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CALF HEALTH AND IMMUNITY

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CONCLUSIONS FROM THE STUDIES

Suckled calves had significantly higher serum IgG 1 concentrations than mart purchased dairy calves.

The marked differences in immunoglobulin levels between suckled calves and dairy calves suggest that these calves received either insufficient quality or quantity of colostral immunoglobulins.

Factors affecting calf serum Ig concentrations are, Ig concentration in colostrum, colostrum intake, Ig mass, calf age at first feeding, nutrition of the dam, method of ingestion, presence of the dam, age of the dam and the calf.

When suckled calves were fed a similar volume of colostrum relative to birth weight (40 ml/kg) and at the same time interval post birth, there was no significant difference across the three suckler herd progeny for IgG_1, IgA and IgM and total Ig serum levels at 28 and 56 days of age. However, serum IgG_2 levels were significantly lower in the Limousin x beef breed when compared with the Charolais x beef breed suckled calves at 28 days of age.

Healthy calves had higher serum immunoglobulins (IgG_1) than calves treated for respiratory disease, enteric disease or for both respiratory disease and enteric disease.

It is well recognised that immunoglobulins are absorbed from the intestine for only a short period post birth and that efficiency of absorption is dependent on ensuring that the calf receives adequate colostrum in the immediate post-partum period.

Low serum IgG_1 concentrations are attributable to failures to obtain adequate colostral immunoglobulins in the period immediately following birth.

The mean IgA and IgM serum levels of suckled calves in the present study were only slightly higher than dairy calves while IgG_1 serum levels were almost approximately twice as high.

Feeding colostrum high in Ig results in higher calf serum Ig concentrations at 48h.

The low serum Ig levels reported in the present study suggest that dairy calves failed to obtain adequate transfer of colostral immunoglobulins.

Calves with a lower immune status are more susceptible to neonatal infection and thus the importance of colostrum in the immediate post partum period cannot be overemphasised. Thus, the identification of calves with low levels of immunity might stimu-

late calf producers to ensure that calves receive adequate levels of colostral immunoglobulins.

The implications of the present findings are that compared with suckled calves, dairy calves are not receiving 1). adequate quantity of colostrum 2). adequate quality of colostrum. 3). Colostrum soon enough post birth 4). or a combination of all of the previous factors.

Rearing calves outdoors using calf jackets had no beneficial effect on calf performance. The incidence of respiratory disease was higher in calves reared indoor when compared with calves reared outdoor with and without jackets. There was an increased incidence of diarrhoea in calves reared outdoors irrespective of calf jacket.

Lymphocytes from calves with respiratory disease manifest an impaired capability to blast *in vitro*.

Chromium (Cr) supplementation (250 mg/kg dry matter intake) enhanced the blastogenic response in healthy calves, while, calves with respiratory had impaired blastogenic responses.

Supplementation with organic Cr (250 mg/kg dry matter intake) for 63 days had no major effect on physiological parameters and had select effects on haematological parameters, namely, the % monocytes. The % monocytes were significantly higher in the standard commercial milk replacer (CMR) (Skim) Cr supplemented calves when compared with the whey based (CMR) + Soya Brand B or whey based CMR + Soya Brand C or whey based enzyme processed soya Brand C + Cr treatment groups.

INTRODUCTION

Promoting positive calf health is difficult particularly with regard to artificially reared calves. Morbidity and mortality of the young calf represent a major cause of economic concern for beef producers. All calves are exposed to infectious organisms from the moment of birth, but natural defence mechanisms usually preclude the establishment of disease. Animals develop disease because of complex relationships between the host (animal), the infectious agent (bacterium, virus, fungus, or toxic agent), and environment. Control of the agent is largely based on prevention of exposure, immunity, and chemotherapeutic (drugs) agents. Viruses that have been predominantly isolated from outbreaks of calf pneumonia are infective bovine rhinotracheitis (IBR), respiratory syncytial virus (RSV), parainfluenza-type 3 virus (PI-3 virus), and bovine virus diarrhoea/mucosal disease (BVD/MD virus). Viral infection will result in extensive, and sometimes fatal, lung damage. Predisposing factors affecting immunocompetence (ability to fight infection) are stress, overcrowding, inadequate ventilation, draughts, fluctuating temperatures, poor nutrition and/or concurrent disease. Poor housing, particularly bad ventilation, will increase the severity of pneumonia outbreaks. The resistance of the calf to these infectious agents depends upon the amount of passive immunity received from colostrum, the "infection"challenge in the environment and the nutrition of the calf in the post-colostrum period (Earley, 1 997a; 1 997d).

Calf immunity

Immunity against infectious diseases is a complex phenomenon, involving interaction between different cell types, each having unique function(s). The immune response is classified into two main categories: (1) humoral immune response, also known as antibody mediated immunity, in which lymphocytes, called B cells produce the antibodies, which circulate in the blood (humoral immunity). These antibodies neutralise antigens by removing them from the body's circulation; and (2) cell-mediated immunity (CMI) where lymphocytes, which mature in the thymus gland are called T cells, are active in cell-mediated immunity, i.e. they activate other cells that cause direct destruction of antigens or assist B cells (Earley, 1 999e).

Colostral immunoglobulins

Calves are born hypogammaglobulinemic and rely on Immunoglobulins (Ig) from colostrum to obtain passive immunity. In

the bovine species, immunoglobulins do not cross the placenta *in utero*, and the new-born calf is, therefore, dependent on antibodies obtained through ingestion of colostrum.

It is well documented that colostrum is an important source of immunoglobulins and provides immunity to disease for the new-born calf (Fallon *et al*., 1986; Fallon *et al*., 1989; Earley *et al*., 1 997a; 1 997b; 1 999c). Immunoglobulins are proteins that bind to and help eliminate foreign agents in the body such as bacteria and viruses. Approximately 80% of all immunoglobulins in colostrum are of the IgG class; thus, IgG plays a critical role in immunoglobulin participation in pathogen clearance. IgA comprise 8 to 10 percent of bovine colostral immunoglobulins, while IgM make up 5 to 12 percent. These two types provide immunity against systemic infections. Each form of immunoglobulin protects the calf against a specific disease or infection.

Experimental Objectives

The main objectives of the series of experiments reported here were:

1. To determine the affect of suckler cow breed type on immunoglobulin concentration of colostrum.
2. To examine serial changes in the serum levels of individual immunoglobulins in suckled calves.
3. To examine the relationship between the serum levels of circulating immunoglobulins and the subsequent health status of mart purchased calves.
4. To examine the Immunological profile of farm and mart purchased Friesian calves exposed to an outbreak of *Salmonella typhimurium*.
5. To investigate the effects on calf health and performance of artificially rearing calves either outdoors with and without calf jackets or indoor without jackets.
6. To examine the effects of dietary supplementation with organic chromium in the calf milk replacer on biochemical, haematological and immunological parameters in calves.

Experiment 1. Effects of quality of maternal colostrum on serum immunoglobulin (Ig) concentrations in suckled calves.

Introduction

Maternal colostrum provides the main source of immunoglobulins for the new-born calf. Considerable variation between cows with respect to immunoglobulin concentration in the colostrum is reported in the literature. In previous studies at Grange it was reported that suckler cows of low milk production potential have lower colostrum yields resulting in lower Ig serum levels in their offspring. A large variation in colostrum (McGee, Drennan and Caffrey, 1995) yield exists between beef and dairy cows. The objective of the present study was to determine the affect of cow breed type on immunoglobulin concentration of colostrum and subsequent serum immunoglobulin concentration and health status of their calves.

Materials and Methods

The spring calving suckler herd at Grange was used as the source of suckled calves. This herd which calved indoors, consisted of upgraded Charolais (Ch) (n = 6 heifers; n = 10 cows), Limousin × Friesian (Li × Fr) (n = 23 heifers; n = 41 cows) and Simmental × Limousin × Friesian cows (Si × (Li × Fr), n = 6). Mature cows were bred to Charolais sires while an easy calving Limousin sire was used on heifers. Following parturition (prior to suckling) a 20ml colostrum sample was obtained from both the front and hind quarters of the udder.

After housing in the autumn, all cows were offered second cut grass silage ad libitum until parturition. All cows received a combined bovine rotavirus and E. coli vaccine (inactivate) (Rotavec K99, Mallinckrodt) by intramuscular injection between 4 and 12 weeks prior to expected calving date.

The suckled calves were fed 40 ml of colostrum per kg of birth weight using an oesophageal feeder and bag (stomach tube) within one hour of birth. The purpose of this procedure was to ensure that suckled calves received adequate immunoglobulin of known quantity and quality in the immediate post-partum period. Serum samples were collected from the suckled calves at 2, 28 and 56 days post partum.

Serum immunoglobulins (IgG_1, IgG_2, IgA and IgM) were measured quantitatively by single radial immunodiffusion (sRID) (Mancini *et al.,* 1965) and calculated via an internal Ig standard (BINDARID, NANORID kits. The Binding site Ltd., R&D, Birmingham, UK.). The zinc sulphate turbidity (ZST) test was performed on all serum samples at 20°C with turbidity readings carried out at 520nm using a spectrophotometer (McEwan *et al.*, 1970).

The data was analysed using analysis of variance procedure and if a significant difference between treatments was observed, Duncan's multiple range test (Duncan, 1955) was applied to determine statistical differences between cow breeds. ($P < 0.01$ was considered statistically significant).

Results

There was no significant difference between breed in colostrum IgG_1 concentrations in either the front or back quarter of the udder (Table 1). However, IgG_1 concentrations were lower in Holstein × Friesian cows compared with beef cows.

Total serum Ig concentration using sRID was significantly correlated with ZST units at 2 days in calves from Ch cows ($r = 0.673$; $P < 0.001$), Li × Fr heifers ($r = 0.769$; $P < 0.001$), Li ×Fr cows ($r = 0.586$; $P < 0.001$) and Si × (Li × Fr) cows ($r = 0.961$; $P < 0.04$). Calves from Charolais cows had significantly lower IgG_1, total Ig serum concentrations and ZST Units than calves from Limousin × Friesian cows at 2 days post-partum (Table 2).

There was no significant difference in serum Ig concentrations in calves from Charolais heifers and Li x Fr heifers. Calves from Li x Fr cows had higher values than the other breeds. Serum Ig concentrations for IgG_1, IgG_2, IgA, IgM and total Ig were significantly lower in calves from Limousin × Friesian cows at 28 days when compared with values obtained at 2 days post-partum. In calves from Charolais cows, IgA and IgM serum concentrations were significantly lower at 28 and 56 days post-partum when compared with values obtained 2 days post-partum. Calf Serum Ig concentrations and ZST units of Si × (Li × Fr) progeny at 2, 28 and 56 days were not significantly different from values obtained for the progeny of Charolais and Limousin × Friesian heifers and cows. There was no outbreak of respiratory disease in the suckled calves (Earley *et al.*, 1998c; 1998d).

Table 1: Colostrum IgG_1 (mg/ml) concentration from the front and Back quarters of the udder. Values expressed as Mean ± s.e.m.

Cow breed	Front Quarter	Back Quarter
Charolais × Beef Breed (n = 16)	163.06 ± 9.386	177.00 ± 17.13
Limousin × Beef Breed (n = 64)	166.29 ± 9.01	164.82 ± 6.95
Hereford × Beef Breed (n = 14)	169.86 ± 21.86	170.77 ± 32.74
Simmental × Friesian (n = 6)	169.42 ± 23.54	168.34 ± 15.78
Holstein × Friesian (n = 80)	85.20 ± 12.10*	88.30 ± 9.78*

* P<0.01 versus beef × cows

Table 2: Calf serum Ig concentration (mg/ml) and ZST (units) at 2, 28 and 56 days post-partum.

	Progeny of	IgG_1	IgG_2	IgA	IgM	Ig Total	ZST
2 days	(Ch) Heifers	36.2••	0.41	0.090	0.92	37.6••	15.1
	(Ch) Cows	35.5••	0.54	0.163	0.79	36.9••	11.9••
	(Li xFr) Heifers	38.6	0.62	0.113	1.086	40.4	15.7
	(Li x Fr) Cows	53.6•	0.89	0.115	1.29	55.9•	19.7
	(Si x (Li x Fr) Cows	44.0	0.50	0.082	0.91	45.5	15.7
28 Days							
	(Ch) Heifers	42.0	0.27	0.033	0.45	42.8	15.6
	(Ch) Cows	40.6	0.57	0.028 *	0.49 *	41.6	13.5
	(Li xFr) Heifers	40.1	0.48	0.028	0.46 *	41.1	16.2
	(Li x Fr) Cows	41.5 †	0.53†	0.052 †	0.38 †	42.5†	18.2
	(Si x (LixFr) Cows	35.3	0.45	0.032	0.32	36.1	14.5
56 Days							
	(Ch) Heifers	43.0	0.29	0.029	0.44	43.8	16.2
	(Ch) Cows	42.0	0.54	0.028 *•	0.49 *•	43.1	14.9
	(Li xFr) Heifers	41.1	0.46	0.029	0.46 *•	42.1	15.9
	(Li x Fr) Cows	42.5 †•	0.51†•	0.053 †•	0.39 †•	44.4†•	17.9
	(Si x (LixFr) Cows	37.5	0.47	0.031	0.32	38.3	15.3

2 days: • P< 0.01 v. LF heifers; •• P < 0.01 Ch cows and Ch heifers v. Li x Fr cows;
28 days: † P< 0.01 IgG1, IgG2, IgA, IgM v. Li x Fr cows at 2 days. * P< 0.01 IgA, IgM v C Cows 2 days; (* P< 0.01 IgM, v. Li x Fr heifers at 2 days
56 days: †• P< 0.01 IgG1, IgG2, IgA, IgM v. Li x Fr cows at 2 and 28 days. * •P< 0.01 IgA, IgM v Ch Cows 48 hours; *•* P< 0.01 IgM, v. Li x Fr heifers at 48 hours

Conclusion

Protection against neonatal infection is dependent on the passive immunity that the calf receives in the immediate post-partum period. There was no significant breed effect in the quality of maternal colostrum. High serum Ig concentrations were obtained by 48 hours post-partum in all calves. By 28 and 56 days post-partum, calves from Limousin x Friesian had lower Ig concentrations when compared with values obtained at 48 hours. The level of serum Ig achieved at 2 days in the calf will depend on the total mass of Ig absorbed, which is additionally a function of the Ig concentration of colostrum and the total amount of colostrum ingested during the period of maximum absorption.

EXPERIMENT 2: EFFECT OF BREED ON THE IMMUNE STATUS OF PURCHASED DAIRY CALVES.

Introduction

Colostral antibodies usually adequately protect calves against disease unless environmental stress is excessive. Failure of passive transfer of immunity is the term used to define the situation where calves have failed to reach a specific serum immunoglobulin concentration following the period of intestinal immunoglobulin absorption. In addition, excessive stress causes production of cortisol by the adrenal glands which in turn further suppresses the immune system, predisposing to bacterial or viral infections. The objective of the present study was to examine the relationship between the serum levels of circulating immunoglobulins and the subsequent health status of mart purchased calves.

Materials and methods

Dairy calves were approximately 28 days of age at arrival in Grange and consisted of Charolais x Friesian (Ch x Fr) (n = 61), Limousin x Friesian (Li x Fr) (n = 39), Friesian (Fr) (n =73) and Belgian Blue (BB X Fr) (n = 9) breeds. They received an individual allowance of 25kg of milk replacer powder offered warm at 38°C by bucket during the first 42 days (as shown) and had *ad libitum* access to a concentrate ration throughout the 56 day experimental period.

Milk Replacer Feeding Programme

Period (days)	1 - 4	5 - 28	29 - 42
Amount (litres/feed)	2	3	3
Number of feeds daily	2	2	1

Serum samples were collected from the dairy calves on days 0 (day of arrival), 28 and 56 after arrival in Grange. Serum immunoglobulins (IgG_1, IgG_2, IgA and IgM) were measured quantitatively by single radial immunodiffusion (sRID). The zinc sulphate turbidity test (ZST) was performed on all serum samples.

Results

There was no significant difference in IgG_1, IgG_2, IgA, IgM, total Ig and ZST units in mart purchased dairy calves either at arrival in Grange on day 0 or on day 28 (Table 3). By day 56, Ch x Fr calves had significantly higher Ig levels (IgG_1, IgG_2, IgA and IgM) compared with day 0 while BB x Fr calves had significantly higher IgA and ZST units compared with day 0, and day 28 values. The values obtained for dairy calves are low compared to those previously reported for single suckled calves.

Mart Purchased Dairy Calves

Table 3: Serum immunoglobulin levels (mg/ml) and ZST (units) in mart purchased dairy calves on days 0 (day of arrival), 28 and 56

Day 0	IgG1	IgG2	IgA	IgM	Total Ig	ZST
Charolais x Friesian	20.0	1.08	0.065	0.88	22.1	8.6
Limousin x Friesian	20.2	0.75	0.059	0.81	21.9	8.8
Friesian	19.0	0.87	0.060	0.79	20.8	10.6
Belgian Blue x Friesian	22.2	0.57	0.059	0.79	23.7	9.6
Day 28						
Charolais x Friesian	21.4	2.7	0.120	1.17	25.5	11.5
Limousin x Friesian	21.6	2.3	0.096	1.19	25.2	13.2
Friesian	24.2	2.2	0.114	1.58	28.1	14.0
Belgian Blue x Friesian	21.1	1.9	0.109	2.12	25.3	12.0
Day 56						
Charolais x Friesian	28.5†	1.9†	0.13†	1.14†	31.6†	16.4†
Limousin x Friesian	29.5 •••	2.4 ••	0.103	1.6 •••	33.6 •••	18.2 ••
Friesian	27.1 •	2.4 •	0.180 •	2.1 |	31.8 •	16.9 •
Belgian Blue x Friesian	20.8	2.0	0.094 *	1.6	24.5	17.9 **

• P < 0.01 versus Friesian on day o and day 28;
| P < 0.01 versus Friesian on day 0;
•• P < 0.01 versus Limousin on day 0 and 28.
* P < 0.01 versus Belgian Blue x on day 0;
** P < 0.01 versus Belgian Blue x on days 0 and 28
† P < 0.01 versus Charolais purchased on days 0 and 28.

EXPERIMENT 3. IMMUNE STATUS OF PURCHASED DAIRY CALVES AND INCIDENCE OF DISEASE.

Introduction

The transplacental transfer of Ig does not occur in calves and for this reason calves are born essentially hypogammaglobulinaemic. Following ingestion of colostrum by the new-born calf, absorption of colostral immunoglobulins occurs in the small intestine by a process of micropinocytosis into the columnar cells of the epithelium. In calves, absorption continues for up to 24 hours but maximum absorption occurs within the first 6 - 8 hours after birth. Thus, the quantitative determination of the serum levels of antibodies is now an important part of the investigation of neonatal disease in the calf (Earley and Fallon, 1 998a). The main objective of this study was to determine the effect of immunoglobulin status on the incidence of diarrhoea and respiratory disease in dairy calves.

Materials and Methods

100 Friesian (Fr), 93 Charolais x Friesian (Ch x Fr) and 30 Limousin x Friesian (Li x Fr) calves were purchased directly from marts (Spring 1998) and were approximately 21 days of age at arrival in Grange. The calves were individually penned and received an individual allowance of 25kg of milk replacer powder offered warm at 38°C by bucket during the first 42 days and had ad libitum access to a concentrate ration throughout the 56 day experimental period. Serum samples were collected on days 0 (day of arrival) and 56. Serum immunoglobulins (IgG_1,) were measured quantitatively by single radial immunodiffusion. The zinc sulphate turbidity test (ZST) was performed on all serum samples. The physiological parameters measured were: red blood cell number (RBC), haemoglobin (Hb), packed cell volume (PCV), mean cell volume (MCV), total white cell count (TWC), platelet number, % lymphocytes, blood Copper (Cu^2), glutathione peroxidase activity (GPx), alkaline phosphatase (ALP), total antioxidant activity, cholesterol, triacyglycerol, betahydroxybutyrate (BHB), non-esterified fatty acids (NEFA), high density lipoprotein and low density lipoprotein.

Haematology parameters were determined for unclotted (K_3-EDTA) whole blood samples using an electronic particle analyser

(Celltac MEK-6 1 0K). Total antioxidant status (TAS - measure of tissue damage) and alkaline phosphatase (ALP) were determined using Randox assay procedures.

Results

Total serum Ig concentration was significantly correlated with ZST units on arrival at day 0 for Ch x Fr ($r = 0.55$; $P < 0.001$), Li x Fr ($r = 0.86$; $P < 0.001$) and Fr calves ($r = 0.79$; $P < 0.001$). Fr calves had significantly lower ZST units on day 0 when compared with ZST units for Ch x Fr and Li x Fr calves (Table 4). Individual incidences of disease were determined by the requirement to treat respiratory disease alone, enteric disease alone or the combination of enteric and respiratory disease. The outbreak of respiratory disease was mainly associated with natural infections of *Pasteurella haemolytica* and *Pasteurella multocida*. The frequencies of antibiotic treatment for bovine respiratory disease are presented in Table 5. Calves with a rectal temperature of >40°C and clinical signs of respiratory disease were administered antibiotic for the treatment of clinical symptoms (defined individually for each animal). Fifty-four out of a total of 223 purchased calves remained healthy throughout the 63 day period indoors. 61 calves (27.3%) required one treatment for respiratory disease, 48 calves (21.5%) required two treatments, 33 calves (14.7%) required three treatments, while 27 calves (12%) required four or more treatments for respiratory disease.

Table 4: Serum immunoglobulin levels (mg/ml) and ZST units (Units) in mart purchased dairy calves on days 0 (day of arrival in Grange) and 56 of the study.

Dairy calves	Number	ZST Day 0	Ig Day 0	Ig Day 56
Ch x Fr	93	9.0	18.4	22.5
Li x Fr	30	9.5	20.0	24.6
Fr	100	7.3 •	21.5	18.4 •

• P < 0.01 versus Ch x Fr and Li x Fr.

Table 5 Frequency of antibiotic treatment for bovine respiratory disease in mart purchased dairy calves[1].

	Frequency of antibiotic treatment				
Breed	0	1	2	3	4 or more
Charolais x Friesian					
Number treated	15	25	20	17	16
% of Total	16	27	22	18	17
Mean ZST (day 0)	10	9	9	8	9
Limousin x Friesian					
Number treated	16	7	4	2	1
% of Total	53	23	13	7	3
Mean ZST (day 0)	10	7	11	10	11
Friesian					
Number treated	23	29	24	14	10
% of Total	23	29	24	14	10
Mean ZST (day 0)	8	7	7	9	6

[1]ZST Units are shown for calves at arrival in Grange on Day 0.

On Day 0, Ch x Fr and Li x Fr calves had significantly higher RBC counts and % haematocrit than Fr calves. NEFA and BHB levels were significantly lower in Ch x Fr and Li x Fr calves on day 0 compared with Fr calves. Li x Fr calves had significantly higher RBC counts, haematocrit, Hb levels, MCV, platelet numbers and ALP activities than Ch x Fr calves (Table 6).

There was no significant breed difference in differential white cell counts, Cu, GPx, cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), and glucose levels on day 0. On day 63 of the study, Fr calves had significantly lower RBC, HCT, Hb, TWC, MCV, platelets, and Cu levels when compared with Ch x Fr and Li x Fr calves (Table 7). Blood haematocrit and Cu levels were significantly higher in Li x Fr calves compared with Ch x Fr calves. Creatinine levels (a measure of muscle mass) and total antioxidant status were significantly higher in Ch x Fr calves compared with Fr calves.

Table 6: Physiological parameters in mart purchased dairy calves ((Charolais x Friesian (n = 93); Limousin x Friesian (n = 30) and Friesian (n = 100)), on Day 0 (Day of arrival in Grange)

Calf Breed	RBC ($X 10^6$/l)	HCT (%)	Hb (g/dl)	TWC ($X 10^3$/l)	MCV (fl)
Charolais x Fr	9.8 •	30.2 •	10.5	13.9	30.7 •
Limousin x Fr	10.9 • *	34.8 • *	11.9 *•	14.4	31.9 *
Friesian	8.2	26.9	10.2	15.1	32.7

	Granulocyte (%)	Monocyte (%)	Lymphocyte (%)	Copper (mol/l)	GPx (IU g/Hb)
Charolais x Fr	6.9	0.43	92.8	17.4	126.3
Limousin x Fr	7.9	0.56	91.6	14.9	139.3
Friesian	7.3	0.53	92.2	14.3	137.2

	NEFA (mmol/l)	BHB (mmol/l)	Creatinine (mol/l)	TAS (mmol/l)	Cholesterol (mmol/l)
Charolais x Fr	0.61 •	0.32 •	89.3 •	1.18•	2.1
Limousin x Fr	0.62 •	0.26 •	90.7	0.99	2.2
Friesian	0.83	0.47	85.2	0.55	2.2

	HDL (mmol/l)	LDL (mmol/l)	Glucose (mmol/l)	Platelets ($X10^3$/l	ALP (IU/l)
Charolais x Fr	1.6	0.33	4.3	887.3 •	557.8
Limousin x Fr	1.7	0.36	4.3	777.7 *	745.2 * •
Friesian	1.7	0.35	4.3	722.4	547.4

- P < 0.05 versus Friesian calves; * P < 0.05 versus Charolais x Friesian calves.

Table 7: Physiological parameters in mart purchased dairy calves ((Charolais x Friesian (n = 93); Limousin x Friesian (n = 30) and Friesian (n = 100)) on day 56 of the study.

Calf Breed	RBC 103/(l)	HCT (%)	Hb (g/dl)	TWC ($X 10^3$/l))	MCV (fl)
Charolais x Fr	11.3 •	31.2 •	11.4•	12.0 •	27.2•
Limousin x Fr	11.4 •	32.6 • *	11.8 •	10.3 •	26.5 •
Friesian	10.3	29.9	10.8	10.0	29.0

	Granulocyte (%)	Monocyte (%)	Lymphocyte (%)	Copper (mol/l)	GPx (IU g/Hb)
Charolais x Fr	1.7	0.62	97.7	14.5 •	131.8
Limousin x Fr	1.8	0.54	97.8	16.1 • *	128.8
Friesian	2.5	0.41	97.1	12.6	132.5

	NEFA (mmol/l)	BHB (mmol/l)	Creatinine (mol/l)	TAS (mmol/l)	Cholesterol (mmol/l)
Charolais x Fr	0.07	0.29	62.1	0.69	2.4
Limousin x Fr	0.08	0.31	64.1	0.71	2.4
Friesian	0.09	0.30	63.1	0.70	2.5

	HDL (mmol/l)	LDL ((mmol/l)	Glucose (mol/l)	Platelets ($X10^3$/l	ALP (IU/l)
Charolais x Fr	1.7	0.49	5.9	998.5•	429.3
Limousin x Fr	1.7	0.51	5.9	968.3 •	434.6
Friesian	1.7	0.53	5.9	810.3	438.4

- P < 0.05 versus Friesian; * P < 0.05 versus Charolais x Friesian.

Conclusion

It is concluded that measuring haematological and immunological parameters are important for evaluating the health status of the calf. Oxidative damage plays an important role in the pathogenesis of disease states and was significantly lower in Friesian calves on Day 0 when values were compared with Ch x Fr calves. Indicators of infection (TWC) can be evaluated and compared with a routine haematological evaluation. Collection of normal and clinical haematological data, aided by analytical methods, should permit specific haemopathological responses to be more closely defined (Earley *et al*., 1998b; 1999c).

EXPERIMENT 4. COMPARISON OF THE IMMUNE STATUS OF SINGLE SUCKLED CALVES VERSUS PURCHASED CALVES.

Introduction

Successful calf rearing depends on minimising disease outbreaks in home-bred and bought-in calves. Passive immunity in the neonatal calf depends on the acquisition of adequate maternal immunoglobulins before closure of intestinal absorption. In the present study, the interactive effects of serum immunoglobulin deficiency and disease on blood and immune characteristics of suckled calves and dairy calves were evaluated.

Materials and Methods

Continental x Beef Breed suckler calves (n = 98) born at Grange and consisted of Charolais x (n = 61), Limousin x (n = 27) and Simmental x (n = 10) breeds. Continental x Friesian (n = 111) and Friesian (n = 73) calves were purchased from auction marts and were approximately 28 days of age at arrival. The Continental x Friesian group consisted of Charolais x (n = 60), Limousin x (n = 40) and Belgian Blue x (n = 11) calves.

Purchased calves received an individual allowance of 25kg of milk replacer powder offered warm at 38°C by bucket during the first 42 days and had *ad libitum* access to a concentrate ration throughout the 56 day study period. Serum samples were collected on days 0 (day of arrival) and 56 of the study. Serum immunoglobulins (IgG_1, IgG_2, IgA and IgM) were measured quantitatively by single radial immunodiffusion. The zinc sulphate turbidity test (ZST) was performed on all serum samples. Serum haptoglobin (measure of acute phase response) was determined on completion of the study (day 56). Haptoglobin is an acute phase protein of infection that is produced by the liver in response to elevated blood tumour necrosis factor (TNF_α) concentrations. Haptoglobin also reduces lymphocyte function and therefore reduces cell mediated immunity. Individual disease episodes were determined by the requirement to treat for either enteric disease or respiratory disease.

Results

The results of the serum Ig concentrations and the zinc sulphate turbidity test are summarised in Table 8 and 9. The IgG_1, total Ig serum levels and ZST units were significantly higher in the suckled calves compared with the dairy calves. There was no outbreak of respiratory disease or enteric disease in the suckled calves or of enteric disease in the Continental x Friesian calves. Continental x Friesian calves that were subsequently treated for respiratory disease (n = 68) had significantly lower IgG_1, total Ig serum levels and ZST units on Day 0 when compared with healthy (n = 43) Continental x Friesian calves (Table 9). Of the 73 Friesian calves, 15 remained healthy, 55 were treated for respiratory disease and 2 were treated for enteric disease. On Day 0, serum IgG_1 and total Ig serum levels were significantly lower in Friesian calves that subsequently were treated for respiratory disease. Serum haptoglobin levels were significantly higher in Continental x Friesian and Friesian calves treated for respiratory disease when compared with corresponding healthy calves

Table 8: Serum immunoglobulin levels (mg/ml) and ZST (units) in suckled calves (28 days of age) and mart purchased dairy calves (28 days of age).

Calf Breed	IgG_1	IgG_2	IgA	IgM	Ig Total	ZST Units
Continental x Beef	38.9	0.65	0.07	0.93	40.6	17.4
Continental x Friesian	20.4•	0.92 *	0.06	0.87	22.3•	8.9 •
Friesian	19.0•	0.87	0.06	0.79	20.8•	10.6 •

• IgG1 ;Total Ig and ZST P < 0.01 versus Continental x Beef calves; * IgG2 P < 0.01 versus Continental x Beef calves.

Table 9: Mean serum immunoglobulin levels (mg/ml), ZST (units) and haptoglobin (g Hb/l) levels of calves treated or not treated for respiratory disease on day 56 of the study.

Calf Breed	IgG_1	Total Ig	ZST	Haptoglobin
Continental x Beef (H)	39.9	40.6	17.4	0.058
Continental x Friesian (H)	22.5	24.6	10.2	0.077
Continental x Friesian (R.D.)	19.1 •	20.8 •	8.2 ••	0.354 •••
Friesian (H)	22.3	24.0	11.7	0.075
Friesian (R.D.)	17.8 †	19.5 †	10.1	0.406 ††

Healthy (H) Respiratory disease (R.D.)

• P ≤ 0.04 IgG_1, Ig total versus Healthy Continental x Friesian;
•• P = 0.01 versus Healthy Continental x Friesian; †† P = 0.001 versus Healthy Friesian calves. ••• P= 0.001 versus Healthy Continental x Friesian;
† P ≤ 0.03 IgG_1, Ig total versus Healthy Friesian;

Conclusion

It is concluded that healthy calves had significantly higher serum immunoglobulins (IgG 1) and lower haptoglobin levels than calves treated for respiratory disease. The identification of calves with low levels of immunoglobulins should stimulate calf producers to ensure that calves receive adequate colostrum (Earley, 1 998e). The incidence of respiratory disease and enteric disease was highest in calves with low Ig levels and ZST units. Calves with low immunoglobulins (< 10 ZST Units; < 21 total Ig) are more susceptible to respiratory disease. Home-bred calves from the suckler herd had significantly higher total Ig concentrations and no incidence of respiratory disease compared to purchased calves. It is concluded that suckled calves at Grange had higher serum immunoglobulins (IgG 1), total Ig and ZST units than mart purchased dairy calves. Young dairy calves purchased from marts were more susceptible to disease than home-bred single-suckled beef calves (Earley et al., 1998d). In order to achieve high Ig values in dairy calves management practices that maximise absorption of immunoglobulins from colostrum that is low in Ig content must be implemented.

Experiment 5. Immunological profile of farm and mart purchased Friesian calves exposed to an outbreak of *Salmonella typhimurium*.

Introduction

Environmental and management stressors such as transportation to and from marts, sales experience and mixing with other bovines have been strongly implicated in the bovine respiratory disease complex. Very few studies have been conducted to ascertain how stressful adverse environments or management practices alter disease defence mechanisms in artificially reared calves. The main objective of this study was to determine the extent of association between calf origin, immunoglobulin status and the incidence of diarrhoea and respiratory disease in purchased Friesian calves.

Materials and Methods

One hundred and sixty-two Friesian male dairy calves were purchased directly from either auction marts or local farms in Autumn 96 and Spring 97 and were approximately 7 days of age at arrival in Grange. The calves were individually penned with a pen size of 1 .55m^2, floor area of 2.1 6m^2 and cubic air capacity of 7.4m3/calf and calves received an individual allowance of 25kg of milk replacer powder offered warm at 38°C by bucket during the first 42 days and had ad libitum access to a concentrate ration throughout the 56 day study period. Serum samples were collected on days 0 (day of arrival) 7, 14, 28 and 56 of the study. Serum immunoglobulins (IgG_1, IgG_2, IgA and IgM) were measured quantitatively by single radial immunodiffusion. The zinc sulphate turbidity test (ZST) was performed on all serum samples. Rectal temperatures were monitored daily.

Results

There was no significant difference in serum Ig concentrations between farm and mart purchased dairy calves on days 0, 14 and 28 (Table 10).

By day 14, IgG_2, IgA, IgM and ZST Units were significantly lower in the farm purchased calves (Autumn 1996). Mart purchased

Friesian calves (Spring 1997) by day 14 had significantly lower IgG_1, IgA, IgM, total Ig and ZST Units compared with day 0 values. IgG_2 and ZST Units were significantly higher on day 28 in mart purchased Friesian calves (Spring 1997) when compared with day 0 and day 14 values.

Of the 162 calves purchased (71 Farm calves; 91 Mart calves) 13 remained healthy (7 farm calves; 6 mart calves), 21 were treated for respiratory disease alone (6 farm calves; 1 1 mart calves), 1 5 for enteric disease (4 farm; 1 1 mart) and 113 for respiratory and enteric disease (54 farm and 59 mart calves) (Table 1 1). *Pasteurella haemolytica* was isolated from nasal swabs of calves with respiratory disease (5%).

The enteric disease was due to an outbreak of *Salmonella typhimurium* which occurred in the calf unit on both occasions (Autumn 1996 and Spring 1997) within 7 days of calf arrival. The duration of the infection varied from 5 to 14 days. Rectal temperatures varied from 39.5°C to 41.5°C at the start of the infection and remained elevated for up to 4 days (40.5 to 41.5°C) during the peak period of the infection. 32 calves died as a result of the infection. Antibiotic resistance developed rapidly throughout the calf house and it was very difficult to obtain a good response to antibiotic therapy. Efficacy of antibiotic treatment was assessed on the basis of reduction in pyrexia, improvement in clinical signs and successful recovery from symptoms.

Overall there was no significant difference in IgG_1 and total Ig serum levels between calves that survived and those that died from the *Salmonella typhimurium* infection (Table 12).

Faecal swabs were taken from each calf in the unit and submitted for bacteriological examination (to the District Veterinary Laboratory). A diagnosis of salmonellosis was based on the presence of the causative organism in faecal samples, together with characteristic clinical signs. Fluorescent antibody techniques (FAT) for respiratory viruses were negative. Enzyme-linked immunosorbent assay (ELISA) for *Mycoplasma bovis* was also negative. A routine post-mortem examination was carried out on the calf carcasses. Gross visible lesions were recorded and samples were taken for bacteriological examination. There was no significant lesions on gross examination except that the mesenteric lymph nodes were enlarged and the intestines were pale with fluid contents. *Salmonella typhimurium* was isolated from the lungs.

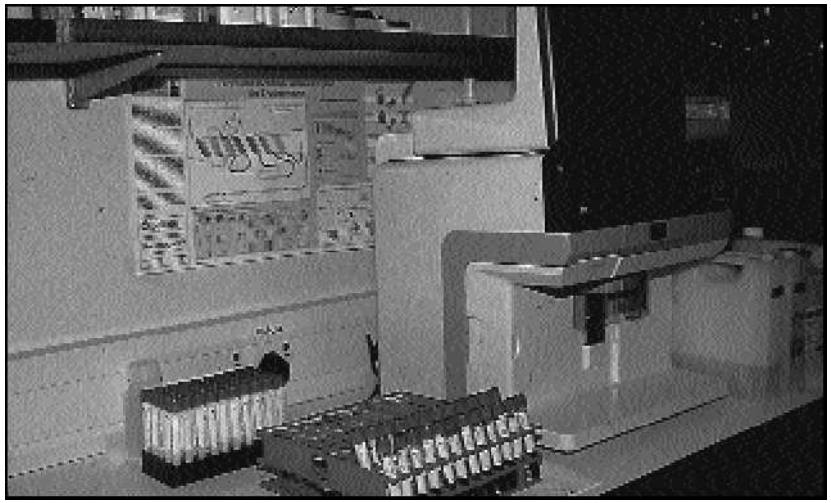
Table 10: Serum immunoglobulin levels (mg/ml) and ZST (units) in mart purchased Friesian dairy calves on days 0 (day of arrival), 14 and 28 of the study.

	IgG_1	IgG_2	IgA	IgM	Ig Total	ZST
Day 0						
Autumn 96 Farm (n = 41)	26.1	1.04	0.12	1.85	29.1	11.9
Mart (n = 41)	25.5	0.86	0.05	1.23	27.6	11.5
Spring 97 Farm (n = 30)	22.2	0.92	0.82	1.36	22.9	9.6
Mart (n = 50)	24.3	0.88	0.06	1.48	26.1	10.1
Day 14						
Autumn 96 Farm (n = 34)	24.2	0.57 *	0.03 *	0.99 *	25.8	7.8 *
Mart (n = 37)	25.1	0.99	0.04	1.75 *	27.9	8.6 *
Spring 97 Farm (n = 26)	20.6	1.01 *	0.04 *	1.77	23.2	8.6
Mart (n = 47)	20.3 *	0.84	0.03 *	1.16 *	21.8 *	8.8 *
Day 28						
Autumn 96 Farm (n = 32)	21.9	0.87 •	0.03 •	1.37	24.1	9.2 •
Mart (n = 35)	22.6	1.38 *	0.04	1.31	25.3 *	10.5 *
Spring 97 Farm (n = 24)	22.1	1.69 • *	0.07	1.09	24.9	14.2 • *
Mart (n = 46)	21.2	1.86 •	0.05	1.17 •	23.7	14.2 •

14 days ZST, IgG_1 ,IgG_2, IgA, IgM * P = 0.05 v day 0; 28 days ZST, IgG_1 IgG_2, IgA, IgM * P = 0.05 versus 14 days
• P = 0.05 versus Day 0; 28 days ZST, IgG_1 IgG_2, IgA, IgM

Table 11: Incidence (Nos.) of respiratory disease and enteric disease in farm and mart purchased Friesian calves exposed to an outbreak of Salmonella typhimurium.

	Healthy	Respiratory disease only	Enteric disease only	Enteric disease & respiratory disease
Autumn 96 Farm (n = 41)	4	4	1	32
Mart (n = 41)	4	4	1	32
Spring 97 Farm (n = 30)	3	2	3	22
Farm (n = 50)	2	11	10	27
Total	13	21	15	113



Haematology Analyser with computer software for the quantitation of 18 different blood parameters in the bovine

Conclusion

Table 12: Serum immunoglobulin levels (mg/ml) and ZST (units) in surviving and dying farm and mart purchased Friesian dairy calves on day 0 (day of arrival).

Surviving calves (Day 0)		IgG1	IgG2	IgA	IgM	Ig Total	ZST
Autumn 96 Farm	(n = 30)	27.4	1.12	0.11	1.92	30.6	12.5
Mart	(n = 36)	25.1	0.89	0.05•	1.21•	27.2	11.4
Spring 97 Farm	(n = 20)	24.2	0.86	0.06•	1.38•	26.5	10.9
Mart	(n = 44)	22.9	0.85	0.05•	1.39	25.2	10.1
Dead calves							
Autumn 96 Farm	(n = 11)	22.3•	0.81	0.14	1.67	24.9•	10.3
Mart	(n = 5)	26.9	0.53	0.10	1.60	29.1	12.1
Spring 97 Farm	(n = 10)	16.4 ••	0.84	0.11	1.29	18.5••	7.1
Mart	(n = 6)	30.3	0.92	0.12	2.02	33.4	10.3
Surviving versus dead calves							
Surviving	(n = 130)	24.8	0.92	0.06	1.46	27.2	11.1
Dead	(n = 32)	22.7	0.79	0.12 *	1.61	25.2	9.6

• P < 0.05 versus (Autumn 96) Farm calves.•• P < 0.05 versus (Spring 97) Mart calves.
* P < 0.003 versus surviving calves

There was no significant difference in serum Ig concentrations between farm and mart purchased dairy calves on days 0, 14 and 28. Calves with low immunoglobulin levels as shown in the present study are more susceptible to neonatal infections. There was a significantly higher incidence of *Salmonella typhimurium* among the farm calves compared with the mart calves and this was reflected in a higher incidence of mortality. However, it was also evident that the *Salmonella typhimurium* outbreak was of sufficient intensity to overcome the passive Ig protection acquired by the calf. In addition it is unlikely that any of the calves would have acquired Ig protection against *Salmonella typhimurium*.

EXPERIMENT 6. EFFECT OF REARING CALVES OUTDOORS WITH AND WITHOUT CALF JACKETS COMPARED WITH INDOOR HOUSING ON CALF HEALTH AND LIVEWEIGHT PERFORMANCE.

Introduction

From an animal health and welfare viewpoint it is important to develop a combination of management procedures which will minimise the adverse effects of respiratory disease on animal performance and health/welfare indicators. The working hypothesis, using controlled comparisons was that, (1) calves reared outdoor using a calf jacket (Hogbak all weather calf jacket) are less susceptible to respiratory disease than calves reared indoors and that (2) calves reared outdoors using calf jackets are more resistant to environmental challenges and disease than calves reared outdoors without jackets.

The Specific objectives were: To evaluate the effects of rearing calves outdoors using the Hogbak calf jacket on performance and immune responses with a view to developing management procedures to improve the health and welfare of calves, and to examine the effects of indoor rearing versus outdoor management practices upon immunocompetence and disease in artificially reared calves.

Materials and Methods

90 male Holstein calves were purchased directly from a dairy farm. On arrival at Grange Research Centre (day 0) the calves were weighed and allocated at random to one of the following treatments (30 calves per treatment).

(a). Jacket Outdoor
(b). No Jacket Outdoor
(c). Indoor group

Each calf received a total of 25kg of milk replacer over the 42 day replacer feeding period. All calves (indoor and outdoor groups), received a concentrate diet consisting (g/kg) of rolled barley (775), soyabean meal (200), mineral/vitamin (25) which was available ad libitum from day 1 to day 63. The indoor group of calves were turned out to pasture on day 63. Clean fresh water was available at all times. Rectal temperature and faecal swabs were taken at arrival and all calves were vaccinated with the Salmonella vaccine - Grovax. For the initial 63 day experimental period three groups of 10 calves (mean age 20 ± 2 days) were housed on straw in a naturally ventilated Monopitch calf house (4.8 x 10.0 m).

Three groups of calves (n = 10 per group) with jackets (mean age 19 (± 2 days) and three groups (n = 10 per group) without jackets (mean age 19 (± 2 days) were reared in plots outdoors (56 x 16 m). The start dates for each group were 9th March, 16th March and the 14th April 1999, respectively. Shelter was provided against adverse weather conditions, such as wind, rain using tildenet double layered fencing (1.2 m high) on three sides. A natural shelter (wooded area) was provided on one side of each plot. A flat"drylying"sanded area (8 x 5 m) and a "mound" of sand in an adjacent area (8 x 5 m) was provided at the back of each plot. The two areas of sand being separated by a wooden fence. A 1.8 m high tildenet fence surrounded this area. For the first 42 days of the experiment the calves had free access to the "flat sanded" area and to the 56x 1 6m plot. The Jackets were removed from the calves on day 42 of the study and the calves were moved to fresh paddocks (1 6m x 1 00m) at the back of the plots, from which they had access to the "mounds" .

Weather conditions including relative humidity and temperature, of the calf house and outdoor paddocks, were recorded continuously throughout the course of the study. At the commencement of the study the temperature ranged from 1.5 °C to 4.0 °C. The lowest temperature recorded outdoors was -1.7 °C on the 26th March at 6:45am and the highest was 25.9 °C on the 1st of May at

1 1:45 am. The corresponding temperatures recorded at the same time indoors were 1.5 °C and 17.7°C, respectively.

All animals were weighed on day 1 of the study and at 7 day intervals thereafter. Rectal temperature for all calves and frequency of antibiotic and electrolyte treatments were recorded daily. Calves with a rectal temperature of >40°C and clinical signs of respiratory disease (moderate to severe respiratory distress on auscultation) were administered antibiotic for the treatment of clinical symptoms (defined individually for each animal).

Calves were bled by jugular venipuncture on day 0, 7, 14, 21, 28, 42, 49, 56 and 63 of the study. Serum was collected for immunoglobulin IgG_1, measurements (sRID) and additionally for the zinc sulphate turbidity (ZST) test.

Haematology parameters using an electronic particle analyser and blood were determined for unclotted whole blood samples. Blood metabolites ((non-esterified fatty acids (NEFA), betahydroxybutyrate (BHB)) were determined on days 0, 14, 28, 42, 63 of the experiment.

Results

There was no significant difference between treatments with respect to liveweight gain (Table 13) from day of arrival (day 0) to day 63 of the study. The incidence of respiratory disease was higher in the indoor group of calves while the incidence of diarrhoea was significantly higher in the outdoor treatments, irrespective of calf jackets (Table 14). There was no significant difference in ZST units or Ig concentrations across the three treatments throughout the study period (Table 15). No significant changes in blood biochemistry were detected up to day 14 of the study. Discrete changes were detected in several biochemical variables throughout the study. By day 21 and 42 of the study, RBC and haemoglobin levels were significantly lower in the indoor group of calves when compared with the two outdoor treatments, while blood haematocrit was significantly lower in the indoor treatment (Table 1 6). There was a significant change in the WBC differential on day 35 and day 49 in the indoor group of housed calves when compared with the outdoor treatments; the % and number of granulocytes

and monocytes were significantly increased while the lymphocyte population was significantly decreased in the indoor calves (Day 35; Granulocytes % Jacket 6.1; No jacket 3.6; Indoor 14.4; Monocyte % Jacket 0.24; No jacket 0.21 and Indoors 0.7; Day 49 Granulocyte % Jacket 2.5; No jacket 3.3 Indoors 10.0; Monocyte % Jacket 0.3; No jacket 0.2 and Indoors 0.4). Changes in the distribution of the WBC differential are normally associated with an underlying disease, in this case, respiratory disease. Lymphopenia (decreases in the % lymphocytes) and changes in the % monocytes and granulocytes are associated with depressed immune responses. Monocytes tend to disappear from blood during the acute phase of disease, returning in increasing numbers as the level of infection increases. By day 63 of the study, BHB levels were significantly lower in the indoor group when compared with the two outdoor treatments.

Table 13: Liveweight (kg) and average daily gain (kg/d) of calves reared outdoors with and without jackets, and indoors.

	Outdoors		Indoors
	Jackets	No Jackets	
Initial weight	55.2	55.0	55.1
Final weight	105.7	106.0	108.2
Average daily gain			
0 - 21	0.51	0.48	0.48
22 - 42	0.75	0.83	0.97
43 -63	1.13	1.11	1.00

Table 14: Incidence and treatment of respiratory disease (RD) and enteric disease (ED) in calves reared indoors and outdoors (Jackets and no Jackets).

	Outdoors		Indoors
	Jacket	No Jacket	
Number of treatments for RD			
0	4	3	1
1	8	11	0
2	7	5	6
3	7	7	5
4 or more	4	4	18
Number of treatments for ED			
0	13	13	23
1	5	5	0
2	2	5	4
3	2	3	2
4 or more	7	4	1

Table 15: Mean serum IgG1 (mg/ml) and ZST (units) in calves artificially reared indoors and outdoors (jackets and no Jackets) from day 0 (day of arrival) to day 63 of the study.

	Outdoors		Indoor
Jacket	No Jacket		
IgG1			
Day 0	15.4	18.2	17.4
Day 21	15.6	18.8	19.0
Day 42	19.9	19.4	17.4
Day 63	25.2	21.6	21.7
ZST			
Day 0	7.4	8.1	9.0
Day 21	11.2	11.2	12.0
Day 42	13.6	13.4	14.1
Day 63	15.7	15.4	15.7

Table 16: Haematological variables of calves reared outdoors with and without jackets, and indoors.

	Outdoors		Indoors
	Jacket	No Jacket	
Total white cell counts (x 10^3/µl)			
Day 0	15.0	14.5	13.8
Day 21	13.7	12.1	12.7
Day 42	14.1	12.1	11.0
Day 63	12.9	10.9	10.7
Lymphocyte (%)			
Day 0	88	87	89
Day 21	92	94	95
Day 42	90	94	93
Day 63	97	97	96
Monocyte (%)			
Day 0	0.54	0.58	0.56
Day 21	0.28	0.39	0.36
Day 42	0.51	0.19	0.31
Day 63	0.19	0.13	0.32
RBC (x 10^6/µl)			
Day 0	9.4	10.1	9.2
Day 21	11.3	11.2	10.5•
Day 42	11.2	11.3	10.3•
Day 63	10.7	10.7	10.4
Hb (g/dl)			
Day 0	10.9	11.4	10.3
Day 21	12.1	12.0	10.9•
Day 42	11.6	11.7	10.3•
Day 63	11.0	10.9	10.5
Haematocrit (%)			
Day 0	31.4	32.8	29.6
Day 21	33.9	33.5	30.1•
Day 42	32.1	32.1	28.3•
Day 63	30.5	30.0	29.1

• P < 0.01 versus Jacket and no Jacket

Conclusion Rearing calves outdoors using calf jackets had no beneficial effect on calf performance. The incidence of respiratory disease was higher in calves reared indoors when compared with calves reared outdoors with and without jackets. There was an increased incidence of diarrhoea in calves reared outdoors irrespective of calf jacket.

EXPERIMENT 7. EFFECTS OF INCLUSION OF ORGANIC CHROMIUM IN THE CALF MILK REPLACER ON IMMUNOLOGICAL RESPONSES OF HEALTHY CALVES AND CALVES WITH RESPIRATORY DISEASE.

Introduction

Chromium (Cr) is an essential component of the glucose tolerant factor (GTF), which potentiates the action of insulin. Cr, therefore, affects insulin, which in turn influences protein synthesis and lipid metabolism. Any of these factors can in turn modify immune responses. The main physiological role of chromium (Cr) is as an integral component of the GTF. Proper Cr nutrition leads to a decreased requirement for insulin and also an improved blood lipid profile. The primary problem related to a deficiency of Cr is impaired glucose metabolism. This can lead to hyperglycaemia, increased blood cholesterol levels and an inability to deal with stress. Deficiencies of specific nutrients have been reported to reduce immune responses and increase disease susceptibility.

The main objectives of the study were: (1) to determine the effect of supplementation of the milk replacer diet with organic Cr on calf performance; (2) to determine the effect of supplemental Cr on the cell-mediated and humoral immune response of healthy calves and calves treated for respiratory disease; and (3) to determine the effect of supplementation with organic Cr on biochemical and haematological parameters in artificially reared calves.

Materials and Methods

One hundred purchased Holstein x Friesian calves, approximately 21 days of age, were used to investigate the effects of supplementation with organic Cr on mitogen induced blastogenesis of isolated peripheral blood mononuclear cells.

On arrival the calves were allocated at random to one of the following treatments (20 calves per treatment: (a) skim milk; (b) whey-based, soyabean isolate, Brand A (c) whey-based, soyabean isolate, Brand B; (d) whey-based, enzyme processed soya, Brand C; and (e) whey-based, enzyme processed soya, Brand D. Within each treatment group, 10 calves received a daily supplementation of organic Cr 250 mg/kg dry matter intake. Calves received an individual allowance of 25 kg of milk replacer, offered warm at 38°C by bucket, during the first 42 days and had *ad libitum* access to a concentrate ration throughout the 63 day study period.

On day 56, 8ml of blood was collected by jugular venipuncture into aseptic vacutainer tubes containing lithium heparin. Serum samples were collected from the dairy calves on days 0 (day of arrival), 28, 56 and 63 after arrival in Grange. Serum immunoglobulins (IgG_1) were measured quantitatively by single radial immunodiffusion (sRID) and calculated via an internal Ig-standard. The zinc sulphate turbidity (ZST) test was performed on all serum samples.

The physiological parameters measured were: red blood cell number (RBC), haemoglobin (Hb), packed cell volume (PCV), mean cell volume (MCV), total white cell (TWC) count, platelet number, % lymphocytes, blood copper (Cu^{2+}), glutathione peroxidase (GPx), alkaline phosphatase (ALP), total antioxidant activity (TAS), glucose, cholesterol, triacylglycerol (TRIG), high density lipoprotein (H DL), low density lipoprotein (LDL) and creatinine. Haematological parameters were determined using an electronic particle analyser. TAS (measure of tissue damage), glucose, cholesterol, TRIG, HDL, LDL and creatinine were determined using Randox assay procedures.

Lymphocyte cells were separated using Ficoll-Paque, diluted to a final concentration of $5x\ 10^6$ cells/ml and grown in Dulbecco's Modified Eagle medium in the presence of 50 g/ml Concanavalin A (Con-A) or phosphate buffered saline. Concanavalin A (Con-A) is thought to primarily stimulate T cells. Following culture for 5 days at 37°C in a 5% CO_2 incubator, cells were pulsed with [^{3}H] Thymidine (50 Ci/ml) and harvested 24 h later. The stimulation index (SI) was calculated for all calves. The frequency of antibiotic treatment for respiratory disease was recorded and correlated with the stimulation index for the lymphocytes.

Results

Mitogen stimulation when lymphocytes from Cr supplemented calves and non-supplemented calves were isolated and compared (Table 1 7). Chromium supplementation enhanced the cell mediated immune response. Suppression in cell mediated immune response was significantly greater in dairy calves treated for respiratory disease (Table 18).

Morbid calves had a lower blastogenic responses than healthy calves to Con-A and supplemental Cr improved these responses.

Table 17: Serum IgG1 immunoglobulin levels (mg/ml) and mitogen stimulation (SI) of lymphocytes from chromium supplemented and non-supplemented calves on day 56 of the study.

	Serum IgG1		Stimulation Index (SI)	
Milk replacer	Chromium	No Chromium	Chromium	No Chromium
Skim	16.0	19.2	34.6 *	17.1
Brand A	18.2	18.6	35.3 *	15.7
Brand B	17.3	18.6	33.1 *	16.8
Brand C	19.4	18.8	28.3 •	20.1
Brand D	20.8	17.5	34.1 *	18.9

n = 10 calves per treatment; • P < 0.03 value versus no chromium
*P < 0.001 versus no chromium

Table 18: Serum IgG1 immunoglobulin levels (mg/ml) and mitogen stimulation (SI) of lymphocytes from healthy calves and from calves treated for respiratory disease on day 56 of the study.

Frequency of antibiotic Treatment	Serum IgG1 Chromium	No Chromium	Stimulation Index (SI) Chromium	No Chromium
0	19.6 (n = 12)	16.9 (n = 11)	40.5 ▲	17.5
1	16.3 (n = 10)	18.3 (n = 19)	30.8 ▲	18.6
2	17.3 (n = 13)	17.6 (n = 11)	32.9 ▲	16.7
3	20.0 (n = 9)	18.3 (n = 5)	30.9 ○	21.9
4	18.8 (n = 6)	26.9 (n = 4)	25.6 •	11.9

• P < 0.05, ▲ P < 0.01, ○P < 0.001 versus no chromium (stimulation index (SI)).

There was no significant difference in physiological and haematological parameters on day 0. Following supplementation with organic Cr in the five calf milk replacers for 63 days, no significant changes were detected in cholesterol, glucose, TRIG, HDL, LDL, NEFA, BHB, creatinine levels. The total white cell (TWC) counts, % granulocytes and % lymphocytes were unchanged by chromium supplementation.

Conclusion

It is concluded that supplementation with organic Cr (250 mg/kg dry matter intake) for 63 days had no major effect on physiological parameters and had select effects on haematological parameters, namely, the % monocytes. The % monocytes were significantly higher in the standard commercial milk replacer (CMR) (Skim) Cr supplemented calves when compared with the whey based (CMR) + Soya Brand B, whey based CMR + Soya Brand C, whey based enzyme processed soya Brand C + Cr treatment groups. The monocytes were significantly higher (P < 0.05) in the blood from calves in the whey-based enzyme processed soya, Brand C treatment without Cr than with Cr. Lymphopenia (decreases in the % lymphocytes) and changes in the % monocytes and granulocytes are associated with depressed immune responses. Monocytes tend to disappear from blood during the acute phase of disease, returning in increasing numbers as the level of infection increases. In calves supplemented with Cr and having no incidence of respiratory disease a significantly higher blastogenic response was measured. Average daily gain, feed intake and frequency of antibiotic treatment for respiratory disease were not affected by Cr supplementation. The humoral immune response as measured by sRID of serum IgG immunglobulin was unaffected by Cr supplementation.

Lymphocytes from calves with respiratory disease manifest an impaired capability to blast *in vitro*. Cr supplementation (250 mg/kg dry matter intake) enhanced the blastogenic response in healthy calves, while, calves with respiratory had impaired blastogenic responses. Cr supplementation during calf husbandry management and/or production associated stress could improve overall immune status and health of artificially reared calves (Earley *et al.* 1 999a; 1999b; 1999d).

Summary

There was no significant difference between beef breed or parity on colostrum IgG1 concentrations in either the front or hind quarters of the udder.

Calves from Limousin x Friesian cows had higher Ig values than the other breeds at 48 hours post-birth.

High serum Ig concentrations were obtained by 48 hours post-partum in all calves but values were generally lower for the progeny of Charolais cows and heifers and Limousin x Friesian heifers than for Limousin x Friesian cows.

Problems of morbidity and mortality are major concerns in calf rearing. A greater incidence of health problems, namely, respiratory disease and diarrhoea are associated with calves having low serum immunoglobulin levels. The implications of the present findings are that compared with suckled calves, dairy calves are not receiving 1). adequate quantity of colostrum 2). adequate quality of

colostrum. 3). colostrum soon enough post birth 4). or a combination of all of the previous factors.

Healthy calves had considerably higher serum immunoglobulins (IgG_1) and lower haptoglobin levels than calves treated for respiratory disease. The higher disease incidence among calves with low levels of immunoglobulins should stimulate calf producers to ensure that calves received adequate colostrum. Calves with low immunoglobulins (< 10 ZST Units; < 21 Total Ig) are more susceptible to respiratory disease.

Low serum immunoglobulin levels affects physiological characteristics that are important in immunological defence to pathogenic challenge. Increases in total white cell counts, shifts in the % Lymphocytes and increased haptoglobin levels are indicators of infection.

Measuring haematological and immunological parameters may be indicative of health and nutritional status of the calf. Oxidative damage plays an important role in the pathogenesis of disease states and was significantly lower in Friesian calves on Day 0 when values were compared with Ch x Fr calves. Measurement of total antioxidant status may identify some risk factors in the different physiological periods in the calf, including nutritional status.

Rearing calves outdoors using calf jackets had no beneficial effect on calf performance. The incidence of respiratory disease was higher in calves reared indoors when compared with calves reared outdoors with and without jackets. There was an increased incidence of diarrhoea in calves reared outdoors irrespective of calf jacket.

Lymphocytes from calves with respiratory disease manifest an impaired capability to blast *in vitro*. Organic chromium (Cr) supplementation (250 mg/kg dry matter intake) enhanced the blastogenic response in healthy calves, while, calves with respiratory disease had impaired blastogenic responses. Supplementation with organic Cr (250 mg/kg dry matter intake) for 63 days had no major effect on physiological parameters and had select effects on haematological parameters, namely, an increase in the % monocytes.

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References

Duncan, D.B. (1955). Multiple range and multiple F-tests. Biometrics 11:1-42.

Earley, B., Fallon, R.J. and Drennan, M.J. (1 997a). An investigation of immunological and physiological events in young calves under both homeostatic and abnormal disease conditions. (Abstract) Irish Journal of Agricultural & Food Research 36:1, 124

Earley, B., McGee, M., Fallon, R.J. and Drennan, M.J. (1997b). Quantitative studies on serum immunoglobulin levels in suckled calves and dairy calves. (Abstract) Irish Journal of Agricultural & Food Research 36:1, 92

Earley, B., Fallon, R.J. and Drennan, M.J. (1 997c). An investigation of immunological and physiological events in young calves under both homeostatic and abnormal disease conditions. In: Agricultural Research Forum, UCD, 03-Apr- 1997, 271-272

Earley, B. (1 997d). Managing calf diarrhoea. Todays Farm 7:5, Jan- Feb, 26-28

Earley, B. and Fallon, R.J. (1 998a). The relationship between immunoglobulin deficiency and disease in calves. (Abstract) Irish Journal of Agricultural & Food Research 37, 1, 118-119

Earley, B. and Fallon, R.J. (1 998b). Health status, immunological and haematological profiles of dairy calves. (Abstract) Irish Journal of Agricultural & Food Research 37, 1, 118

Earley, B., Fallon, R.J. and Drennan, M.J. (1998c). Effects of quality of maternal colostrum on serum immunoglobulin levels in suckled calves. (Abstract) Irish Journal of Agricultural & Food Research 37, 1, 103-104

Earley, B. and Fallon, R.J. (1998d). Immunological status of suckled calves and mart purchased dairy calves. In: Proceedings 49th Meeting of European Association for Animal Production, Warsaw, 23-Aug-1 998, p 71

Earley, B. (1 998e). Calf pneumonia - control and prevention. Todays Farm 8, 5, 20-22.

Earley, B. and Fallon, R.J. (1 999a) Effect of organic chromium on immunological responses of healthy calves and calves with respiratory disease. In: Agricultural Research Forum, UCD, 25-26 March, 1999 (p 255-256).

Earley, B. and Fallon, R.J. (1 999b) Effect of dietary supplementation with organic chromium in the calf milk replacer on biochemical and haematological parameters in calves. In: Agricultural Research Forum, UCD, 25-26 March, 1999 (P 217-218).

Earley, B. and Fallon, R.J. (1999c) Haematological profiles of calves reared outdoors v. calves reared indoors. In: Agricultural Research Forum, UCD, 25-26 March, 1999 (P 2 19-220).

Earley, B. and Fallon, R.J. (1 999d) Inclusion of organic chromium in the calf milk replacer: Effects on immunological responses of healthy calves and calves with respiratory disease. EAAP ñ 50th Annual meeting, 22-26th Aug, Zurich 1999.

Earley, B. (1999e) Calf Health and Immunity, in Animal Health and Diagnostic Workshop, Demonstration of haematological and biochemical tests. September 9th, 1999.

Fallon, R.J., Harte F.J. and Harrington, D. 1986. The effect of calf purchase weight, serum Ig level and feeding systems on the incidence of diarrhoea, respiratory disease and mortality. Proceedings 14th World Congress on Disease of Cattle. 1: 288-281.

Fallon, R.J., Harte F.J. and Keane M.G. 1989. Methods of artificially feeding colostrum to the new-born calf. Irish Journal of Agricultural and Food Research. 26: 1-7.

Mancini, G., Carbonara, I. and Heremans, J.F. 1965. Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry, 2: 235-241.

McEwan, A.D. Fisher, E.W., Selman, I.E. and W.J. Penhale 1970. A turbidity test for the estimation of immune globulin levels in neonatal calf serum. Clinica Chemica Acta, 27; 155-163.

McGee, M., Drennan, M.J. and Caffrey, P.J. 1995. Suckler cow colostrum yield and immunoglobulin concentrations and their calvesí serum immunoglobulin concentrations (Abstract). Animal Science, 60 (Part 3): 565 and Proceedings British Society of Animal Science (BSAS),