NUTRITIONAL AND HORMONAL INFLUENCES ON IMMUNOGLOBULIN ABSORPTION BY THE PRERUMINANT NEONATE

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(ABSTRACT)

Two studies were conducted to investigate factors involved in absorption of immunoglobulins in the preruminant neonate. In the first study 26 Angus cows were fed 57% or 100% of their NRC recommended requirements for protein and energy for the last third of gestation in each of 2 years. Resulting calves were fed measured amounts of colostrum from their dam or from a cow of the reciprocal nutritional treatment group. Cows from the restricted intake treatment lost weight and body condition ($P < .05$). Gestation length, birth weight, calving ease, days open and weaning weight, however, were not affected by treatment. Calves born to

restricted fed dams had higher serum cortisol and lower serum T_3 concentrations ($P < .05$), but absorption of IgG was not affected. Calves fed colostrum from restricted cows did have lower serum IgG concentrations ($P < 0.2$); although, none of the calves were considered hypogammaglobunemic.

In the second study, 2 trials were conducted to evaluate the effect of varying cortisol concentrations on Ig absorption in lambs. Treatments consisted of control (CO), high cortisol (HC), single peak of cortisol (SP) and low cortisol (LC). Lambs in trial 1 were obtained on d 136 to 138 of gestation by caesarean operation. HC and SP lambs tended to have a faster rate of Ig absorption through 24 h, but did not differ from CO lambs in serum Ig concentration by 36 h. Precocious closure to Ig absorption had occurred for LC lambs by 20 h and they had lower Ig concentrations at 36 and 48 h ($P < 0.05$).

In trial 2, lambs were obtained on d 140 to 142 of gestation. Lambs had a shorter Ig absorptive period than trial 1 (24 vs 36 h for CO). Premature closure for the LC lambs occurred by 16 h postpartum ($P < 0.05$) and they tended to have depressed Ig concentrations post 24 h.

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Chapter I

INTRODUCTION

Significant losses occur shortly after birth with livestock. These losses adversely affect the profitability of the perspective enterprises. Thus, research leading to prevention of mortality and morbidity of newborns is warranted.

Preruminants are born with little or no circulating immunoglobulins and must rely on absorption of colostral immunoglobulins for passive immunity. However, a large percentage of calves receiving colostrum remain hypogammaglobunemic (McEwan et al., 1970; Logan and Gibson, 1975; Frerking and Aikens, 1978). This can be the result of differences in rate and extent of immunoglobulin absorption, as well as differences in time of gut closure to such absorption. Since preruminants utilize passive immunity for protection against pathogenic organism for the first few weeks of life, optimization of immunoglobulin absorption is necessary. These studies were conducted to further the understanding of the regulation and factors affecting immunoglobulin absorption. The knowledge of this absorption is necessary to assure passive immunity is maximized, and morbidity and mortality are minimized in the neonate.

Chapter II

LITERATURE REVIEW

Passive Immunity

Calves and lambs are born with no maternal immunoglobulins (Ig) due to an absence of transplacental transfer of maternal Ig (Brambell, 1970). At birth, the preruminant immune system is functional so any circulating Ig found prior to colostrum consumption are of fetal origin (Kruse, 1983). Since antibody synthesis is slow by the neonate due to the lack of B-memory cells, the newborn is unable to respond to pathogenic invasion (Hohenboken et al., 1986) and must therefore rely on passive immunity for temporary protection. Passive immunity is obtained by ingestion of colostrum and absorption of colostral Ig by the intestinal epithelium. This absorptive process is finite in duration, occurring during the first 24 to 36 h of life. Maximum Ig concentrations in neonatal blood serum are obtained at the time of cessation of absorption (Husband et al., 1972). The concentration of colostrally-derived Ig then starts to decline due to equilibration into extra-vascular spaces and catabolism of the molecules (Sasaki et

al., 1977).

Ten to 40% of the calves which receive colostrum remain hypogammaglobulinemic, and a variety of factors influence the extent of passive immunity obtained (Waggoner, 1987). The two most important variables in absorption of Ig are the amount of colostral Ig fed and the time of ingestion. Stott and Fellah (1983) observed a linear relationship between colostral Ig concentration and calf serum Ig concentration. The volume of colostrum fed was also an important factor in absorption and as volume ingested increases the efficiency of absorption is decreased. Therefore, if Ig concentration of the colostrum is low, inadequate Ig absorption occurs regardless of volume.

Some reports indicate that yield of colostrum and its Ig concentration may be affected by parity and heredity. Devery-Pocius and Larson (1983) reported colostral Ig yield increased through the 4th lactation and declined in subsequent lactations. The volume of colostrum from the first milking is negatively correlated with Ig concentration (Kruse, 1970b). Muller and Ellinger (1981) reported that Jersey cows had the highest Ig concentration followed by Ayrshires, Brown Swiss and Holsteins. Norman et al. (1981) reported heritabilities of serum IgG_1 and IgM concentrations for beef calves at 24 h of age was .52 and .30 respectively.

However, Muggli et al. (1984) calculated heritability of IgG_1 concentration to be much lower at .23.

The other major factor involved in serum Ig concentration in preruminant neonates is the relationship of colostrum feeding to time postpartum. Maximum absorption of Ig occurs if colostrum ingestion is immediately postpartum (Bush et al., 1971). Frerking and Aeikens (1978) observed a correlation between signs of diarrhoea and hypogammaglobulinemia with increasing time between birth and intake of first colostrum. Kruse (1970a) found the absorption coefficient to be reduced linearly by delaying feeding from 2 to 20 h.

Macromolecular Absorption in the Neonate

Immunoglobulins are absorbed by the intestinal epithelium through receptor mediated endocytosis and are vacuolized in the cytoplasm. These vacuoles are then transported to the baso-lateral membrane where release into the extracellular spaces by exocytosis takes place (Staley and Bush, 1985). The receptor binding of Ig appears to be highly specific and dependent on the Ig Fc fragment. It has been demonstrated that only the Fc fragments are bound to receptors while Fab and $F(ab)_2$ fragments appear to have no

binding (Borthistle et al., 1977; Wallace and Rees, 1980). The affinity for binding various classes of immunoglobulins appears to be different. In a study with neonatal mice, MacKenzie and Keeler (1984) observed that the binding affinity of IgG was higher than IgM or IgA. They also found that maximum binding was between pH 6.2 and 6.6. Rodewald (1980) reported that no binding takes place at or near pH 7.4. Therefore, at the pH for serum, receptors do not function suggesting that IgG remains bound during transit across the epithelial cell and is released at the basal cell surface (Staley and Bush, 1985).

However, non-selective absorption has also been demonstrated to occur in the bovine neonate. Young calves can absorb a variety of macromolecules including conalbumin, ovalbumin, dextran, gelatin and insulin (Deutsch and Smith, 1957; Balfour and Comline, 1959). Brandon and Lascelles (1971) found no significant difference in the absorption efficiency of IgG_1, IgG_2, IgM or IgA. Staley et al. (1972) examined the transport of rabbit antihuman IgG conjugated to ferritin and ferritin alone. The cells of the jejunum did not take up ferritin but did transport the ferritin-IgG conjugate into the apical tubular complex. However, cells of the ileum did not demonstrate the same selectivity, and both ferritin and the ferritin conjugate were taken up.

Release of ferritin from the basal vacuoles was not observed and ferritin was not detected in the peripheral blood sera. This data would suggest that nonselective uptake occurs in the preruminant but transport into the extracellular spaces appears to be regulated by the cell. Intracellular digestion by the absorbing cell appears to serve as the mechanism for regulation of macromolecular absorption (Bamford, 1966; Morris and Morris, 1977). Staley and Bush (1985) suggested that Ig attached to receptors are protected from proteolytic digestion, whereas macromolecules unbound and free in the phagolysosome are degraded. The attached, vacuolized Ig can then be transported and released into the intercellular space and enter the capillaries.

The distribution of IgG uptake along the small intestine has been shown to be different. James et al. (1979) reported that duodenum uptake of ^{125}I IgG was negligible. They also found that uptake was greatest in the jejunum after .5 h exposure and the ileum after 1.5 h exposure. Staley and Bush (1985) stated that the jejunum was the most active site of transport and that the ileal epithelial cells appear largely nonfunctional in the transport of colostral-derived Ig to circulation. Since ileal cells of neonate have a large supranuclear vacuole containing acid phosphatase, an indicator of lysosomal

enzymes, intracellular digestion activity may be higher in ileal cells suggesting reduced transport of Ig into circulation (Cornell and Padykula, 1969).

Gut Closure

Gut closure refers to the termination of transport of immunoglobulins out of intestinal absorptive cells. Clarke and Hardy (1969 a, b) defined closure from a histological standpoint in the rodent intestine. The villi on the terminal part of the small intestine have tall, columnar, highly vacuolated epithelial cells that readily uptake maternal antibodies. These cells were progressively replaced by more mature cells which were not efficient in absorption and were therefore associated with declining rates and eventual closure to macromolecular absorption. In the calf cessation of absorption of antibody occurs shortly after 24 h postpartum and between 24 and 36 h postpartum for the lamb (Lecce and Morgan, 1962; Marx and Stott, 1980). Hormonal regulation of the maturation of the gut has been proposed by Halliday (1959).

Enterocytes of the fetus do not migrate along the villi before birth. Simpson-Morgan and Smeaton (1972) reported enterocytes synthesizing DNA at birth reached the tip of

jejunal villi by about 3 days of age. Also, Moon and Joel (1975) found that replacement time of intestinal epithelial cells of the newborn lamb and calf to be "well in excess of 48 h". Since closure to absorption of immunoglobulins occurs between 24 and 36 h postpartum in the lamb and calf, cessation of absorption can only in part be explained by cell replacement.

Intracellular digestion appears to be the major reason for the closure phenomenon. Clark and Hardy (1971) observed uptake of polyvinyl pyrrolidone (PVP) occurred for 14 d postpartum in the neonatal pig; although, it was not transported into circulation. The supranuclear vacuole capable of proteolytic and mucolytic digestion develops in the epithelial cells of the jejunum but at a slower rate than the ilium (Morris and Morris, 1976). This results in digestion of Ig and failure of the Ig to be transported to the baso-lateral membrane for exocytosis.

Penhale et al. (1973), feeding pooled colostrum to calves, concluded that closure for the different classes of immunoglobulins occurred independently. Closure was reported to be 16, 22 and 27 h for IgM, IgA and IgG, respectively. However, Stott et al. (1979) were unable to substantiate these observations and found no significant difference in efficiency or closure time between IgG, IgA or

IgM.

Interaction Between Glucocorticoids and Ig Absorption

Glucocorticoids play an important role in final maturation of fetuses before parturition (Liggins, 1976) and have been implicated as having a role in intestinal epithelium absorption and gut closure to macromolecular absorption. The suggested involvement of glucocorticoids in the absorption of colostral Ig has been alluded to in early work by Halliday (1959). Administering large doses of exogenous corticosteroids (5 mg cortisone acetate) to rat pups, ranging in age from 9 to 16 d, initially increased absorption of antibodies in some cases. However, precocious closure of the intestine of nursing pups to macromolecular absorption became apparent at 24 h with complete cessation after d 2.

Daniels et al. (1972) measured corticosterone levels in rat pups during the first 28 d postpartum and related the levels to the intestinal uptake of ^{125}I-labelled PVP. Plasma corticosterone levels remained low (2 ug/100 ml) from d 5 to 18. However, concentrations rose to 15 ug/100 ml between 18 and 28 d. This rise in corticosterone levels was closely correlated with declining absorption of PVP after d

18, with closure occurring at d 21. This relationship was also observed by Hough et al. (1987) in work with mouse pups. Daniels et al. (1973) investigated the effect of administering large doses of exogenous corticosteroids (cortisone acetate) at 5 or 12 d postpartum on macromolecular absorption. Injections of 2.5 mg at d 5 or 5 mg at d 12 were shown to lower PVP uptake. The reduction in uptake was transient and returned to control levels 5 d post injection. This temporary reduction in uptake did not appear to be associated with changes in histological character of the small intestine when examined by light microscopy. Injections of 5 mg of corticosteroid at 5 or 12 d resulted in precocious closure to PVP uptake at 6 and 4 d post injection, respectively. A progressive displacement of vacuolated villi cells of the distal small intestine occurred in this time frame as would be associated with normal gut closure in the rodent.

In a similar experiment, Morris and Morris (1976) orally administered 5 mg of corticosterone or cortisone at d 12 postpartum and studied the effect on absorption of labelled IgG. A marked reduction in IgG absorption was observed 3 d following corticosterone injection with some recovery of transport function 5 d post treatment. However, 3 d after cortisone treatment, there was a noted gut closure

to labelled IgG transport. Morris and Morris (1980) studied the effects of exogenous corticosteroids on the histological changes in the gut and subsequent absorption of ^{125}I-labelled IgG. Cortisol, cortisone acetate, deoxycorticosterone acetate, corticosterone or control (vehicle alone) were injected at d 12, 13 and 14 postpartum. On d 16, ^{125}I-labelled IgG was injected into a ligated segment of the proximal small intestine and the amount of radioactivity in the vascular compartment was measured 2 h post injection. The results of treatments were significantly different, but all treatments were significantly lower in IgG absorption than control. Gut closure had taken place with injections of cortisol, cortisone acetate and deoxycorticosterone acetate, while only partial closure was observed with corticosterone. To assess the histological changes, similar treatments were administered on d 14, 15 and 16 to a different group of newborn rat pups. Pups were sacrificed on d 18 and intestinal linings were examined. Villi on the ileum lining from the control group exhibited tall, columnar, vacuolated epithelial cells. Cortisone acetate and deoxycorticosterone acetate were shown to be effective in producing replacement of these cells, while cortisol resulted in partial replacement at the time of observation. Corticosterone

treated pups had vacuolated cells comparable to those of controls.

Hough et al. (1987) designed an experiment to investigate the impact of a corticosterone surge induced by ACTH administration on the rate of IgG and IgM absorption and subsequent gut closure to such absorption in the mouse pup. Treatments of ACTH (10 IU/pup) or control (saline) were administered IM on d 10 postpartum. ACTH treated pups had an increased rate of absorption through 4 d post-treatment. However, precocious closure to absorption of IgG was induced, and no increase in IgG concentration was observed at 6 or 8 d post treatment. Control pups continued to absorb IgG through d 18 and no net difference in circulating IgG concentration was observed between treatments on d 18. ACTH treated pups had higher serum concentrations of IgM on d 12 postpartum, but closure to absorption was not induced. This study supported the hypothesis that the mechanism for absorption for various immunoglobulin classes is different.

Serum cortisol levels in the calf are high at birth (80 to 140 ng/ml), followed by a rapid decline and then a stabilization of levels (Johnston and Oxender, 1979; Stott, 1980; Nightengale and Stott, 1981; Schlagheck, 1983). Stott (1980) artificially elevated serum cortisol levels in the

bovine neonate to study the effect on Ig absorption. Treatments consisted of injecting newborn calves with ACTH or Predef (a potent synthetic glucocorticoid) as well as a no hormone control treatment. Even though ACTH significantly increased serum cortisol levels, and Predef was a synthetic glucocorticoid of high potency, no differences in Ig absorption among treatments as measured by total serum Ig concentrations were observed. The author concluded that elevated serum glucocorticoid concentrations had no effect on immunoglobulin absorption in the preruminant calf. However, in a field study by Boyd and Hogg (1981), utilizing forty-eight bull calves injected with ACTH within the first few hour postpartum, serum Ig concentrations were enhanced at 12 and 24 h postpartum. Intake in this experiment was not controlled and could serve as a potential confounding effect.

Johnston and Oxender (1979) treated newborn calves with synthetic ACTH or metyrapone (an inhibitor of cortisol synthesis) or control. A 6 h bleeding regime was utilized for sampling. Treatments were significant with regards to ACTH increasing and metyrapone decreasing serum glucocorticoids when compared to control. No significant differences in serum IgG levels could be detected due to the high within treatment variability. However, metyrapone

calves had lower mean IgG serum concentrations when compared to the similar values obtained by ACTH or control calves.

Exogenous corticosteroids have been demonstrated to be effective in inducing premature parturition in cattle and sheep, but Husband et al. (1973) questioned the use of corticosteroids to induce parturition. They treated pregnant cows with Opticortenol (dexamethasone trimethylacetate), which is slowly released and would cross the placenta and enter the fetal circulation. Calves born prematurely from treated cows had half the circulating Ig concentration of calves born from untreated cows with normal gestation periods. In a similar experiment, Muller et al. (1975) induced parturition with a more rapid acting glucocorticoid (dexamethasone). Calves were allowed to nurse naturally and were sampled at 3 d of age. No difference in Ig absorption was observed between premature calves and control calves. These results could suggest that premature calves have delayed gut closure when compared to calves from a normal gestational period.

Patt and Eberhart (1976) performed cesarean sections on sows, and treated the resulting pigs with ACTH, metyrapone or control. Treatment was effective in altering serum cortisol levels, with ACTH treated pigs having elevated and metyrapone treated pigs having depressed cortisol levels

when compared to control. Pigs were fed pooled bovine colostrum, and metyrapone treated pigs absorbed significantly lower amounts of bovine IgG. The authors concluded that maximal absorption of immunoglobulins required adequate levels of cortisol.

Bate and Hacker (1985), considered the sow's adrenal activity on the ability of the piglet to absorb bovine IgG from colostrum. Sows were treated with ACTH, metyrapone or saline solution between d 104 and 114 post breeding. Pigs were force fed bovine colostrum at 30 min, 2, 4 and 6 h postpartum and a bleeding regime of 6 h, 1, 2, 4, 8, 12, 16 and 21 d was carried out. Pigs from ACTH and metyrapone treated sows had significantly higher serum IgG levels at 6 h, with metyrapone treated pigs maintaining consistently higher IgG levels throughout the experiment.

Johnston and Stewart (1986) studied the effects of glucocorticoids and prematurity on the absorption of colostral Ig in the calf. Their treatments consisted of: 1) glucocorticoid-induced premature calving utilizing dexamethasone trimethylacetate, 2) prematurity alone via caesarean operations or 3) control, full-term calves. Control calves exhibited the fastest rate of absorption of immunoglobulins and obtained the highest serum concentrations, while calves delivered by caesarean section

exhibited the slowest rate of absorption. They concluded that prematurity is responsible for the slower uptake of colostral immunoglobulins, and the presence of glucocorticoids enhances the immunoglobulin absorption in the premature calf.

Adrenal response of the neonate to colostral feeding has also been an area of research interest. Nightengale and Stott (1981) studied the interaction between the time of first colostrum feeding and cortisol concentration. In calves subjected to delays of 12 h or more, cortisol release in response to feeding was observed. The increase in cortisol concentration was enlarged with longer periods of deprivation, up to 24 h. Schlagheck (1983) fed calves pooled colostrum, whole milk plus immunoglobulin extract or whole milk (control) at 0 or 12 h postpartum and measured cortisol concentration in response to feeding. A cortisol peak was elicited when pooled colostrum or whole milk plus immunoglobulin extract was fed at 0 or 12 h. However, feeding only whole milk resulted in depressed cortisol levels at either feeding time. The author suggested that colostral immunoglobulins acted as a "primary messenger" initiating a cortisol surge. However, when similar treatments were utilized by Waggoner (1987) a cortisol surge was not elicited by colostrum feeding. Cortisol

concentration in fact tended to decline when calves were fed colostrum or milk.

In summary, glucocorticoids are involved with the maturation of the intestinal epithelium. High levels of corticosteroids have been demonstrated to cause precocious closure of immunoglobulin absorption in the neonatal rodent. However, several studies with the rat and mouse pup have also shown increased the rate of absorption in response to elevated corticosteroid concentrations. In calves, high fetal concentrations are associated with parturition, and elevated cortisol levels postpartum do not cause precocious closure of absorption. However, if cortisol levels are depressed or the animal is premature, colostral immunoglobulin absorption is negatively affected. This indicates that relatively high cortisol levels are necessary to assure maximum immunoglobulin absorption.

Interaction Between Thyroxine and Ig Absorption

Thyroid hormones have also been implicated as having a role in the maturation of intestinal epithelium and its absorption of Ig. Chan et al. (1973) examined the effect of T_4 administration at 5 d postpartum of ^{125}I-PVP uptake and gut closure in the rat pup. T_4 treatment resulted in

precocious closure to macromolecular absorption 4 to 5 d post treatment. However, immediately preceding the T_4-induced closure the serum corticosterone concentration rose quickly as would be associated with normal closure in the pup. This suggests that the effect of the T_4 treatment was mediated by adrenal secretion.

Cabello and Levieux (1978) observed a negative correlation between the plasma thyroxine level and the length of the absorptive period. This was supported in subsequent studies (Cabello and Levieux, 1980 and 1981). However, post absorptive Ig concentration was not affected by T_4 treatment at birth. In another experiment Cabello et al. (1980) conducted a study to examine prepartum fetal T_4 concentration on Ig absorption. One mg of T_4 was injected through an intra-amniotic catheter each wk starting 30 d before the expected parturition of pregnant goats. Kids were fed 2.5% of their body weight of pooled colostrum every 4 h for 32 h postpartum. Maximal serum IgG_1 concentration was not affected by treatment, but the absorptive period was shortened by 10 h (20.7 h for T_4 treated kids and 30.7 h for control kids).

Constituents of Bovine Colostrum Involved in Ig Absorption

Colostrum may contain many compounds involved in the absorption of Ig. Hardy (1969) administered PVP in water to newborn calves which resulted in little PVP uptake. However, when a duodenal infusion of colostrum was administered 3 h later, PVP crossed the ileal epithelium and passed into the lymph almost immediately. As accelerating factors, several organic acids present in colostrum were found effective including lactate, pyruvate, formate, acetate, propionate, butyrate and isovalerate.

Histamine has been shown to increase capillary permeability and is in higher concentration in colostrum than milk (Zarkower, 1967). However, Patt et al (1972) observed no effect on Ig absorption when histamine was orally supplemented to newborn calves.

Guth (1959) detected another compound found in colostrum but not milk which may impact immunoglobulin absorption by neonatal enterocytes. This peptide was found to be a kinin and was named colostrokinin. Kinins have been demonstrated to cause vasodilation, spasmatic motility of smooth muscle, an increase in capillary permeability and increased ion transport across enterocytes. Schlagheck (1983) stated that colostrokinin was found in colostrum in a

precursor form, colostrokininogen, and was activated by the salivary enzyme kallikrein. He compared a newborn diet of whole milk containing Ig and colostrokinin with a similar control diet without colostrokinin, and found that colostrokinin increased immunoglobulin absorption. However, absorption was not enhanced if ingestion was delayed for 12 h. The author suggested that colostrokinin was involved in both the transfer of Ig into circulation and in cessation of Ig uptake.

Colostrum also contains substances which help prevent proteolysis of Ig in the intestinal lumen, thus increasing the concentration of Ig molecules available for absorption. IgG_1 is split by trypsin and at a slower rate by chymotrypsin, while IgM is a attacked by chymotrypsin only (Brock et al., 1977 a,b and 1978). Two types of trypsin inhibitor have been identified in colostrum of the cow, sow and ewe (Laskowski and Laskowski, 1957; Sandholm and Honkanen-Buzalski, 1979). The inhibitors are small glycoproteins with a molecular weight of about 12,000 to 14,000 and are resistent to digestion by pepsin (Kassell and Laskowski, 1956). The inhibitor in bovine colostrum binds and inhibits trypsin and alpha-chymotrypsin. Since the concentration of immunoglobulin available for absorption is a major factor in the extent of passive immunity, the amount

and activity of trypsin inhibitors would play an important role in assuring adequate absorption by the neonate.

The Influence of Prepartum Nutrition in the Beef Cow on Passive Immunity of the Calf

The effect of prepartum nutrition of the dam on the survivability of the neonate has been a major topic of research interest. Bull et al. (1974), in a field study, observed that protein consumption and incidence of weak calf syndrome was negatively correlated (-.74). They found that for every .1 lb decrease in the dam's protein intake below 2 lb, the incidence of weak calf syndrome increased approximately 1%. Corah et al. (1975) studied the effect of prepartum nutrition on the incidence of calf scours. They placed 43 second-calf Hereford cows on an energy deficient ration (8.4 Mcal DE/d) 100 d prior to scheduled parturition. Thirty days prior to parturition, the cows were allotted to 2 nutritional treatments. One group remained on the restricted diet while the second group was elevated to an energy level of 18.3 Mcal DE/d. The incidence of calf scours was higher for calves born to cows on the restricted diet compared to control (52% vs 33%). Also, 19% of the calves from restricted cows died between birth and weaning

due to scours, while no calves from control cows died due to scours. The authors hypothesized that maternal nutrition may have altered the gamma globulin levels in the colostrum or hindered the ability of the calf to absorb gamma globulin.

Loh et al. (1971) studied the effect of protein restriction of the rat during gestation on the development of the progeny's gut, and the gut's ability to absorb protein. Pups from restricted dams had a decreased number of differentiated villi in the jejunum. Furthermore, existing villi were shorter in length and smaller in diameter indicating that fewer cells had been produced in the associated crypt areas. This resulted in decreased protein uptake by the enterocytes of newborn pups from deprived females.

Prepartum nutrition has also been reported to have an effect on colostrum yield of cows. Logan (1978) compared cows wintered in a barn which were fed a on silage and concentrates with those wintered outside with no supplementary feed. Housed cows gave 3 times as much colostrum in the first milking as the cows wintered outside. He reported that a large proportion of the outwintered cows gave insufficient colostrum to ensure survival of their calves. Mellon and Murry (1985 a,b) observed that ewes

maintained on a low plane of nutrition for the last half of gestation yielded significantly lower colostrum volumes and lower total quantities of colostral lactose, lipid and protein. Olson et al. (1981b) studied the effect of feeding a protein and energy restricted diet to beef cows on Ig concentration of colostrum. They utilized diet combinations that were either 100 or 33% of NRC requirements for crude protein and either 100 or 72% of NRC requirements for metabolizable energy for the last 156 d of gestation. Diet treatment did not significantly affect colostral Ig concentration; although, Ig concentration tended to be higher for cows restricted in metabolizable energy.

Halliday et al. (1978) fed varying percentage levels of ME required for maintenance in each of 2 years (75.2 to 171.5% the first year and 65 to 125% the second year) to 3 breeds of beef cattle for the last 12 wk of pregnancy. The concentration of Ig in the colostrum was not affected by energy intake. Mean calf serum Ig concentration was not affected the first year but the second year calves from cows fed the 65% diet had the lowest Ig concentration in each breed. Delong et al. (1979) restricted protein levels to beef cows during the last 4 mo of gestation and were unable to demonstrate an effect on colostrum Ig concentration or 24 h calf serum Ig concentration.

In most studies, calves suckled their dams ad-libitum so intake was a potential confounding effect. Blecha et al. (1981) conducted an experiment in which calf colostral intake was constant. They fed beef heifers various levels of protein (.52 to .98 kg CP/d) for the last 100 d of gestation. Colostrum was collected from each dam for analysis of Ig concentration. Each calf was separated from its dam immediately postpartum before suckling occurred. Calves were fed 1 l of pooled, first milking colostrum from dairy cows at 1 h of age. At 8 h intervals the calves were then fed 1 l of whole milk from dairy cows. Pooled colostrum was utilized to assess the effect of prepartum protein restriction on the calf's ability to absorb Ig. Colostrum concentration of IgG_1, IgG_2 and IgM was not affected by treatment. However, the calf ability to absorb Ig was altered. The serum IgG_1 concentration of the calves was significantly higher for the higher maternal protein intakes, and serum IgG_1 concentration exhibited a positive correlation with maternal protein intake. The mean 24 h serum IgG_1 concentration for the .98 kg CP/d calves was 5.91 mg/ml while the .52 kg CP/d calves had .66 mg/ml. Despite this effect on IgG_1 absorption, no effect on IgG_2 or IgM absorption was observed. The authors suggested that there was a selective decrease in Ig absorption in calves from

dams fed the low-protein diet. In a similar experiment, Olson et al. (1981a) fed calves from cows restricted in energy and (or) protein intake, pooled dairy colostrum at 1 h postpartum followed by a whole milk diet. However, absorption of IgG_1, IgG_2 and IgM was not affected by treatment.

Burton et al. (1984) fed 26, 2 yr old Holstein heifers 66 or 115% of the NRC requirements for crude protein during the last trimester of pregnancy. Calves were removed from their dams and bottle-fed measured amounts of their dams' colostrum. The yield and Ig concentration of the colostrum were not significantly affected by treatment. However, 24 h postpartum serum IgG_1, IgG_2, IgA and IgM concentration was significantly lower for calves born to protein restricted cows. This data indicates the calf's ability to absorb Ig is negatively affected by prepartum protein restriction.

A recent study by Holland et al. (1987) did not support the hypothesis that prepartum protein restriction decreases passive immunity. Twenty-four 2 yr old beef heifers with either an identical twin or full-sib embryo were fed an adequate (approximately 100% of NRC) or restricted (approximately 60% of NRC) crude protein diet for the last trimester of gestation. Calves were fed .9 l of their dams colostrum via esophageal feeder immediately postpartum.

Calves remained separated from their dams for 14 h after birth at which time they were reunited and allowed to nurse. Colostrum yield was lower in restricted heifers (1931 vs 2683 ml), but colostral IgG_1 and IgM concentrations were higher (7157 vs 5810 mg/dl and 639 vs 511 mg/dl, respectively). Calf serum IgG_1 and IgM concentrations were higher in restricted calves at 12 h (1467 vs 1059.5 and 175 vs 125.3 mg/dl, respectively) and 24 h (1683.8 vs 1111.8 and 181.2 vs 112.8 mg/dl, respectively).

In summary, nutritional stress during gestation has been shown to negatively affect colostral yield. The colostral Ig concentration is not lowered by restricted gestational intake and is increased in some cases. Protein restriction in the rat has been shown to affect the newborn pup's intestinal epithelium resulting in abnormal development and maturation. However, studies with the ruminant have been inconclusive. In some cases protein and (or) energy intake negatively affects absorption, but in one case absorption was actually enhanced. The calf's ability to absorb Ig does appear to be affected if the maternal nutrition restriction is severe enough. The possibility of colostrum from nutritionally stressed cows having unmeasured factors affecting colostral immunoglobulin absorption in the neonate has not been investigated.

Prepartum Nutrition and Cow Productivity

Prepartum nutrition can have a significant effect on the productivity of a cow herd. In early work, Wiltbank et al. (1962) investigated the role of prepartum TDN intake on production traits in 88 Hereford cows. The cows receiving restricted energy lost weight during gestation, delivered calves with lighter birth weights and had a longer interval to first estrus and conception. Dunn et al. (1969) conducted a similar experiment in which calving difficulty was not influenced by diet. However, time to first estrus was delayed and conception rate at the first service was lower for heifers receiving a restricted energy intake prepartum.

Corah et al. (1975) conducted 2 experiments investigating gestational nutrition on cow and progeny productivity. In their first experiment, 59 Hereford heifers with known breeding dates were allotted to two nutritional planes. Test diets consisted of 65 or 100% of the NRC recommended levels for dietary energy for the last 100 days of gestation. Control cows gained weight (36.1 kg) while 65% cows lost weight (-5.8 kg). Birth weight was also lower for calves from restricted cows (28.6 vs 30.6 kg), but

gestation length and percentage of assisted births was not affected (283 vs 282 d and 28 vs 27%, respectively). However, there was a decrease in calves alive at birth from restricted cows (90 vs 97%). Weaning weight for calves nursing these restricted cows was also lowered (147.6 vs 160.6 kg), but milk production and days to first estrus was not affected (5.0 vs 4.8 kg/d and 52 vs 51 d respectively). In the second experiment, 43 cows were fed 50% of NRC recommended levels of energy from 100 to 30 d prepartum. For the last 30 d of gestation, cows either remained on the restricted diet or were elevated to 117% of the NRC recommended energy level. Cows on the restricted level lost more weight (-64.7 vs -9.9 kg) and had shorter gestation lengths (279 vs 284 d). Also, birth weight of the calves was lower (26.7 vs 34.4 kg) and calf survivability was reduced at birth and weaning (90 vs 100% and 71 vs 100%, respectively). In this experiment, milk production was negatively affected for the restricted cows (4.1 vs 5.5 kg/d) as was weaning weight of their calves (133.8 vs 145.5 kg). The days to first estrus was also longer (50 vs 42 d). This data suggests that the critical period of gestational nutrition is the last 30 d.

In a subsequent study, Bellows and Short (1978) reported similar results. Cows were fed high or low energy

diets for the last 90 d of gestation. The lower precalving energy intake significantly affected precalving body weight, precalving condition score, postpartum interval from calving to first estrus, birth weight of the calf and percentage pregnant at weaning. However, diet did not affect pelvic area, calving difficulty score or percentage, or weaning weight of the calf.

Bellow et al. (1982) compared diets of 3.6 (low) and 6.8 (high) kg TDN daily for 102 cows and heifers during the last third of gestation. This study had similar responses in that low cows had significantly lower weight gains (.05 vs .71 kg/d), precalving condition score (3.7 vs 6.7) and precalving fat thickness (1.8 vs 3.8 mm). However, gestation length, calf birth weight, dystocia score and calf weaning weight was not affected by diet. The low cows did have longer times to first estrus and had an increase in postpartum interval to conception. Thompson et al. (1983) supported these observations. Feeding either 12.9 or 18.0 Mcal ME$\cdot hd^{-1} \cdot d^{-1}$ for the last third of gestation affected body weight, and empty body fat. Birth weights, calving ease scores and weaning weights were not affected by treatment; although, they tended to be lower for the calves born to cows with the lower energy intake. In a recent study, Boyd et al. (1987) fed a moderate and high energy

diet to Angus cows for the last 50 d of gestation. They observed prepartum energy intake significantly affected birth weight and calf gains through weaning.

Warrington et al. (1985) studied the effect of energy intake on body composition of large and small mature size heifers. A cottonseed hull based diet (ME = 1.9 Mcal/kg) was fed at a rate of 1.0 or 1.5% of body weight daily from d 90 of gestation through parturition. Body composition was determined at 90 d of gestation and at 1 wk postpartum. The empty body weight for heifers fed the moderate energy intake diet decreased 68.5 kg while the high energy intake heifers gained 2 kg. Moderate heifers also lost more empty body fat (-49 vs -28.5 kg) and empty body protein was negatively affected (-6.5 vs 3.0 kg). These data indicate that repartitioning occurs during gestation in heifers, and nutrient restriction causes mobilization of maternal protein in addition to retrieval of fat. Richards et al. (1986) reported that cows with inadequate body condition (4) at calving were slower to exhibit estrus and took more days to pregnancy.

Waldman et al. (1979) studied the effect of prepartum protein restriction alone. They fed .37 kg CP/d or .96 kg CP/d, and observed that gestation length was shortened (274 vs 282 d) and calf mortality was increased by restricting

prepartum protein intake. In contrast, Holland et al (1987) reported that protein restriction during the last trimester of gestation did not affect gestation length or birth weight. However, they did observe that calves from restricted cows did tend to take longer to stand after calving (97.4 vs 66.0 min).

Prepartum nutrition has also been investigated in the ovine. Nordby et al. (1987) fed 70 or 100% of the NRC requirements from 30 d prior to breeding through parturition. Ewes on the 70% diet had lower weight gains and lambed fewer lambs. The lambs were lighter, weaker, had a higher incidence of mortality and took 18 d longer to reach slaughter weight. Mellor and Murray (1985 b) reported that lambs born from ewes that experienced nutritional restriction during gestation, had lighter birth weights, and lower glycogen and lipid body reserves.

The studies reviewed demonstrate that when maternal nutrition restriction occurs during gestation, body weight gain is negatively affected. The female mobilizes protein and fat to compensate for the decreased intake. The effect on gestation length is inconclusive, but birth weights and weaning weights tend to be lowered after prepartum undernutrition has occurred. Although the birth weight tends to be lower, the incidence of dystocia does not appear

to be affected. If body condition is insufficient at calving, reproductive performance is also hindered.

Chapter III

INFLUENCE OF NUTRITIONAL STRESS DURING LATE GESTATION ON PRODUCTION MEASURES AND PASSIVE IMMUNITY IN BEEF CATTLE.

Abstract

A 2 yr study was conducted to examine the effects of nutritional stress during the last 90 d of gestation on neonatal immunity and production measures. Treatments used in this study were: 1) control (CO), 100% of the NRC requirements for protein and energy; or 2) stressed (ST), 57% of the NRC requirements for energy and protein. All cows received a similar diet postpartum. Each yr, 26 Angus cows were grouped by age and weight-height ratio (WT:HT), and randomly allotted to treatments. Calves born to dams within each nutritional treatment group were allotted to one of two colostrum treatments: 1) colostrum from their dam, or 2) colostrum from a cow from the reciprocal nutritional treatment group. Stressed cows lost weight, had lower WT:HT and lower CS at the end of the last trimester of gestation ($P < .01$). Gestation length, birth weight, and days open were unaffected by treatment. Calves from restricted dams

had higher cortisol (33.77 vs 26.07 ng/ml) and lower T_3 (3.82 vs 4.01 ng/ml) concentrations ($P < .05$). Maternal nutrition did not affect the calf's ability to absorb immunoglobulins (19.06 vs 20.17 mg/100 ml IgG at 24 h for ST and CO, respectively) and colostrum IgG concentration (43.0 vs 39.5 mg/100 ml for ST and CO, respectively), but calves fed colostrum from restricted cows tended to have decreased absorption (17.23 vs 21.99 mg/ml IgG at 24 h). There were also no differences in calf hypogammaglobulinemia, mortality, morbidity, or weaning weight (mean 241.3 kg).
(Key Words: Cow, Nutrition, Passive Immunity, Production)

Introduction

Drought, unsatisfactory harvesting conditions or poor management can result in inadequate nutrient availability for wintering beef cows. Producers often decrease cow nutrient intake compared to normal wintering diets when faced with inadequate feed supplies. However, limiting nutrients causes a decrease in body condition, and inadequate body condition at calving can severely affect production traits such as rebreeding and calf growth (Corah et al., 1975; Bellow et al., 1982; Richards et al., 1986). Calves may also be affected having less vigor and reduced

passive immunity when precalving nutrient availability is low (Bull et al., 1974; Blecha et al., 1981; Burton, 1984). Studies with rat pups indicate that gut development may be impaired and macromolecular absorption lowered when protein is restricted for the dam during gestation (Loh et al., 1971). Yield or chemical makeup of the colostrum may also contribute to decreased passive immunity of the neonate (Logan, 1978; Olson et al., 1981). Therefore, this experiment was designed to evaluate the impact of prepartum nutrient stress on passive immunity for the calf and production traits of the beef cow.

Materials and Methods

Twenty-six Angus cows were used in each year of a two-year study. Cows were grouped yearly by age and weight-height ratio (WT:HT), and randomly allotted to one of two treatment groups. The cows ranged in age from 4 to 8 yr and were evaluated as being physically sound prior to being placed on the study. All cows had been artificially inseminated at known breeding dates and pregnancy had been checked prior to treatment allotment. Cows were placed on their respective corn silage, soybean meal diets (Table 1) at 90 d prior to the predicted calving date. Treatments

were: 1) control (CO), 100% of NRC requirements for protein and energy, or 2) stressed (ST), 57% of NRC requirements for protein and energy. Cattle were individually fed and received a free-choice mineral mix during the entire study. Feed was sampled monthly to monitor nutrient content of the diets. Dry matter intake was adjusted according to dry matter and chemical analyses. Cows were given condition scores (1 to 9, from thinnest to fattest according to the system described by Richards et al., 1986) prior to being placed on the trial and again before calving (Richards et al., 1986). Hip heights were obtained at the beginning of the study and weights were recorded every 2 wk for the duration of the study. Weight:height ratios were calculated as an objective measure of body fleshing.

Since prepartum nutrition could affect colostral quality or the ability of the calf to absorb colostral immunoglobulins, a factorial experiment was conducted. At birth and prior to suckling, calves were weighed and separated from their dams. The calves remained separated for 48 h. Treatments were implemented following jugular catheterization and an initial blood sample was obtained (0 h). Calves born to dams within each nutritional treatment group were allotted to one of two treatments according to colostral source: 1) colostrum from their dam, or 2)

colostrum from a cow from the reciprocal nutritional treatment group. Calves were fed 1 l of colostrum via an esophageal feeder at 0, 12, 24 and 36 h. Blood samples were obtained prior to feeding and at 1 h intervals thereafter for 6 h. An additional sample was obtained at 48 h postpartum before the calf was reunited with its dam. Blood samples were refrigerated, allowed to clot for 20 h and centrifuged at 2000 X g to harvest the serum. Serum and colostrum samples were stored in sealed tubes at -20 C until analysis.

Colostrum and serum were assayed for IgG by single radial immunodiffusion (sRID) based on techniques of Mancini et al. (1965) as modified by Fahey and McKelvey (1965). Samples were diluted with phosphate buffered saline (PBS), pH 7.4, to 1:20 for serum and to 1:40 for colostrum. Rabbit antibovine IgG was mixed with 1.5% agarose in PBS for serum and colostrum analyses (ICN ImmunoBiologicals). Whole colostrum was used in sRID analysis to assure maximum accuracy (Fleenor and Stott, 1981). Serum was also assayed for the concentration of cortisol and T_3 (sensitivity to .1 ug/dl for cortisol and .2 ug/dl for T_3) (Amersham Corp.). A pooled bovine serum sample was used to calculate inter- and intra-assay coefficients of variations, which were found to be 9.8% and 3.3% for cortisol, and 8.6% and 2.5% for T_3,

respectively.

Immediately postpartum, all cows were placed on a similar diet and the cows and calves from two treatments were managed together. Weaning weights of calves were measured at approximately 7 months of age and weights were adjusted for age of calf, age of dam and sex of calf.

Production data was analyzed with ANOVA procedures. A model considering the effect of treatment and year on previously defined production measures was fit. Data from serum analysis for IgG was analyzed as repeated measures with independent variables time, nutritional treatment, colostrum treatment, year and specific two- and three-way interactions among time and treatments. All analyses were conducted using the general linear model routine from the Statistical Analysis System (SAS, 1985).

Results and Discussion

The reduction in protein and energy intake by 43% caused cows on the stressed treatment to lose 22 kg (- 3.7% BW) during the last 90 d of gestation (Table 2). During this same period of time control cows gained 34.8 kg (5.7% BW). The result was a net 56.8 kg difference in weight changes for the treatment groups ($P < .01$). In conjunction

with the weight reduction, stressed cows also exhibited declines in their WT:HT ratio (- .17 kg BW/cm HT) and in condition score ($P < .01$). Warrington et al. (1985), in work with pregnant heifers, reported that nutrient restriction causes retrieval of maternal fat and mobilization of protein. Though, body composition was not measured in the experimental cows of our study, declines in WT:HT ratios and condition scores indicate a mobilization of body fat and muscle to be used as energy and protein sources.

Several studies have reported that poor prepartum nutrition decreases weight and condition of the dam and birth weight of the calf but does not alter calving difficulty (Corah et al., 1975; Bellows and Short, 1978). Bellows et al., 1982 were not able to lower birth weight when TDN was restricted. The results of the present study demonstrated similar results in that body condition was decreased, although, gestation length, birth weight and dystocia score were not affected by nutrient restriction (Table 2). There appears to be no benefit to lowering the precalving nutritional plane to lower the incidence of calving difficulty. Depressed gestational nutrient intake has been shown to decrease the survivability of the calf (Corah et al., 1975). However, no difference in calf

mortality, morbidity or vigor was observed in the present study.

Production measures evaluating reproductive performance and calf growth are shown on Table 2. Although we were able to demonstrate a treatment difference in weight, WT:HT ratios and condition scores of the cows, our data did not indicate a treatment difference for days to rebreed for the cows or weaning weights of the calves. It has been demonstrated that nutrient restriction during the last third of gestation can negatively affect reproductive performance of cows and weaning weights of calves (Corah et al., 1975; Bellows and Short, 1978; Boyd et al., 1987). Richards et al. (1986) reported that cows with inadequate body condition (< 4 condition score) at calving were slower to exhibit estrus and took more days to pregnancy. The effect of treatment in this study apparently was not severe enough to lower condition below this critical level.

The effect of prepartum maternal nutrition on the resulting progeny was also investigated. Cortisol and T_3 have been shown to be necessary for maturation of the intestinal epithelium (Halliday, 1959; Chan et al., 1973; Cabello et al., 1980; Johnston and Stewart, 1986). Serum cortisol was increased and T_3 decreased in calves born to dams with restricted nutrient intake (Table 3) suggesting

that endocrine compensation may occur with calves, in response to nutritional stress to their dams during late gestation. Carstens et al. (1987) observed lower metabolic rates in calves born to dams restricted in protein intake during the last third of gestation. This observation is consistent with the decrease in T_3 observed in the present study. Mellor and Murray (1985) reported that lambs born from ewes that experienced nutritional restriction during gestation had lower liver glycogen reserves. Though, hepatic glycogen concentration was not analyzed in the present study, it could be hypothesized that the increased cortisol concentration in calves from restricted intake dams was due to decreased liver glycogen. This endocrine response would serve to increase gluconeogenesis if glycogen reserves were inadequate.

Colostrum feeding has also been reported to affect cortisol concentration. Nightengale and Stott (1981) elicited a serum cortisol rise in response to colostral feeding by delaying ingestion for at least 12 h. Schlagheck (1983) fed calves whole milk or whole milk plus immunoglobulin extract or colostrum. Feeding whole milk only depressed serum cortisol concentration while whole milk plus immunoglobulin extract or colostrum both caused a peak in serum cortisol to occur. The author suggested that

colostral immunoglobulins acted as a "primary messenger" initiating a cortisol surge. Our study does not support the observations of Schlagheck as serum cortisol concentrations appeared to be depressed by colostrum ingestion (Figure 1). This is consistent with the findings of Waggoner et al. (1988). Furthermore, serum cortisol and T_3 concentrations were not affected in the calves by the colostrum source treatment (Table 3).

Burton (1984) observed depressed serum Ig concentrations when calves were born to dams restricted in protein intake during the last trimester. Blecha et al. (1981) fed beef calves, from dams fed either restricted or adequate crude protein diets, pooled dairy colostrum at 1 h postpartum followed by whole milk. Calves from restricted intake dams absorbed significantly less IgG_1. This suggests that prepartum nutrition developmentally affects the calf's ability to absorb immunoglobulins. This is supported by the work of Loh et al. (1971) in which rat pups from restricted intake dams had a decreased number of differentiated villi in the jejunum. The results of the present study do not support these findings (Figure 2). Thus, the calves altered cortisol and T_3 concentrations did not appear to affect absorption of Ig.

Colostral IgG concentration was not effected by nutritional treatment; although, the colostrum from stressed cows tended to have a higher concentration (Table 2). Other workers have reported similar results (Holliday et al., 1978; Blecha et al., 1981; Burton, 1984). Calves in the present study fed colostrum from ST cows had less ($P < .2$) circulating IgG (Figure 3); although, the colostrum from these cows had a 8.1% higher concentration of IgG. Despite this, none of calves could be considered hypogammaglobulinemic, and calf morbidity and mortality was not different for treatments. Our data suggests that some unmeasured factor may be involved with Ig absorption from the colostrum, and that this factor could be reduced in concentration or altogether missing thus affecting the rate and extent of Ig absorption. Several compounds whose concentration was not determined in the present study have been demonstrated to enhance Ig absorption. Hardy (1969) reported organic acids present in colostrum served as accelerating factors for immunoglobulin absorption. Colostrokinins are also present in colostrum but not whole milk. They have been implicated in enhancing macromolecular absorption (Schlagheck, 1983). It is possible that these or other constituents of colostrum affecting immunoglobulin absorption may be affected by the maternal nutritional

stress.

Several studies have demonstrated that when cows are in poor condition at calving, production is impaired. However, our data indicate that cows in at least average condition beginning the third trimester of gestation can actually lose body weight prior to calving without impairing rebreeding or calf growth. Additionally, calves born from stressed cows did not have altered immunoglobulin absorption and survivability was not affected. Calves from restricted dams also had higher cortisol and lower T_3 concentrations. Furthermore, colostral immunoglobulin concentration was not affected by treatment, but the colostrum from the stressed cows appeared to be altered in some manner that decreased the availability of the immunoglobulins for absorption into circulation by the calf. Despite this, none of the calves appeared to hypogammaglobulinemic and morbidity and mortality were not affected by colostrum source.

Table 1. Composition of Diets[a]

Item	Year 1	Year 2
Diet ingredients, %		
Corn silage	95.6	96.8
Soybean meal	4.4	3.2
Chemical composition		
Dry matter, %	34.9	37.9
Crude protein, %	9.9	9.6
Acid detergent fiber, %	24.0	21.8
Neutral detergent fiber, %	47.6	46.3
ME (Mcal/kg), estimated	2.49	2.54
Dry matter intake, % body weight		
Control	1.38	1.35
Stressed	.79	.77

[a]All items on a dry matter basis except dry matter.

Table 2. The effect of nutritional stress on cow weight, cow weight:height ratio, condition score, gestation length, birth weight, calving ease, colostrum IgG, days open and weaning weight.

Item	Treatment		SE
	Control	Stressed	
Starting wt, kg	607.9	597.0	13.0
Ending wt, kg[a]	642.7	575.0	13.1
Starting WT:HT, kg/cm	4.57	4.52	0.09
Ending WT:HT, kg/cm[a]	4.83	4.35	0.09
Starting COND score	5.3	5.1	0.2
Ending COND score[a]	5.3	4.4	0.2
Gestation length, d	280.4	280.2	0.9
Birth weight, kg	39.0	39.0	1.0
Calving ease score[b]	1.1	1.1	0.01
Colostrum IgG, mg/ml	39.5	43.0	3.6
Days open	85.8	83.4	4.5
Weaning weight, kg[c]	240.1	242.5	3.5

[a] Means differ between treatments ($P < .01$).
[b] 1 = No assistance; 2 = Easy pull; 3 = Hard pull; 4 = Mechanical assistance; 5 = Caesarean section.
[c] Adjusted for age of dam, age of calf and sex of calf.

Table 3. The effect of prepartum nutrition and colostrum source on mean serum cortisol and T_3 concentration[a] in calves.

Item	Treatment		SE
	Control	Stressed	
Prepartum Nutrition			
Cortisol[b]	26.07	33.77	.98
T_3[b]	4.01	3.82	.03
Colostrum source			
Cortisol	29.41	30.00	1.03
T_3	3.92	3.91	.06

[a] ng/ml
[b] Means differ between treatments ($P < .05$).

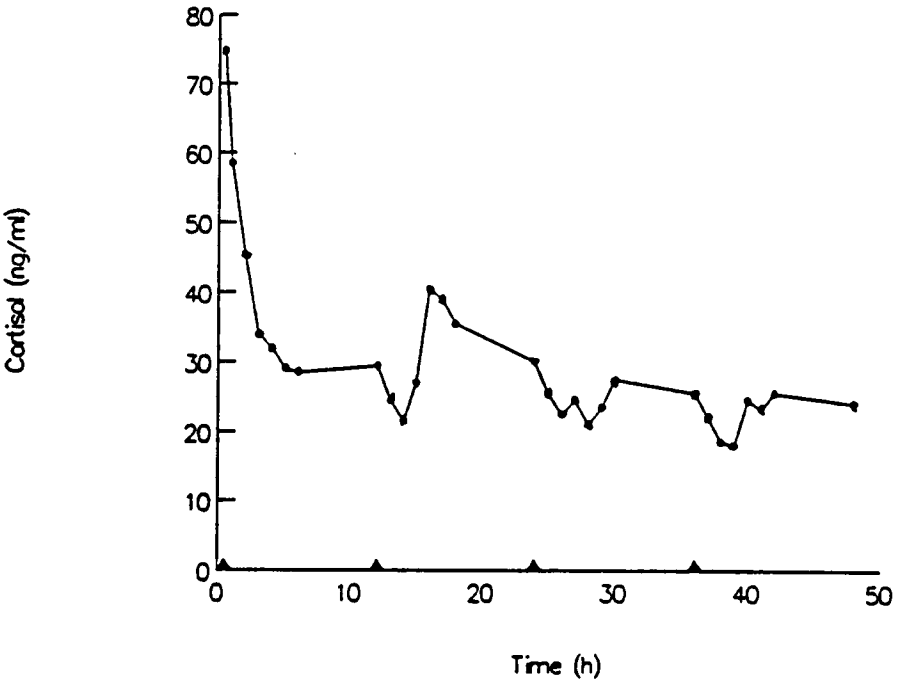

Figure 1. Serum cortisol concentration of calves fed (▲) at 0, 12, 24 and 36 h postpartum. Each point represents the mean of 44 calves. Pooled SE = 3.12.

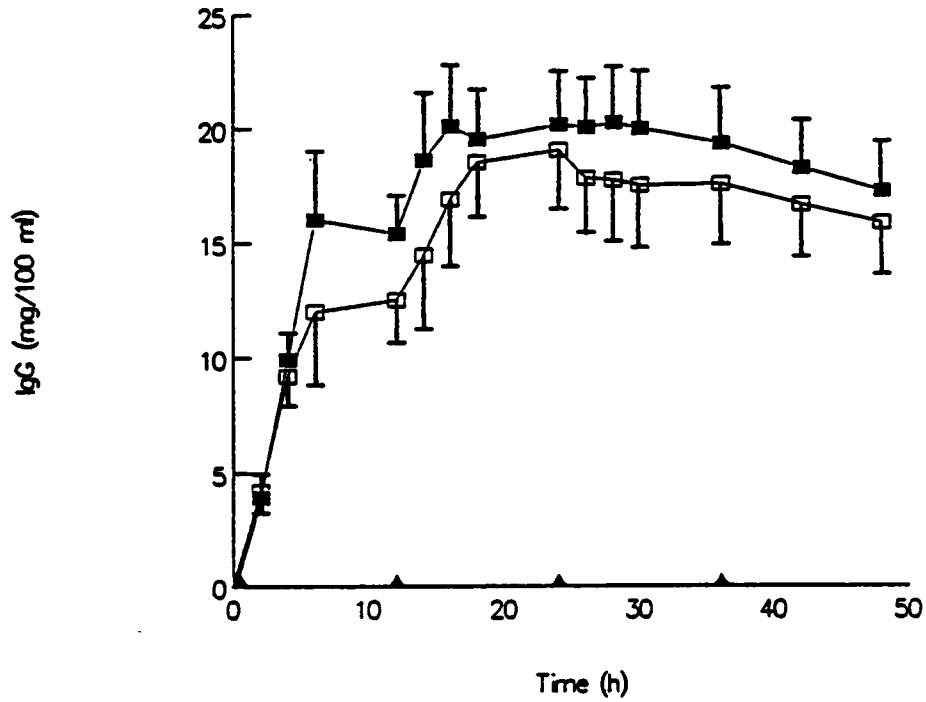

Figure 2. Mean IgG concentration for calves born to CO cows (■) or ST cows (□). Each point represents the mean ± SE for 22 calves. Time of feeding is denoted by ▲.

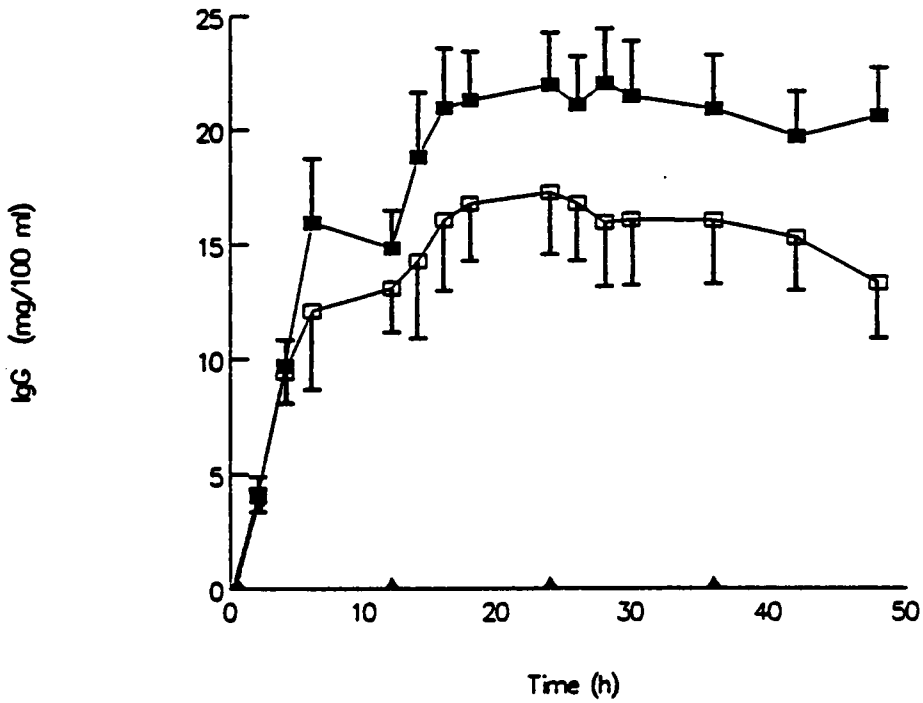

Figure 3. Serum IgG concentration for calves fed (▲) colostrum from CO cows (■) or ST cows (□). Each point represents the mean ± SE for 22 calves.

Chapter IV

INFLUENCE OF GLUCOCORTICOID ON MACROMOLECULAR ABSORPTION AND PASSIVE IMMUNITY IN NEONATAL LAMBS

Abstract

The effect of cortisol on immunoglobulin absorption and gut closure in cesarean derived neonatal lambs was evaluated in two trials. In trial 1, 21 lambs were obtained on d 136 to 138 of gestation and in trial 2, 17 lambs were obtained on d 140 to 142 of gestation. At birth, lambs were randomly assigned to 4 treatments: 1) control (CO), 1 ml saline/kg body weight (BW) every 4 h; 2) low cortisol (LC), 5 mg metyrapone/kg BW every 4 h; 3) single peak cortisol (SP), 10 IU ACTH/kg BW at 0 h; or 4) high cortisol (HC), 5 mg cortisol/kg BW every 4 h for trial 1 or 10 IU ACTH/kg BW every 4 h for trial 2. Lambs were fed an aliquot of pooled bovine colostrum every 4 h at 2 and 3.5% BW for trial 1 and 2, respectively. Compared to CO, HC increased cortisol, LC decreased cortisol and SP had elevated cortisol levels through at least 8 h for both trials. In trial 1, HC and SP exhibited elevated IgG, IgM and IgA concentrations by 20 h

compared to CO. However, no difference in immunoglobulin concentration was observed at 36 h between CO, HC and SP. Conversely, LC had the lowest immunoglobulin concentration at 36 and 48 h, and precocious closure to immunoglobulin had occurred by 20 h ($P < .05$). No treatment effects were observed in trial 2 for serum immunoglobulin concentration; although, LC tended to have depressed immunoglobulin concentrations post 24 h. Premature closure was also observed in trial 2 for LC lambs, and closure had occurred by 16 h.

(Key Words: Lamb, Cortisol, Passive Immunity, Absorption)

Introduction

Lambs are born with little serum antibody due to a lack of cross-placental transfer of immunoglobulins (Campbell et al., 1977). Thus, they must depend on colostral immunoglobulin absorption for passive immunity and the resulting temporary protection against pathogenic organisms (Hohenboken et al., 1986).

Glucocorticoids have been implicated as having a role in the intestinal epithelium absorption of immunoglobulins and gut closure to macromolecular absorption (Halliday, 1959). The majority of the research conducted has utilized

rodents. However, the rodent pup is born in a less mature state than the preruminant neonate, and gut closure in the pup does not occur until 18 to 21 d postpartum. In contrast, gut closure to immunoglobulin absorption in the lamb normally occurs between 24 and 36 h postpartum. Therefore, the objectives of these studies were to investigate in the neonatal lamb: 1) the influence of cortisol levels on absorption of colostral immunoglobulins; and 2) the interaction of cortisol with the natural maturation of intestinal epithelial cells as reflected by onset of gut closure to immunoglobulin absorption.

Materials and Methods

Two trials were conducted to evaluate the effect of circulating cortisol on immunoglobulin absorption in premature lambs. Cesarean sections were performed on ewes with known breeding dates at 136 to 138 d of gestation for trial 1 and 140 to 142 d gestation for trial 2. The cesarean section procedure was utilized to minimize the prepartum fetal glucocorticoid surge associated with parturition (Drost et al., 1973; Magyar et al., 1980). At birth, lambs were towelled vigorously, injected with doxapram hydrochloride (.1 ml/lamb) and administered oxygen

to stimulate breathing. Lambs then were weighed and ear tagged for identification.

Treatments were randomly administered in this repeated measures study following jugular catheterization of the newborn lambs and after an initial blood sample (0 h) was obtained. Trial 1 utilized 21 lambs with treatments consisting of the following: 1) control (CO), saline only; 2) low cortisol (LC), 5 mg of metyrapone/kg BW; 3) single peak cortisol (SP), 10 IU of ACTH/kg BW at 0 h only, saline at other times; 4) high cortisol (HC), 5 mg of cortisol/kg BW. Treatments were administered at 0, 4, 8, 12, 16, 20 and 24 h postpartum. Treatments LC, SP and CO were suspended in a phosphate buffered saline solution and administered via the jugular catheters. The HC treatment was suspended in corn oil and administered subcutaneously. Blood was sampled just prior to the administration of all treatments with additional samples obtained at 2, 36 and 48 h. The SP treatment was used to investigate the effect of a cortisol peak on closure of intestinal epithelial cells to macromolecular absorption. The other treatments were utilized to assess the continuous high and low concentrations of glucocorticoids on the rate of absorption of immunoglobulins and on rate of maturation of the intestinal epithelium as reflected in gut closure.

Treatments for trial 2 were administered randomly to 17 lambs and were similar to trial 1 with the exception of the HC treatment, where 10 IU ACTH/ml at each treatment time was substituted for the cortisol treatment. For trial 2, sampling and treatment times were at 0, 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44 and 48 h. Again, a 2 h sample was obtained to determine cortisol release from the SP treatment.

Lambs were fed pooled bovine colostrum via stomach tube at a rate of 2% of their body weight every 4 h for 48 h in trial 1 and 3.5% of their body weight in trial 2 [Al-Jawad and Lees, 1985; Clarkson et al., 1985]. Colostrum IgG, IgM and IgA concentrations were 3007, 153 and 167 mg/ml, respectively, for trial 1 and 2986, 139 and 186 mg/ml, respectively, for trial 2. Blood samples were obtained and treatments administered prior to each feeding.

Collected blood was refrigerated, allowed to clot for 20 h and then centrifuged at 2400 x g for 20 min for serum harvest. Serum and colostrum were analyzed for IgG by single radial immunodiffusion based on techniques of Mancini et al., [1965] as modified by Fahey and McKelvey [1965]. Analyses for IgM and IgA were performed utilizing kits [ICN ImmunoBiologicals]. Whole colostrum was utilized for analysis of immunoglobulin concentration based on the

techniques of Fleenor and Stott (1981). Calf serum was also assayed for the concentration of cortisol (sensitivity to .1 ug/dl; intra-assay coefficient of variation, 2.8%) (Amersham Corp.)

Cortisol and immunoglobulin data were analyzed as repeated measures with independent variables time, treatment and the time-treatment interaction. Gut closure was determined utilizing a Helmet transformation to test when a plateau had been reached. Analyses were conducted utilizing the general linear model routine of the Statistical Analysis System (SAS, 1985).

Results

Gestation lengths were different for both trials, so lambs were assumed to have been at slightly different stages of development. Furthermore, colostral intake, sampling regime and the HC treatment were different for the two trials, thus the results are presented as separate and distinct studies.

Trial 1. Lambs were born on the d 136 to d 138 of gestation, and appeared premature and weak at birth. Cortisol concentration was 101.8 ng/ml at birth, indicating that the stress of the cesarean operation had elevated the

levels and (or) the prepartum fetal glucocorticoid rise had begun. In either case, lambs had high cortisol concentrations at the initiation of the study. However, treatments administered to alter serum cortisol concentrations were effective (Figure 1). When compared to CO, the HC treatment maintained greater cortisol concentrations throughout the trial ($P < .05$), while SP lambs exhibited greater cortisol concentrations through 16 h postpartum ($P < .05$). Conversely, LC had lowered serum cortisol by 4 h, and cortisol continued to be lower through 24 h when compared to CO. For CO lambs, cortisol was high initially, declined to a plateau until 24 h, then declined further to a concentration similar to LC and SP at 48 h postpartum.

Manipulation of the cortisol concentration did affect immunoglobulin absorption in trial 1. Control lambs absorbed IgG through 48 h, with IgM and IgA being absorbed through 36 h. Absorption of immunoglobulins was inhibited by LC when compared to CO (Figure 2), and LC lambs did not exhibit an increase in serum IgG or IgM concentration after 20 h and after 16 h for IgA ($P < .05$). The HC treated lambs continued to absorb IgG, IgM and IgA through 36 h, 36 h and 48 h, respectively; while SP lambs absorbed IgG, IgM and IgA through 48 h, 24 h and 36 h, respectively. Lambs given the

single ACTH injection (SP) tended to have the highest IgG concentration at 16 h, while both SP and HC had the highest IgG concentration at 20 h (Figure 2a). Similar results were observed for IgM and IgA with SP lambs, where IgM and IgA concentrations tended to be greater at 16 h, while both SP and HC lambs displayed an apparent elevation in concentrations at 20 and 24 h when compared to CO (Figure 2b, 2c).

Trial 2. Lambs in this trial were obtained on d 140 to d 142 of gestation. Compared to the lambs in trial 1, cortisol levels of 124.8 ng/ml at birth were higher. The elevated cortisol concentrations most likely were the result of lambs being taken 2 to 6 d later in gestation for trial 2 compared to trial 1. Though our objective of taking the lambs cesarean section was to minimize the cortisol surge, data from both trials suggests that the glucocorticoid concentration at birth was not minimized. However, the prepartum duration of elevated cortisol would presumably be shorter for trial 1 since lambs were obtained in a more premature state (Magyar et al., 1980).

Treatments altered the serum cortisol concentrations ($P < .05$) for the lambs in trial 2 (figure 3). Lambs receiving ACTH throughout the trial (HC) had the highest cortisol concentrations overall ($P < .05$). Lambs receiving a single

injection of ACTH (SP) exhibited a cortisol peak somewhere between 2 and 4 h, with concentrations declining to levels comparable to those of CO by 12 h postpartum. The CO lambs had similar cortisol concentration patterns to CO lambs in trial 1, with cortisol declining rapidly during the first several hours then plateauing until about 28 h postpartum when an additional decline occurred to plateau again after 32 h. Cortisol concentrations for lambs on the LC treatment exhibited a rapid decline shortly after birth to plateau at approximately 65 ng/ml between 2 and 4 h postpartum. This depressed concentration was maintained throughout the sampling period.

The HC and SP treatments did not enhance IgG or IgA absorption; although, HC did tend to have higher concentrations of IgM (Figure 4). No increases in mean IgG, IgM or IgA concentration were observed after 24 h for HC and SP lambs ($p < .05$). Similar results were observed for CO lambs with no increase in IgG, IgM and IgA by 24 h, 20 h and 28 h, respectively. Conversely, immunoglobulin concentrations did not increase after 16 h for LC treated lambs and they tended to have lower IgG and IgM concentration after 30 h when compared to all other treatments.

Discussion

Lambs in trial 1 had lower concentrations of immunoglobulins than lambs trial 2. This can largely be explained by the 75% increase in colostral intake for lambs in trial 2, as well as the prematurity of the lambs in trial 1. Stott and Fellah (1983) observed a linear relationship between colostral immunoglobulin concentration ingested and serum immunoglobulin concentration. Furthermore, Johnston and Stewart (1986) observed that premature calves obtained by cesarean operation absorbed colostral immunoglobulins at a slower rate and obtained lower serum immunoglobulin concentrations when compared to calves with normal gestational periods.

Lambs administered the SP and HC treatments in trial 1 had enhanced immunoglobulin absorption initially. However, the CO lambs reached the same concentration by 36 h but at a slower rate. Hough et al., (1987) reported the rate of IgG and IgM absorption was initially enhanced by ACTH treatment in the mouse pup, but no net effect in serum concentration was observed by d 18 postpartum. Johnston and Stewart (1986) reported that calves obtained by glucocorticoid induced premature parturition had enhanced immunoglobulin absorption when compared to premature calves obtained by

caesarean operation. In trial 2, in which the lambs were 2 to 6 days longer in gestation and had higher cortisol levels at birth, treatment of lambs to elevate cortisol levels did not affect absorption of IgG or IgA. This suggests that gestation length differences between the two trials affected the intestinal enterocytes response to elevated serum cortisol concentrations. The lambs in trial 1 presumably would have been exposed to prepartum elevated cortisol concentrations for a shorter period since they were obtained in a more premature state.

When metyrapone was administered to newborn pigs (Patt and Eberhart, 1976) and calves (Johnston and Oxender, 1979) to lower serum glucocorticoid concentrations, the neonates absorbed less colostral immunoglobulins than controls. ACTH treatment to these pigs and calves resulted in similar serum Ig concentration as controls. In the present study, LC lambs tended to have lower immunoglobulin levels after 24 h; although, the effect of inhibiting cortisol synthesis on immunoglobulin absorption was more pronounced in trial 1. These data indicate that elevated cortisol levels are probably necessary prior to and during the macromolecular absorptive period.

Administration of ACTH or corticosteroids to neonatal rats has been demonstrated to induce premature cessation of

immunoglobulin absorption (Halliday, 1959). However, precocious closure has not been demonstrated in the preruminant by glucocorticoid administration (Stott, 1980). The normal duration of macromolecular absorption in lambs is 24 to 36 h (Leece and Morgan, 1962). In the present study, gut closure appeared to be induced prematurely if cortisol synthesis and release is inhibited in the neonatal lamb. The LC treatment initiated precocious closure to immunoglobulin absorption by 20 h in trial 1 and 16 h in trial 2. Conversely, high levels of cortisol in the newborn lamb does not appear to cause precocious closure to immunoglobulin absorption, as has been demonstrated in the rodent.

The duration of the absorptive period was shorter for trial 2 suggesting that gut closure may be delayed by prematurity. That HC treatment tended to increase IgM in trial 2 as well as differences in time of gut closure for different immunoglobulin classes in trial 1 suggests that regulation of immunoglobulin by class (i.e. IgG, IgM and IgA) absorption may be different. These differences possibly reflect selectivity of transport (Staley and Bush, 1985) and the molecular weight difference of the various immunoglobulins (150,000; 465,000; 950,000 for IgG, secretory IgA and IgM, respectively).

Absorption of immunoglobulins by the neonatal lamb appear to be affected by the serum cortisol concentration. The data from both trials suggest that the prepartum exposure to cortisol enhances immunoglobulin absorption. Furthermore, cortisol is necessary to obtain maximum immunoglobulin absorption and to prevent premature closure to absorption.

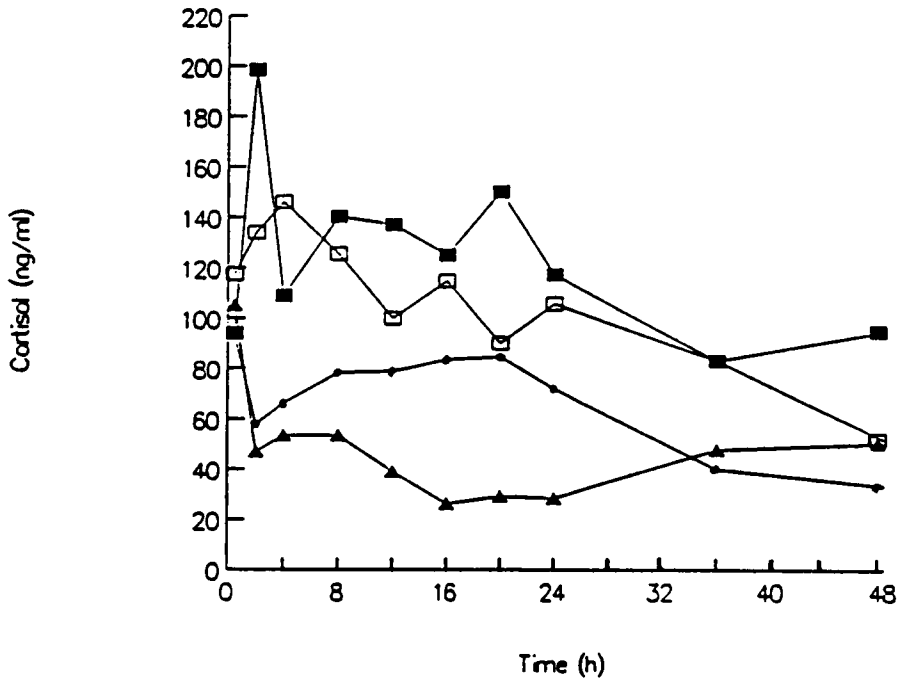

Figure 1. Serum cortisol concentration for CO (●), HC (■), SP (□) and LC (▲) treated lambs. Pooled SE = 8.51.

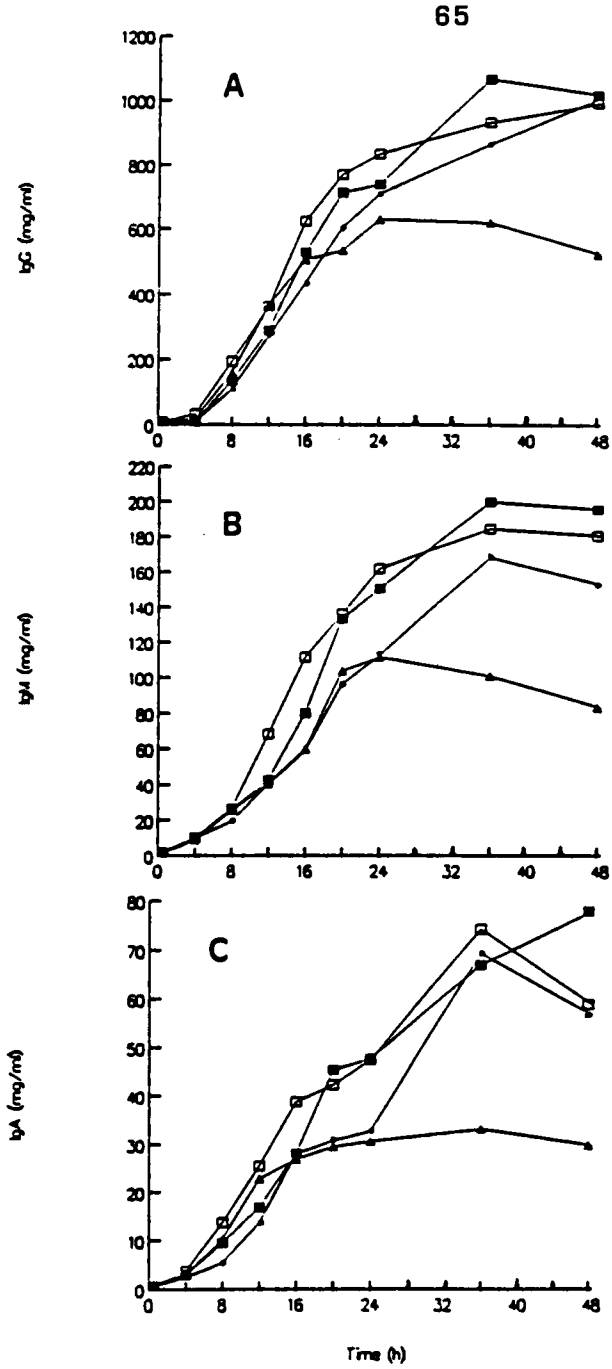

Figure 2. Serum concentration of IgG (Panel A), pooled SE = 71.4; IgM (Panel B), pooled SE = 10.4; and IgA (Panel C), pooled SE = 3.4 for CO (●), HC (■), SP (□) and LC (▲) treated lambs.

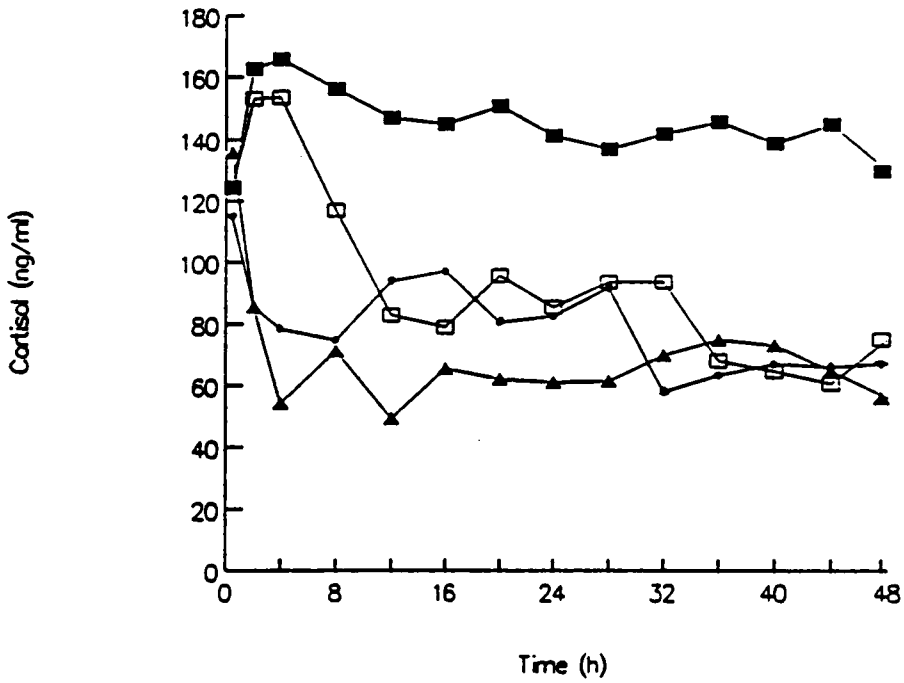

Figure 3. Serum cortisol concentration for CO (●), HC (■), SP (□) and LC (▲) treated lambs. Pooled SE = 9.4.

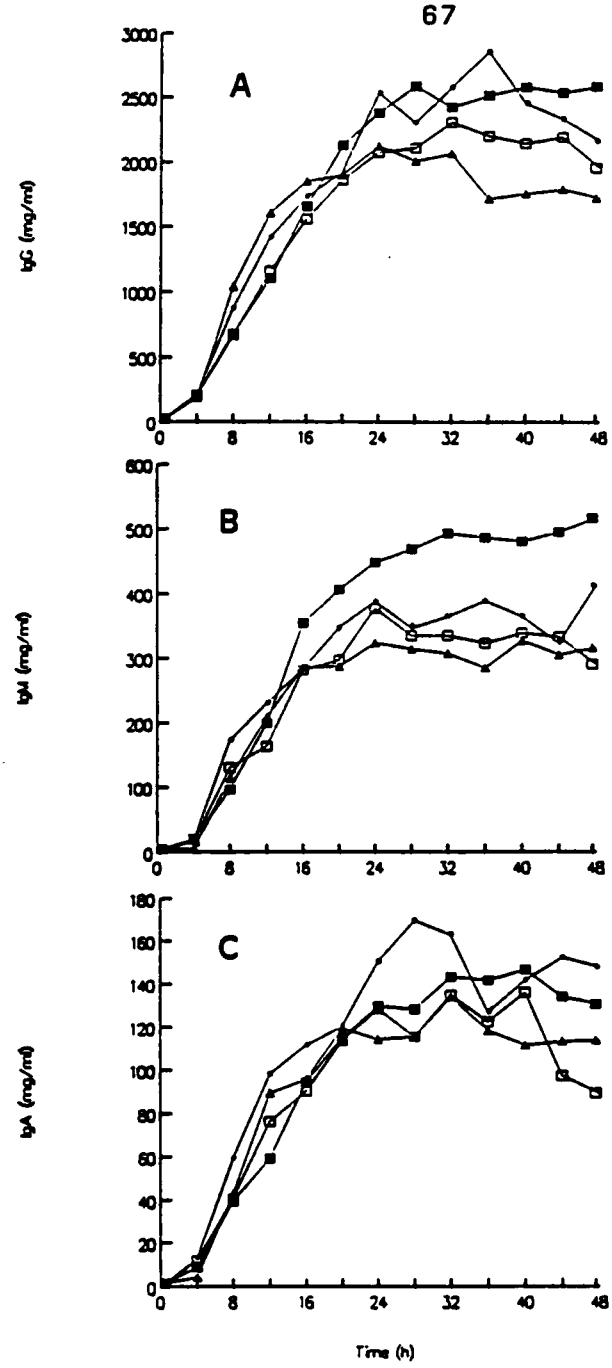

Figure 4. Serum concentration of IgG (Panel A), pooled SE = 130.7; IgM (Panel B), pooled SE = 38.2; and IgA (Panel C), pooled SE = 12.6 for CO (●), HC (■), SP (□) and LC (▲) treated lambs.

Chapter V

DISCUSSION

Neonatal survivability is important to any livestock operation. Since transplacental transfer of Ig does not occur in the ruminant, the absorption of colostral immunoglobulins is necessary for passive immunity of the newborn. The rate and extent of this uptake and transport of Ig will affect the incidence of morbidity and mortality of the neonate for the first few weeks of life. Factors affecting the absorption were studied including prepartum nutrition and endocrine regulation.

Prepartum nutrition did not affect the calf's ability to absorb IgG. However, the calves had adapted from an endocrine standpoint to the maternal nutritional stress and had higher cortisol and lower T_3 concentration during the first 48 h postpartum. These changes suggest that gluconeogenesis would be increased and metabolic rate decreased in these calves. This would be a logical response to a decrease in lipid and glycogen body reserves at birth resulting from altered in-utero nutrient availability. The effect of cortisol effect on gut maturation and absorption of macromolecules has been widely researched. However, the

elevated cortisol concentration of the calves in the present study did not result in altered absorption of IgG or gut closure to such absorption. Birth weight also was not altered by treatments. This indicates that although endocrine adaptation had occurred, growth and development was not changed.

The dam's colostrum appeared to be changed by nutritional stress despite there being no difference in colostral IgG concentration. Calves fed colostrum from restricted intake dams absorbed less of the available colostral immunoglobulins. Therefore, some factor of the colostrum was changed other than Ig concentration. The observation of Schlagheck (1983) that colostral Ig was a "primary messenger" for cortisol release would be an explanation since cortisol is an important factor for absorption. However, this study did not find colostral Ig acting as a messenger for cortisol release, and colostrum source did not affect cortisol concentration. That cortisol concentration decreased when colostrum was ingested would be a logical endocrine response to decrease gluconeogenesis in a fed state. The possibility of other factors being changed in the colostrum exists. Organic acids have been shown to be accelerating factors for macromolecular absorption. Presumably, their mode of action is by serving as an energy

supply to the enterocytes necessary for the active transport of the Ig. The organic acid concentration of the colostrum being decreased by restricted prepartum nutrient intake would be a plausible explanation for the decreased absorption by the calves.

In the studies conducted on the neonatal lambs the role of cortisol in macromolecular absorption was investigated. High levels of cortisol in the premature lamb served as an accelerating factor for Ig absorption. Conversely, if cortisol synthesis was inhibited, premature closure of Ig absorption occurred. Research with the rodent has demonstrated that high levels of cortisol after 10 d postpartum results in precocious closure to absorption. However, the pup is born in a much more premature state than the preruminant. Since cortisol is elevated at parturition in the preruminant, it could be hypothesized that the maturation process has already been initiated at birth. This process is finite in length and thus would not be further enhanced by additional cortisol elevation. This is supported by the observation that lambs born prematurely (136 to 138 d gestation) had a longer absorptive period than lambs obtained in a more mature state (140 to 142 d gestation). The premature lambs presumably would have been obtained before high fetal cortisol concentrations

associated with parturition had occurred.

The lambs with the lower cortisol concentrations having premature closure to Ig absorption is less clearly understood. It could be hypothesized that cortisol exerts an action on the regulation of the Ig receptors. Low cortisol lambs might have had a lower number of Ig receptors which were exhausted at an earlier time. Therefore, non-specific uptake and cellular digestion of Ig would have been occurring after the time closure was observed. This hypothesis is supported by the observation that Ig absorption did not differ from control in the times before closure.

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Appendix A

IgG DETERMINATION

Preparation of Gel Plates. One and four tenths g of agarose powder[1] was added to 200 ml of phosphate buffered saline (PBS)[2] in a 1000 ml Pyrex flask. The agarose solution was placed in a 90 C water bath and continually stirred via a magnetic stirrer until the agarose powder was completely dissolved. Aliquots (11.5 ml) of the dissolved agarose solution (1.5%) were pipetted into 20 ml test tubes containing 335 ul of rabbit anti-bovine IgG_1[1]. The tube was then vortexed and suspended in a 56 C water bath until the solution had cooled to this temperature. The mixture was then poured onto a 10 X 9 cm plate of glass. The glass plate was set atop a leveling table[3] that was kept warm by a hot plate that had been placed underneath the leveling table. After the agarose solution had been poured onto the glass plate, the heat source was removed and the gel allowed to solidify. Wells (3 mm in diameter) were then punched into the agarose gel utilizing a tubular cutter and a

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[1] ICN ImmunoBiologicals, Lisle, IL 60532.

[2] 0.25 M (7.4 g) NaCl, 0.02 M (1.8 g) NaH_2PO_4, 0.372 g EDTA, 0.5 g NaN_3, filled to volume (1 L) with deionized H_2O; final conc. 0.01 M, pH 7.4.

[3] Bio-Rad Laboratories, Richmond, CA 94804.

plastic template. To remove the gel cylinders after cutting, vacuum was applied to the same tubular cutter.

Preparation of Standards. Standards were prepared by reconstituting 100 mg of bovine IgG_1 with 2 ml of phosphate buffered saline. This was diluted 20 fold and stored in 200 ul aliquots in sealed tubes at -20 C. For each assay a tube was thawed and serially diluted to obtain standards.

Inoculation of Gel Plates. To each well was added 3 ul of a standard, colostrum or serum sample. The serum samples were first diluted 20 fold and colostrum samples were diluted 40 fold. Pipetting samples into the wells was performed using a capillary tube pipettor[1]. After wells were filled, plates were placed in a humidifying chamber and incubated for 36 h at 4 C. After 36 h the plates were placed on a colony counter[2] and the diameter of the opaque precipitin rings were measured using a calibrated ruler.

Calculations. Linear regression analysis was used to establish unknown concentration by regressing the

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[1]Helena Labs, Beaumont, TX 77704.

[2]American Optical Corp., Buffalo, NY 14215.

concentration of standards (50, 25, 12.5, 6.25 and 3.13; or 100, 50, 25, 12.5 and 6.25 mg/ml for serum and colostrum analysis, respectively) against the log transformed precipitin ring diameters of the standards. Concentration of unknowns were then determined when their log transformed ring diameters were fitted into the linear regression equation.

Appendix B

IgM AND IgA DETERMINATION

Procedure. Standards[12], serum and whole colostrum aliquats were pipetted (10 ul) onto immunodiffusion plates[12]. Four hundred ul of H_2O was then pipetted into a moisture trough bordering each plate. The plates were then covered and allowed to incubate for 28 h at room temperature. After the incubation period, the plates were placed on a colony counter[3] and the diameter of the opaque precipitan rings were meesured using a calibrated ruler.

Calculations. A prediction equation was used to establish unknown IgM and IgA concentration. The prediction equation was derived by linearly regressing the concentration of standards against the log transformed precipitin ring diameters of the standards. Concentration of unknowns were then determined when their log transformed ring diameters were entered into the prediciton equation.

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[1]Bovine IgM Quantitative Immunodiffusion Kit. ICN ImmunoBiologicals, Lisle, IL 60532.

[2]Bovine IgA Quantitative Immunodiffusion Kit, ICN ImmunoBiologicals, Lisle, IL 60532.

[3]Beckman model 5500

Appendix C

SERUM CORTISOL QUANTITATION

Procedure. Standards[1] and serum samples were pipetted (25 ul) into 12 x 75 mm borosilicate tubes[2] in duplicate. Cortisol ^{125}I derivate[1] (100 ul) was added to the tubes using a hamilton repeating syringe[2]. Again utilizing the hamilton repeating syringe, a cortisol antibody suspension[1] (100 ul) was added to each tube. Samples were then mixed thoroughly with a vortex mixer[2]. They were then covered with a plastic film and incubated for 1 h in a 37 C waterbath. After incubation tubes were centrifuged for 25 min at 2000 Xg. Tubes were then carefully placed into decanting racks and the supernatant was poured off. The tubes were maintained in an inverted position and allowed to decant on absorbant paper for at least 2 h. After decanting, the tubes were reinverted and placed in a gamma counter[3] and read.

Total count tubes were also read in the gamma counter. These tubes contained 100 ul) of the ^{125}I cortisol derivative.

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[1]Amersham Cortisol RIA Kit, Amersham Corp., Arlington Heights, IL 60005.

[2]Fisher Scientific, Raleigh, NC 27604.

[3]Beckman model 5500.

Calculations. Calculations were computed by the stored computer program within the Beckman gamma counter. The computer utilized a log-logit plot analysis to determine the unknown sample concentrations:

% Bound = B/Bo x 100

B = Sample counts

Bo = Average zero standard counts

Appendix D

SERUM T_3 QUANTITATION

Procedure. Standards[1] and serum samples were pipetted (25 ul) into 12 x 75 mm borosilicate tubes[2] in duplicate. T_3 ^{125}I derivative[1] (200 ul) then T_3 antibody suspension (200 ul) were added to the tubes using a hamilton repeating syringe[2]. Samples were then mixed thoroughly with a vortex mixer[2]. Tubes were then covered with a plastic film and samples were incubated for 1 h in a 37 C waterbath. After incubation, samples were centrifuged for 25 min at 2000 X g. Tubes were then carefully placed into decanting racks and the supernatant was poured off. The tubes were maintained in an inverted position and allowed to decant on absorbant paper for at least 2 h. After decanting, the tubes were reinverted and placed in a gamma counter[3] and read.

Total count tubes were also read in the gamma counter. These tubes contained 200 ul of the T_3 ^{125}I derivative.

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[1]Amersham T_3 RIA Kit, Amersham Corp., Arlington Heights, IL 60005.

[2]Fisher Scientific, Raleigh, NC 27604.

[3]Beckman model 5500.

Calculations. Calculation were computed by the stored computer program within the Beckman gamma counter. The computer utilized a log-logit plot analysis to determine the unknown sample concentration:

% Bound = B/Bo x 100

B = Sample counts

Bo = Average zero standards counts.

Appendix E

CALF CATHETER PREPARATIONS AND PROCEDURES

Catheters were inserted into the jugular vein of the calves. The neck was first sheared and scrubbed, and a 12 gauge needle was inserted into the jugular vein. Approximately 12 cm of a 25 cm segment of Tygon microbore tubing[1] was then introduced into the vein through the needle. The needle was then removed, and an adapter and stopper was attached to the tubing. The tube was then flushed with a 3.5% sodium citrate[2] solution (0.5 ml). The catheter was the sutured to the skin at the point of entry. This was performed by folding a 5 cm piece of cloth tape around the tube so that the tube was located in the center. The tape was the sutured to the skin and 0.2% nitrofurazone[3] was applied liberally. The necks of the calves were then wrapped with an elastic, adhesive bandage[4] and a gauze pocket was formed for storage of the stopper, adapter segment of the catheter between samples. Catheters were

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[1]Fisher Scientific, Raleigh, NC 27604.

[2]35 g Sodium Citrate (Fisher Scientific)/l deionized H_2O; Autoclaved.

[3]Clay-Parks Labs, Bronx, NY 10469.

[4]Elastikon, Johnson and Johnson, Inc., New Brunswick, NJ 08903.

maintained by flushing with 0.5 and 3.5% sodium citrate solution after each blood sample.

Appendix F

TABLE F.1. Least-square means of serum IgG concentrations (mg/ml) for calves born to cows fed 100 or 57% of their NRC requirements for energy and protein for the last 90 d of gestation.

Hours Postpartum	Treatment		
	Control	Stressed	SE
0	0.03	0.08	0.02
2	3.92	4.20	0.72
4	9.93	9.20	1.22
6	16.02	12.00	3.08
12	15.41	12.50	1.76
14	18.64	14.45	3.18
16	20.10	16.92	2.83
18	19.53	18.55	2.28
24	20.17	19.06	2.45
26	20.04	17.83	2.28
28	20.25	17.74	2.58
30	19.99	17.53	2.62
36	19.33	17.59	2.56
42	18.26	16.67	2.16
48	18.20	16.66	2.22

TABLE F.2. Least-square means of serum IgG concentrations (mg/ml) for calves fed colostrum from cows fed 100 or 57% of their NRC requirements for energy and protein for the last 90 d of gestation.

Hours Postpartum	Treatment		SE
	Control	Stressed	
0	0.04	0.07	0.02
2	4.01	4.11	0.71
4	9.72	9.42	1.24
6	15.95	12.07	3.11
12	14.84	13.07	1.78
14	18.81	14.28	3.04
16	20.96	16.06	2.81
18	21.31	16.77	2.26
24	21.98	17.23	2.46
26	21.11	16.75	2.25
28	22.05	15.95	2.63
30	21.47	16.06	2.62
36	20.89	16.03	2.61
42	19.67	15.26	2.61
48	19.17	14.00	2.24

TABLE F.3. Least-square means of serum cortisol concentrations (ng/ml) for calves born to cows fed 100 or 57% of their NRC requirements for energy and protein for the last 90 d of gestation.

Hours Postpartum	Treatment		
	Control	Stressed	SE
0*	70.77	78.77	8.00
1	49.25	67.95	6.62
2	39.58	51.06	6.24
3	29.45	39.18	4.60
4	28.36	36.31	4.35
5	27.22	31.45	3.37
6	24.03	33.35	3.48
12	29.17	30.13	3.73
13	24.50	25.12	2.47
14	18.79	25.81	4.16
15	22.91	32.22	3.98
16	37.46	44.70	5.84
17	31.45	47.11	6.18
18	30.91	41.57	4.55
24	27.18	33.69	3.81
25**	20.98	30.55	4.05
26**	15.12	30.37	4.09
27	18.13	31.39	3.67
28	18.04	24.17	3.24
29*	19.21	28.14	4.29
30	19.93	35.21	4.48
36**	21.49	30.71	3.91
37	16.76	28.17	3.00
38	14.99	23.52	3.84
39	14.65	22.41	3.85
40*	20.21	29.73	4.55
41	17.55	29.49	4.11
42	20.84	30.37	4.09
48	21.93	26.37	3.62

*Means within the same row differ ($P < 0.05$).
**Means within the same row differ ($P < 0.01$).

TABLE F.4. Least-square means of serum cortisol concentrations (ng/ml) for calves fed colostrum from cows fed 100 or 57% of their NRC requirements for energy and protein for the last 90 d of gestation.

Hours Postpartum	Treatment		SE
	Control	Stressed	
0	72.88	76.67	8.01
1	57.15	60.05	6.68
2	41.24	49.39	6.27
3	32.69	35.93	4.61
4	33.30	31.37	4.37
5	28.40	30.27	3.41
6	29.90	27.49	3.45
12	29.84	29.45	3.74
13	25.57	24.05	2.37
14	20.37	24.22	3.95
15	24.70	30.44	3.99
16	42.27	39.90	5.84
17	40.21	38.35	6.21
18	35.01	37.48	4.56
24	27.88	32.99	3.84
25*	21.22	30.31	4.06
26	21.33	24.17	4.09
27	22.62	26.91	3.67
28	22.27	19.93	3.28
29	22.14	25.22	4.30
30	26.96	28.18	4.49
36*	22.05	30.16	3.93
37	18.09	26.83	2.99
38	14.87	23.63	3.85
39*	15.69	21.63	3.91
40	17.93	32.00	4.57
41	20.84	26.20	4.13
42	21.31	29.90	4.12
48	21.20	27.12	3.66

*Means within the same row differ ($P < 0.05$).

TABLE F.5. Least-square means of serum T_3 concentrations (ng/ml) for calves born to cows fed 100 or 57% of their NRC requirements for energy and protein for the last 90 d of gestation.

Hours Postpartum	Treatment		
	Control	Stressed	SE
0	3.24	3.51	0.36
1	4.57	4.37	0.27
2	4.82	4.86	0.32
3	5.17	5.01	0.31
4	4.90	4.63	0.31
5	4.91	4.73	0.28
6	4.73	4.56	0.30
12	4.48	4.01	0.29
24	3.80	3.70	0.27
36	3.37	3.23	0.28
48	2.97	2.84	0.22

TABLE F.6. Least-square means of serum T_3 concentrations (ng/ml) for calves fed colostrum from cows fed 100 or 57% of their NRC requirements for energy and protein for the last 90 d of gestation.

Hours Postpartum	Treatment		SE
	Control	Stressed	
0	3.80	3.38	0.38
1	4.39	4.55	0.28
2	4.79	4.90	0.33
3	5.00	5.18	0.31
4	4.76	4.77	0.31
5	4.65	4.98	0.29
6	4.64	4.65	0.30
12	4.27	4.22	0.29
24	3.77	3.73	0.27
36	3.17	3.43	0.27
48	2.72	3.09	0.22

Appendix G

TABLE G.1. Immunoglobulin concentration (mg/ml) of pooled bovine colostrum.

Item	Trial 1	Trial 2
IgG	3007.15	2986.24
IgM	153.61	139.11
IgA	167.61	186.34

Table G.2. The effect of saline (CO), cortisol (HC), ACTH (SP) and metyrapone (LC) treatments on serum cortisol concentration (ng/ml) in the new born lamb.[a]

	Treatment				
Hours Postpartum	CO	HC	SP	LC	SE
0	93.35[b]	94.19[c]	117.52[d]	105.42[b]	7.43
2	57.90[b]	197.47[c]	133.95[d]	46.85[b]	8.96
4	66.03[b]	109.02[c]	145.89[c]	53.29[b]	7.06
8	78.32[b]	140.31[c]	125.47[c]	53.33[b]	7.32
12	78.74[b]	137.02[b]	100.07[b]	38.89[c]	8.13
16	83.48[b]	124.97[b]	114.87[b]	26.18[c]	8.84
20	84.51[b]	150.22[c]	90.04[b]	29.10[d]	9.87
24	71.95	117.22	105.87	28.54	15.31
36	39.99[b]	83.18[c]	82.98[c]	47.63[b]	4.45
48	33.19[b]	94.72[c]	51.57[bc]	50.20[bc]	6.54

[a]Trial 1

[b,c,d]Least-square means in the same row with different superscripts differ (P < .05)

Table G.3. The effect of saline (CO), cortisol (HC), ACTH (SP) and metyrapone (LC) treatments on serum IgG concentration (mg/100 ml) in the new born lamb[a].

Hours Postpartum	Treatment CO	HC	SP	LC	SE
0	0	0	0	0	---
4	0.09	0.08	0.32	0.19	0.06
8	1.09	1.34	1.94	1.60	0.25
12	2.73	2.90	3.64	3.68	0.41
16	4.36	5.26	6.24	5.06	0.55
20	6.05	7.13	7.70	5.34	0.65
24	7.10	7.38	8.34	6.30	0.81
36	8.65	10.67	9.34	6.21	1.15
48	10.11	10.18	9.93	5.23	1.16

[a]Trial 1

Table G.4. The effect of saline (CO), cortisol (HC), ACTH (SP) and metyrapone (LC) treatments on serum IgM concentration (mg/ml) in the new born lamb[a].

Hours Postpartum	Treatment CO	HC	SP	LC	SE
0	0	0	0	0	--
4	10.90	10.42	10.31	8.70	1.03
8	19.11	25.85	26.29	25.15	1.97
12	40.39	42.26	67.93	40.25	5.11
16	58.29	79.74	111.70	59.40	8.46
20	96.10	133.17	136.29	103.59	12.16
24	112.69	150.31	161.74	111.61	12.89
36	167.84	199.77	184.15	100.81	16.72
48	152.43	195.51	180.48	82.92	13.63

[a]Trial 1

Table G.5. The effect of saline (CO), cortisol (HC), ACTH (SP) and metyrapone (LC) on serum IgA concentration (mg/ml) in the new born lamb[a].

	Treatment				
Hours Postpartum	CO	HC	SP	LC	SE
0	0	0	0	0	--
4	2.48	3.08	3.76	2.89	0.27
8	5.44	9.39	13.60	10.24	1.20
12	13.44	16.66	25.34	22.71	2.48
16	27.99	27.98	38.80	26.84	2.74
20	30.78	45.39	42.27	29.44	2.57
24	32.72	47.76	47.45	30.54	2.80
36	69.20	66.90	74.16	33.09	6.08
48	56.67	77.77	58.88	29.67	5.05

[a]Trial 1

Table G.6. The effect of saline (CO), continuous ACTH (HC), ACTH at 0 h (SP) and metyrapone (LC) treatments on serum cortisol concentration (ng/ml) in the new born lamb[a].

	Treatment				
Hours Postpartum	CO	HC	SP	LC	SE
0	114.72	124.26	124.51	135.79	12.56
2	85.61$_{bc}$	162.77$_{b}$	153.10$_{b}$	85.61$_{c}$	11.08
4	78.45^{bc}	165.04^{b}	153.50^{bc}	54.19^{c}	11.17
8	74.70^{b}	156.16^{c}	117.02^{bc}	71.19^{b}	6.44
12	93.92^{b}	146.98^{c}	82.91^{b}	49.30^{d}	6.13
16	97.21^{b}	144.37^{c}	79.00^{b}	65.54^{b}	7.58
20	80.59$_{bc}$	150.98$_{b}$	95.75$_{bc}$	62.15$_{c}$	11.03
24	82.65^{bc}	141.31^{b}	85.59^{bc}	61.02^{c}	9.68
28	91.93$_{b}$	137.07$_{c}$	93.79$_{b}$	61.50$_{b}$	11.62
32	57.91^{b}	142.01^{c}	93.72^{b}	70.09^{b}	7.63
36	63.41^{b}	145.74^{c}	68.14^{b}	78.95^{b}	8.32
40	67.11^{b}	138.86^{c}	64.75^{b}	73.99^{b}	7.02
44	66.25^{b}	144.98^{c}	60.56^{b}	66.40^{b}	7.78
48	67.42	129.79	75.04	57.45	10.05

[a]Trial 2

[b,c,d]Least-square means in the same row with different superscripts differ ($P < .05$)

Table G.7. The effect of saline (CO), continuous ACTH (HC), ACTH at 0 h (SP) and metyrapone (LC) treatments on serum IgG concentration (mg/100 ml) in the new born lamb[a].

Hours Postpartum	Treatment CO	HC	SP	LC	SE
0	0	0	0	0	--
4	2.16	2.09	2.06	1.82	0.35
8	8.73	6.79	6.65	10.39	0.93
12	14.21	11.01	11.63	16.08	1.19
16	17.32	16.56	15.62	18.51	1.03
20	19.32	21.27	18.60	19.01	1.13
24	25.36	23.82	20.76	21.26	1.63
28	23.06	25.91	21.11	20.06	1.42
32	25.79	24.25	23.08	20.66	1.34
36	28.57	25.18	22.02	17.18	1.71
40	24.56	25.82	21.46	17.56	1.60
44	23.32	25.37	21.90	17.90	1.40
48	21.65	25.84	19.54	17.23	1.35

[a]Trial 2

Table G.8. The effect of saline (CO), continuous ACTH (HC), ACTH at 0 h (SP) and metyrapone (LC) treatments on serum IgM concentration (mg/ml) in the new born lamb[a].

	Treatment				
Hours Postpartum	CO	HC	SP	LC	SE
0	0	0	0	0	--
4	19.04	20.50	16.64	4.99	3.99
8	172.83	96.68	130.61	113.52	15.83
12	230.19	199.14	163.01	208.72	20.15
16	283.76	354.18	282.06	285.55	39.84
20	347.05	405.61	297.48	286.57	35.11
24	386.49	447.08	376.57	323.47	44.92
28	346.81	468.80	334.64	313.63	41.20
32	364.97	492.69	334.55	307.23	45.30
36	388.08	485.49	323.27	285.11	47.61
40	364.97	479.67	338.31	327.03	41.22
44	323.65	494.68	332.50	304.98	43.94
48	411.59	515.15	291.22	315.75	48.41

[a]Trial 2

Table G.9. The effect of saline (CO), continuous ACTH (HC), ACTH at 0 h (SP) and metyrapone (LC) treatments on serum IgA concentration (mg/ml) in the new born lamb[a].

Hours Postpartum	Treatment CO	HC	SP	LC	SE
0	0	0	0	0	--
4	13.20	8.89	11.85	4.46	1.98
8	59.08	39.36	40.78	42.77	5.89
12	97.88	59.23	76.06	89.17	8.91
16	111.36	94.47	90.15	95.53	6.31
20	120.15	115.01	113.60	119.33	8.12
24	150.64	129.46	128.41	114.12	9.20
28	169.35	128.19	115.27	115.59	10.82
32	162.40	143.14	134.80	134.22	10.01
36	116.63	141.70	122.29	118.07	7.98
40	141.77	146.76	135.91	111.32	8.97
44	152.15	133.91	97.20	113.15	8.35
48	147.81	130.26	89.28	113.81	9.34

[a]Trial 2

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