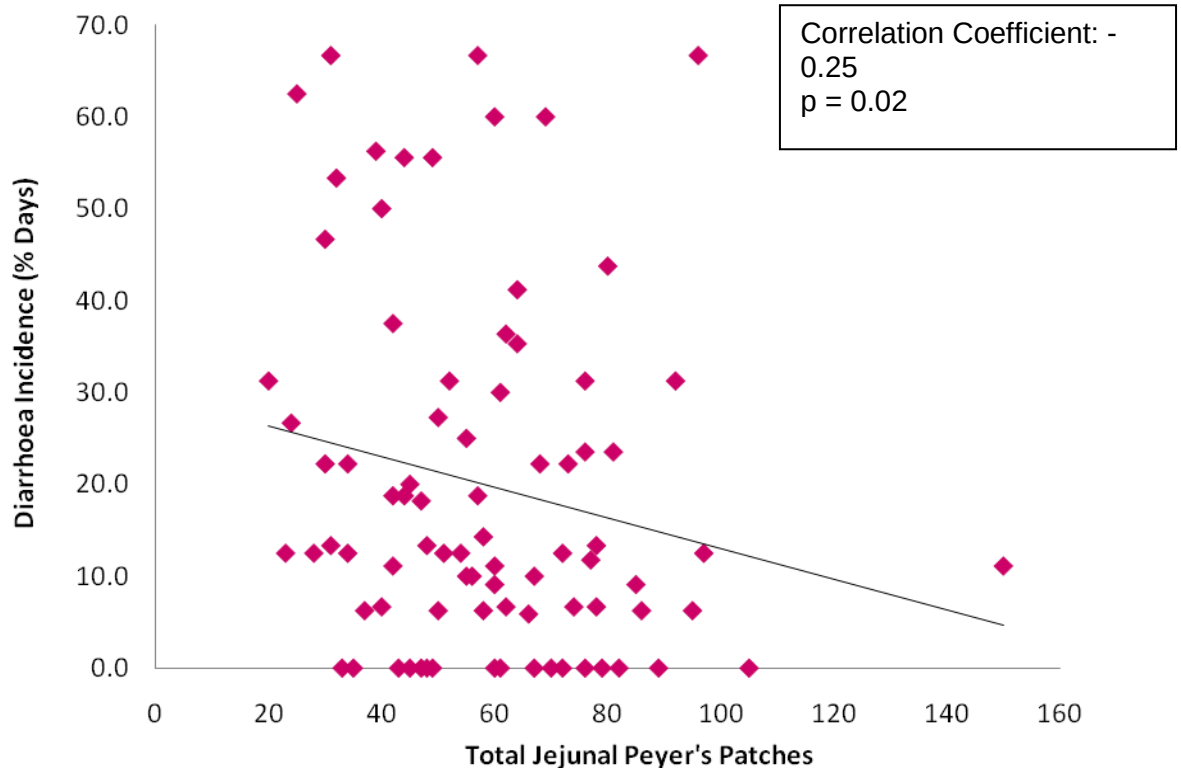
There was a strong positive correlation between serum IgG concentration at 5 days of age and final serum IgG concentration measured on day of slaughter, (coefficient = 0.8, p = 0.004). However, there was no correlation between initial serum IgG concentration at 5 days of age and thin, watery, or overall abnormal faeces

throughout the study period. There were also no correlations between any of these disease variables and final IgG concentration at the conclusion of the study period.

Experiment 5 Combined Data peyer's patches

Gross Morphology

Figure 5.1 Negative correlation between increasing numbers of total Jejunal Peyer's patches and decreasing diarrhoea incidence expressed as a percentage of study days in hand-reared Friesian bull calves aged 2 and 3 weeks. Y = -0.187x + 29 (Spearman's rho coefficient -0.25, p = 0.02)



Seventy-three percent of H calves and 85% of L calves scoured during rearing. Calves in the H group displayed reduced diarrhoea incidence in comparison to L calves; presenting with diarrhoea on 15.0 ± 2.6% of study days. This was significantly different (p = 0.03) to L calves who presented with diarrhoea on 23.9 ± 3.0% of study days (Figure 8.4).

Figure 5. 2 Incidence of diarrhoea as a percentage of days on treatment (% of study days) in 2 and 3 week old hand-reared Friesian bull calves with high (H: >57), or low (L: ≤ 57) numbers of JPPs. Data expressed as means ± SEM

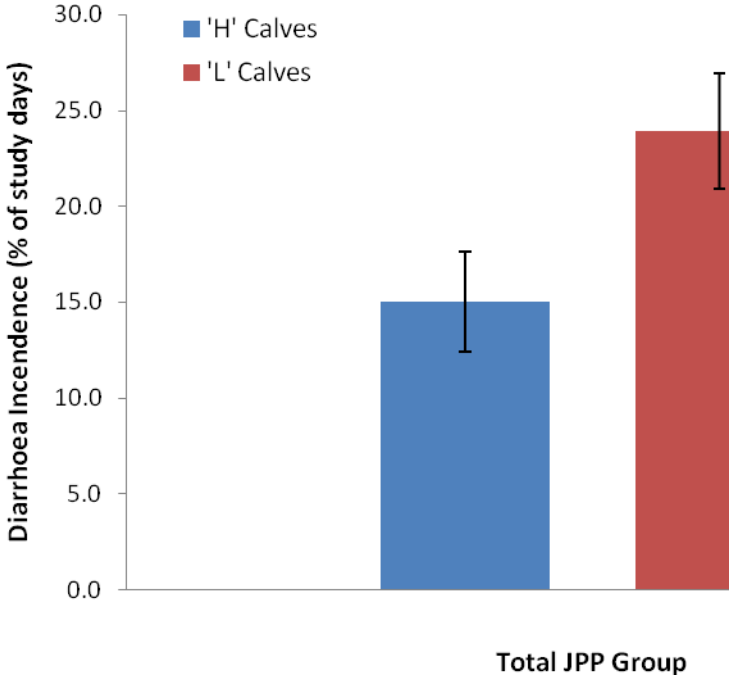


Table 5. 1 Numbers of Jejunal Peyer's Patches (JPPs) in different length categories in 2-3 week old hand-reared Friesian bull calves with high (H: > 57) or low (L: ≤ 57) numbers of JPPs (expressed as means ± SEM)

Total JPP Group	<0.5cm	0.5-3.0cm	3.5-6.0cm	6.5-9.0cm	9.5-12cm	>12.5cm
H (N = 41)	27.9 ± 2.0[a]	20.1± 1.1[a]	15.0 ± 0.6[a]	10.8 ± 0.6	2.1 ± 0.4[a]	0.4 ± 0.1
L (N = 46)	6.3 ± 0.9[b]	10.7 ± 1.1[b]	10.9 ± 0.7[b]	10.4 ± 0.5	3.8 ± 0.5[b]	0.6 ± 0.1
H (% of total)[+]	36.6	26.4	19.7	14.2	2.8	0.4
L (% of total)[+]	14.8	25.1	25.5	24.4	8.9	1.4

For each category, means within columns followed by different letters are significantly different at $p < 0.01$

\+ length as % of total PP length, calculated from means.

Table 5.2 Total length (cm) of the small intestine, lengths (cm) of the jejunum, ileum, ileal Peyer's Patch involution and of all jejunal Peyer's Patches and distance (cm) between the last jejunal Peyer's Patch and the beginning of the ileal Peyer's Patch in 2-3 week old hand-reared Friesian bull calves with high (H: > 57) or low (L: ≤ 57) numbers of JPPs (expressed as means ± SEM)

	Measure (cm)	
	H (N = 41)	**L (N = 46)**
Total Length (jejunum + ileum, cm)	2081.8 ± 42.1[a]	2246.6 ± 55.0[b +]
Jejunal Length	1834.0 ± 41.4[a]	2004.5 ± 58.6[b +]
Sum of JPP Lengths	198.2 ± 4.4	188.1 ± 5.0
Distance (last JPP to IPP)	66.3 ± 6.2[a]	90.2 ± 7.8[b]
Ileal Length	207.9 ± 4.7	200.0 ± 6.2[+]
Length (Ileal Tapering)	51.7 ± 2.8	55.3 ± 3.5[+]
Length JPP (% of jejunal length)	10.9 ± 0.2[a]	9.6 ± 0.3[b]
Ileal length (% of total J and I lenth)	10.1 ± 0.2 [a]	9.0 ± 0.3 [b]
Ileal taper (% of ileal length)	25.1 ± 1.6	28.5 ± 2.1
PP tissue in J and I as % of total	19.6 ± 0.3 [a]	17.6 ± 0.4 [b]

As a percentage of liveweight, total length of the small intestine was greater in L calves (4.6 ± 0.2%) compared to H calves (4.3 ± 0.1%), p = 0.04. Ileal length as a percentage of total length was greater in H calves (10.1 ± 0.2%) than L calves (8.6 ± 0.4%), p = 0.00. The percentage of PP tissue in the small intestine of H calves (19.6 ± 0.3%) was also greater than that in L calves (16.8 ± 0.7%), p = 0.00. Interestingly, the percentage of normal, non-PP tissue in the small intestine was also greater in H calves (80.4 ± 0.3%) than L calves (78.8 ± 2.5%), p = 0.00.

Initial IgG Concentration and Disease Prevalence

(Based on Control calves ONLY)

Seventeen of the 44 calves were assessed as having low initial (Low) serum IgG, with a mean concentration (± SEM) of 5.05 ± 0.86mg/ml, (range: 0 – 9.79mg/ml). Mean serum IgG concentration (± SEM) of the high initial (High) group (N = 27), in contrast, was 22.22 ± 2.56mg/ml, (range: 10.82 – 65.30mg/ml).

Table 5.3 Incidence of abnormal, thin and watery faeces, as well as abnormal temperatures (>39.5^{O}C) as a mean percentage of study days (± SEM) in Friesian bull calves hand-reared for 2-3 weeks with Low (N = 17) or High (N = 27) initial serum IgG concentration

	Total % of study days presenting with disease symptom	
	Low	**High**
Abnormal Faeces	27.06 ± 5.12	19.25 ± 4.28
Thin	11.51 ± 3.08[a]	5.19 ± 1.70[b]
Watery	4.92 ± 1.77	5.52 ± 1.84
Abnormal Temperature	7.30 ± 1.92	5.88 ± 1.74

Different letters represent significant differences at the level of $p < 0.05$

There was a significant correlation between initial serum IgG concentration and the percentage of days calves presented with moderate diarrhoea ($p = 0.032$). However, there was no correlation between initial serum IgG concentration and duration of diarrhoea episodes in calves. There was also no correlation between serum IgG concentration and the percentage of days calves presented with abnormal rectal temperatures. There was also no correlation between the percentage of days calves presented with diarrhoea and the percentage of days calves presented with abnormal temperatures throughout the study period.

5 Discussion/conclusion

This project specifically researched the development of the gut-associated immune system of calves in the first three weeks of life through the influence on the health and immune responses of calves of two different forms of additives to calf milk replacer. In particular, observations and analyses were made on the Peyer's patches of the small intestine. These specific and important immune structures are critical for the calf against pathogens. The first experiment investigated the effects of the addition of 5% sunflower seed oil (poly unsaturated fatty acids) and 5% palm fat (saturated fatty acids) on health status of bull calves and the gut associated lymphoid tissue (specifically Peyer's patches) that is important in protecting young calves against disease entry through the gut. The second and third experiments investigated the effects of a commercial preparation of nucleotides on health status and immunity of bull calves. Unlike early work where replacing all the fat in calf milk replacer with unsaturated fats caused increased mortalities, the addition here of 5% unsaturated fatty acids did not cause increased mortality or morbidity in two or three week old calves. Addition of a commercial nucleotide to calf milk replacer appeared in the first experiment to be of benefit at 2 weeks of age but at 3 weeks it appeared to be associated with an increased rate of diarrhoea and a decreased weight gain. Because solid feed intake increases between 2 and 3 weeks it was thought that

increased availability of substrates from the solid feed and availability of nucleotides for microbial development could have caused an increase in microbial proliferation to the detriment of calf health. A second experiment using nucleotides was designed to determine whether amount of solid feed intake could influence the effects of nucleotides on health of calves. The responses of calves to nucleotides differed from those in the first nucleotide experiment and no advantage of addition of nucleotides to calk milk replacer was seen at two weeks of age. There was no significant effect of amount of solid feed on health of the calves. During the experiments on fatty acids and nucleotides it was noted that the appearance of the Peyer's patches in the intestinal tract varied considerably from calf to calf. A correlation between Peyer's patch numbers and incidence of diarrhoea showed that calves with a greater number of Peyer's patches in the small intestine had a lower incidence of diarrhoea. This important finding could be exploited further if a genetic marker for Peyer's patches could be found so that genetic selection on the basis of Peyer's patch number could be made. There is evidence from others to suggest that the numbers of Peyer's patches in other species of animals is predetermined by the genetic make-up but the function can be modified to some degree after birth. If a genetic marker could be found it would benefit not only the dairy bull beef industry but also the beef industry as the prevalence of diarrhoea in beef breed calves reared by their dams is about 3% and this could increase with an increase in early weaning practices. As a spin-off from the third experiment in which the effects of an interaction between nucleotides and solid feed was investigated, the effects on methanogens and selected other microorganisms in the developing rumen were also assessed. Both nucleotides and amount of solid feed eaten in young calves have the potential to increase rate of development of fermentation reactions in the rumen and the numbers or types being established. In calves given the nucleotide supplement methanogens were reduced compared to calves without the nucleotide supplement. This suggests that there is a potential to influence the early establishment of methanogens in calves and that this, in view of interactions between gut microorganisms and the immune function in the gut, could have potential for influencing the lifetime concentrations of methanogens in the rumen.

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