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THE INFLUENCE OF PASTURE CRUDE PROTEIN INTAKE ON DAIRY CATTLE CONCEPTION EFFICIENCY

A THESIS PRESENTED IN PARTIAL FULFILMENT
OF THE REQUIREMENTS FOR THE DEGREE
OF MASTER OF PHILOSOPHY IN VETERINARY
CLINICAL SCIENCES AT MASSEY UNIVERSITY

EDITH CRISTINA FERNANDEZ-BACA

1992

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ABSTRACT

Excessive dietary rumen degradable protein and high blood urea nitrogen (BUN) levels are reported to depress conception efficiency in dairy herds. High protein levels occur in New Zealand dairy pastures. This study explores the association of pasture protein and energy levels, and cow BUN, protein and energy measures with conception efficiency in dairy cows in the Manawatu region of New Zealand.

A survey study was conducted on 10 dairy farms on which samples of blood and vaginal mucus were collected from 745 cows within 2 hours before artificial insemination. Blood was analyzed for BUN, serum albumin (ALB), glucose (GLUC), ß-hydroxybutyrates (BOHB) and non-esterified fatty acids (NEFA) while vaginal mucus was analyzed for urea nitrogen content (MUN). Pasture samples were collected weekly during the animal sampling period and analyzed for crude protein, metabolizable energy and dry matter content. Reproductive records, including results of pregnancy diagnosis conducted 8 to 15 weeks after services at which samples were collected, were obtained from the farms and entered into the DairyCHAMP computer program.

Pasture crude protein levels ranged from 13% to 28%. The associations between dietary crude protein levels classified as high, medium and low and weekly conception rates indicated that as pasture crude protein increased, fertility decreased ($P<0.005$) in cows in their fourth or greater lactation. There was little variation in pasture metabolizable energy content and no significant association existed with conception rate. The association of the probability of conception with BUN and MUN, and blood levels of ALB, GLUC, BOHB and NEFA when tested by logistic regression analysis, revealed a significant negative association between BUN and fertility ($P<0.005$). MUN was positively associated with the probability of conception ($P<0.05$).

Dietary crude protein from pasture and BUN had a similar negative influence on fertility in pastured cattle as was observed in heavily supplemented or fully fed cattle in the northern hemisphere.

ACKNOWLEDGEMENTS

I would like to thank my supervisor, Prof. Norm Williamson for his guidance, patience and help during the whole length of my research. I would also like to thank my co-supervisor, Dr. Max Merrall for his very helpful suggestions and his assistance during the sampling period and during the preparation of the thesis.

I would like to thank the New Zealand Government for their financial support through the ODA scholarship fund.

Special thanks to Jeff Wichtel for his help during the sampling period.

My acknowledgement to Dr. Hugo Varela- Alvarez for the considerable statistical guidance he offered me in the analysis of my work.

To all the farmers that participated in the survey, I am very grateful for their cooperation and assistance in making this research possible.

To the Veterinary Clinical Pathology laboratory, the Department of Physiology and Anatomy and the Department of Animal Science, for their assistance in the blood analyses.

I wish to express my appreciation and sincere gratitude to my friends Pim, Pinthita, Gerardo, Louise and Mrs. Gay Eustace who in some way or the other helped me during my research work and thesis preparation.

Finally I would like to thank and dedicate this thesis to my parents, brothers and MSE with all my love.

EDITH FERNANDEZ-BACA
AUGUST, 1992

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3-OHB	3-hydroxybutyrate
AB	Artificial breeding
AI	Artificial insemination
ALB	Albumin
ASAT	Aspartate amino transferase
BOHB	ß-hydroxybutyrate
BOHBA	ß-hydroxybutyric acid
BUN	Blood urea-N
CIDR	Intravaginal progesterone device
CP	Crude protein
CPDM	Crude protein dry matter
CR	Conception rate
dg	Degradability
DCP	Dietary crude protein
DIP	Degradable intake protein
DM	Dry matter
GLUC	Glucose
GnRH	Gonadotropin releasing hormone
MConD	Mean conception date
ME	Metabolizable energy
MFID	Mean first insemination date
MUN	Vaginal mucus urea-N
NEFA	Non-esterified fatty acids
PBSEG	Phosphate buffer solution in EDTA and gelatine
PGF	Prostaglandin F_{3b}
PSB	Planned start of breeding programme
PUN	Plasma urea-N
RDP	Rumen degradable protein
SE	Starch equivalent
SUN	Serum urea-N
UIP	Undegradable intake protein
VFA	Volatile fatty acids

I

INTRODUCTION

Nutrition and reproduction are closely linked in dairy cattle. It is commonly accepted that nutrition has a large influence on dairy cow fertility.

Until recently, only severe nutritional deficiencies were thought to limit reproductive performance. Early research indicated that nutrient requirements for high milk production were greater than those for reproduction, therefore it was believed that cows fed for high milk yields could receive adequate nutrients for reproduction. However, it is possible that the systems of feeding which are suitable for milk production may cause nutritional imbalances that can be detrimental to herd fertility.

The profits of a dairy farm are largely determined by milk production and the number of calves born. The initiation of milk production depends on the establishment of pregnancy and the accomplishment of normal parturition. Thus, the goal of the dairy farmer is to maintain reproductive efficiency in his herd that results in the production of maximum milk yield during the cow's herd life. For this, among other things, it is important to achieve a correct nutritional balance that can be adequate for both reproduction and high milk yield.

In recent years, a number of studies have been done overseas that have suggested that protein levels in excess of approximately 20% of the ration's dry matter, in the diets of high producing cows, play an important role in reducing fertility. It seems to be that not only excess is an important factor but also the degradability of the protein in the rumen and the amount of energy available in the diet for maintaining a proper protein - energy balance in the animal can contribute to the relationship between conception efficiency and dietary protein levels.

Dairy production in New Zealand is totally based on the conversion of pasture into milk by grazing cows. New Zealand pastures are high in crude protein content, commonly surpassing the 20% crude protein level and reaching levels as high as 30% crude protein. These levels are above those reported by overseas studies as detrimental to fertility. However, conception efficiency in New Zealand dairy herds is reported to be very high by world standards.

The present study investigated if the negative influence of high dietary protein on fertility observed overseas, occurred in New Zealand where pasture protein levels are high but conception rates are also low.

II

LITERATURE REVIEW

1.0 EFFECTS OF NUTRITION ON REPRODUCTION IN DAIRY CATTLE

The high producing cow cannot meet her peak nutrient requirements unless an adequate energy supply is provided via proper nutrition and accompanying gluconeogenesis (Sommer, 1975).

Nutrient deficiencies, imbalances or excesses can contribute to poor reproductive performance (Thatcher, 1986; Swanson, 1989; Ducker, 1984). Usually a single nutrient deficiency or excess is not the sole cause of reproductive problems. However, interactions between nutrition, disease, stress, environment, genetics and overall reproductive management can influence reproduction (Otterby and Linn, 1983; Thatcher, 1986).

Many studies have been done in which relationships between reproduction and nutrient deficiencies, excesses or imbalances have been found. Signs and effects of nutritional imbalance vary, depending on the phase of the reproductive cycle (Otterby and Linn; 1983, Ducker, 1984).

Nutrition will affect calving patterns through its effect on post-partum anoestrus, conception rate, duration of mating and artificial induction of calving (McGowan, 1981).

Ducker *et al* (1985a) suggested that levels of feeding in late pregnancy and around the time of insemination can greatly influence the reproductive performance of dairy cattle, especially the occurrence of first ovulation. Decreased conception rates in cows losing weight are associated with decreased episodic LH peaks and lower progesterone concentrations and these have been associated with unsuccessful insemination (Weaver, 1987).

Otterby and Linn (1983) and Ducker *et al* (1985b), report an inverse relationship between levels of albumin and potassium with the number of services per pregnancy and associated longer calving to conception intervals with low sodium. Ducker *et al* (1985a) observed that patterns of feeding which are suitable for milk production, could be detrimental to herd fertility.

Deficiency of phosphorus has been related to an increased number of services per conception as have overfeeding and obesity (Otterby and Linn, 1983). Phosphorus deficiency has also been related to anoestrus, impaired ovarian function and early embryonic death (Weaver, 1987). There is also a relationship between the calcium-phosphorus ratio. In herds in which the calcium-phosphorus ratio was wide and phosphorus intake was low, breeding efficiency was significantly lower than in those with a narrow ratio and high intake of phosphorus. (King, 1971).

Severe total digestible nutrient deficiencies can cause abortion as well. A low nutritional plane during late pregnancy apparently delays oestrus after parturition while deficiency of B-carotene is reported to cause anoestrus following parturition. Vitamin deficiency such as that of vitamin A has been observed to lead to sterility in some cases (King, 1971) as well as cause short gestation periods and retained placenta as has the deficiency of vitamins D and E (Weaver, 1987).

Energy deficiencies just before or after parturition have been linked with anoestrus or suppression of oestrus signs. On the other hand, excesses of energy or unbalanced rations after peak production and/or during dry periods, lead to development of the fat cow syndrome (Otterby and Linn, 1983),with subsequent negative effects on reproduction (Morrow, 1976).

High levels of urea have been associated with a high incidence of retained placenta in cows at second calving. In Holstein heifers in mid pregnancy, an association with an increase in abortions and shorter gestation periods for non-aborting heifers was observed (Erb *et al*, 1976; Otterby and Linn, 1983).

Energy-protein imbalances and excessive protein intakes are thought to contribute to infertility and poor reproductive performance. Studies on the relationship that may exist between protein and reproduction have increased, demonstrating a growing interest in the subject (Chalupa, 1984; Ferguson and Chalupa, 1989; Otterby and Linn, 1983).

There are several methods that are currently used to detect nutritional deficiencies such as analysis of feedstuffs, and identification of soil mineral deficiencies. In the animal blood, analysis to assess the levels of blood metabolites and the observation of changes in body weight can be of use (King, 1971).

2.0 EFFECTS OF PROTEIN ON REPRODUCTION

2.1 General protein metabolism

To meet net requirements for protein, dairy cattle must receive sufficient nitrogen in protein or non-protein nitrogen form in their diet (Holmes and Wilson, 1987). Protein is required for formation of amino acids used in milk production, maintenance, reproduction and tissue growth and repair (Chalupa, 1984; Edwards *et al*, 1980).

2.1.1 Protein metabolism in the rumen

Protein metabolism in ruminants is a complex, dynamic process as can be seen from figure 1. Ruminal microorganisms require nitrogen obtained from dietary crude protein (DCP), in the form of ammonia, amino acids, and peptides which are mainly used in dairy cattle to produce protein for maximum milk production (Chalupa, 1984). An insufficient supply in the rumen of nitrogen substrate for microbial growth, limits microbial activity and potentially impairs digestion (Oldham, 1984).

Ruminal bacteria can use non-protein nitrogen in addition to protein for amino acid formation; thus non-protein nitrogen (primarily as ammonia) is trapped as bacterial protein. Protein incorporated into bacteria is passed to the small intestine and subsequently digested by the animal. The bacterial protein is used to supply amino acids for production of animal protein that will deposit in milk, animal or foetal tissues (Chalupa.1984; NRC, 1985).

Dietary crude protein is in the form of degraded intake protein (DIP) which breaks down in the rumen and undergoes partial or total conversion to bacterial and protozoal crude protein (CP), or is passed from the rumen to the omasum as undegraded intake protein (UIP) (NRC, 1985). Once ingested, DCP is separated into pools that are insoluble or soluble in ruminal fluid and into pools that are degradable or undegradable by ruminal microorganisms (Chalupa, 1984).

Solubility of protein is an important factor in the diet. Soluble proteins are degraded more rapidly and completely than insoluble ones (Chalupa, 1984). Solubility is partly determined by the relative amount of soluble albumins and globulins. Feeds with a greater amount of albumin and globulin have higher protein solubility than those that

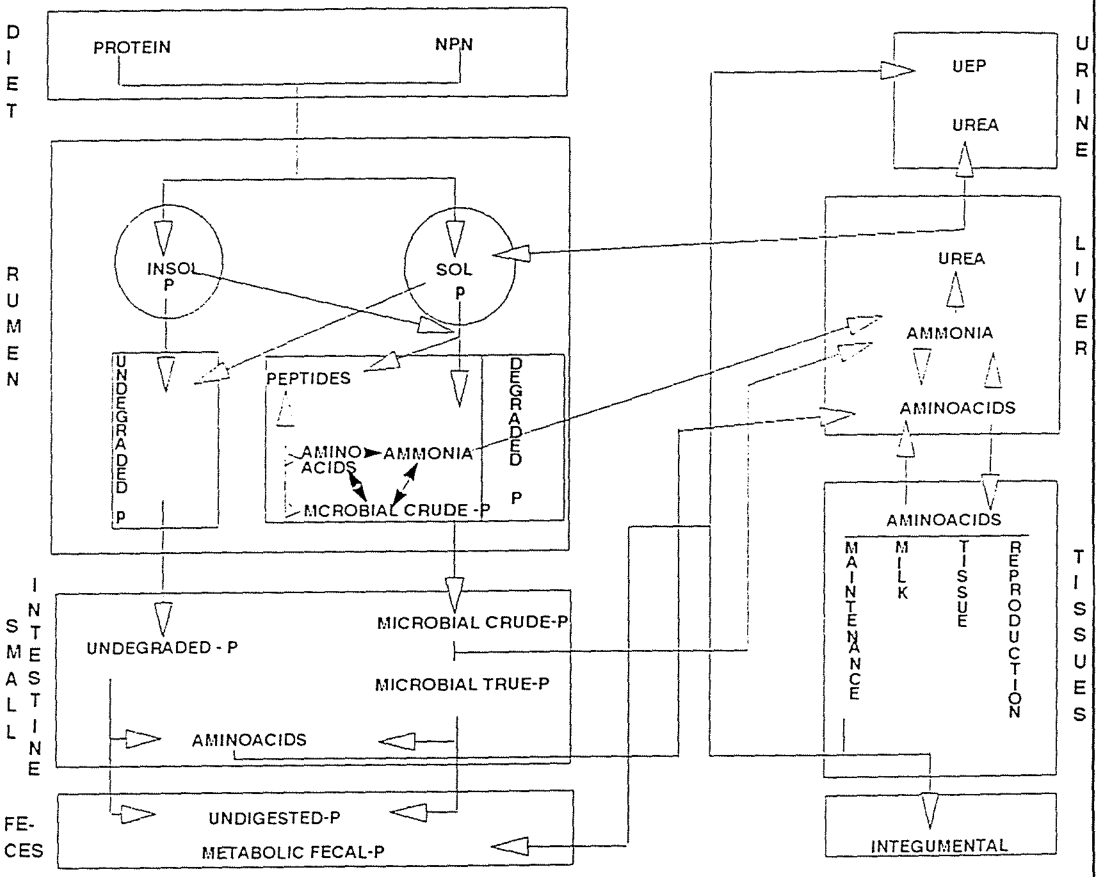

Figure 1. Protein digestion and metabolism in lactating dairy cattle.

From: Chalupa, 1984.

contain mainly prolamins and glutamines in their proteins (Smith, 1979).

Even though solubility is an important determinant of protein degradation in the rumen, it cannot be taken as a guarantee of degradability since both characteristics are not identical (Smith, 1979; Chalupa, 1984).

Each type of protein varies in the degree of degradability. Protein in most forages is susceptible to ruminal degradation. Evidence obtained from different studies suggests that small grains, such as barley and oats, have protein that is more degradable than the protein in corn. Soyabean meal protein is a relatively degradable protein while brewers grains, distilled grains, corn gluten meal, fish meal, blood meal and meat and bone meal are more resistant than most feed grains and oil meals to ruminal degradation, since almost 50% or more of the protein escapes rumen degradation (Chalupa, 1984; NRC, 1985).

Researchers have developed various methods, both *in vivo* and *in vitro*, to be able to measure the degradability of protein contained in these and other feedstuff in the forestomach of ruminants (Tamminga,1979). In vitro methods include those based on either the release of ammonia after incubation with fluid from the rumen, or on the estimate of the proportion of nitrogen which goes into solution after incubation at body temperature for a definite period of time. Incubation media, such as diluted NaOH, artificial saliva, autoclaved ruminal fluid or a diluted solution of pepsin in 0.1 N HCl have been used for this purpose (Tamminga, 1979, Shirley, 1986). This methodology is useful for providing a relative ranking of feedstuffs on the basis of protein degradation rather than in providing absolute estimates of protein degradation (Satter, 1986).

In vivo methods measure dietary nitrogen intake, and the non-ammonia nitrogen and microbial nitrogen passing to the duodenum to calculate the proportion of protein escaping breakdown in the rumen (McDonald *et al.*, 1988). One of the methods most often used to estimate degradation in the rumen is by incubation of the food in synthetic fibre (dacron) bags suspended in the rumen. The degradability is equal to the difference between the nitrogen initially present in the bag and that present after incubation (McDonald *et al*, 1988, Tamminga, 1979). However, this method has its drawbacks. Protein in the dacron bags is not entirely subjected to the dynamic system characteristic of the digestive metabolism in the ruminant animal. Furthermore, some soluble proteins may be washed out without actually being degraded (Tamminga, 1979). Not all researchers agree on this point. Mertens (1977) proposes that in vivo, all of the soluble protein is degraded in the rumen.

Some insoluble protein is also degraded in the rumen. Mertens (1977) observed that 40 to 50% of the insoluble protein is degraded in the rumen while in another study, Jarrige *et al* (1978), calculated that around 35% of insoluble protein is degraded.

Using in vivo methods, the estimated amount of dietary protein from individual feedstuffs that escapes microbial degradation in the rumen has been calculated. Satter (1986) reports values ranging from approximately 20% for protein in barley, oats, wheat and alfalfa silage to 65 to 70% for protein in fish meal and animal by-products.

Degradation consists of: 1) Hydrolysis of peptide bonds, production of peptides and amino acids (proteolysis) and 2) Deamination and degradation of amino acids (NRC, 1985; Tamminga,1979).

After proteolysis, liberated peptides or amino acids may leave the reticulo-rumen, be used for microbial growth, or degraded to ammonia and fatty acids. Since amino acids are rapidly degraded in the rumen, only small amounts of true amino acids are available for absorption or passage from the reticulo-rumen (NRC, 1985).

The major end product of protein and non-protein nitrogen degradation is ammonia. All amino acids and nucleic acids are potential sources. Ammonia is produced from glutamine by the kidney. It is also produced by both endogenous and bacterial enzymes within the alimentary tract. Almost 75% of the alimentary tract ammonia is produced by means of urea hydrolysis (Visek, 1984; NRC, 1985).

Ruminal ammonia not used for microbial growth is largely absorbed through the wall of the reticulo-rumen and converted to urea in the liver. Excess degradable intake protein increases the concentration of ruminal ammonia and sometimes the production of ammonia exceeds the capacity of the bacterial population to use it. As a consequence, part of this excess ammonia may elevate concentrations in the liver to toxic levels and the rest is excreted in the urine after its conversion to urea. Therefore there must be a proper balance of degradable and undegradable protein to maintain ammonia below toxic levels (Visek, 1984; Chalupa, 1984; NRC, 1985).

2.1.2 Protein metabolism in small intestine

Dietary protein that escapes rumen degradation passes to the small intestine. Intestinal nitrogen consists of microbial, undegradable feed and endogenous fractions (Chalupa, 1984; Ferguson and Chalupa, 1989). Approximately 80-90% of nitrogen entering the small intestine has been observed to be truly digested (Shirley, 1986).

Digestion of protein in the abomasum and small intestine appears to be the same for ruminants as for nonruminants, except for the slow neutralization of digesta in the small intestine and abundance of pancreatic ribonuclease. The mucosa of the small intestine contains uptake systems for free amino acids, peptide, nucleotide and nucleoside (Chalupa, 1984; NRC, 1985).

2.1.3 Nitrogen metabolism in large intestine

This metabolism occurs when nitrogen enters the caecum and large intestine from the ileum and by diffusion through the intestinal walls. Undigested feed protein and bacterial protein, indigestible feed protein, and endogenous nitrogen secreted from the upper sections of the intestinal tract are the various types of proteins that come from the ileum (NRC, 1985). The quantity of nitrogen entering the caecum amounts to about 20% of the total nitrogen intake (Bondi, 1987). The amounts of free amino acids or peptide entering the large intestine are insignificant (NRC, 1985).

Nitrogen absorption from the caecum and large intestine into the blood stream or through diffusion to other organs is enhanced by the high intestinal pH (7 to 8) and primarily consists of ammonia (NRC,1985).

2.1.4 Faecal and urinary excretion

Faeces contain undigested dietary protein and metabolic faecal protein that is protein originating in the gastrointestinal tract that is not reabsorbed (Chalupa, 1984).

Waste nitrogen, principally as urea, proceeding from deamination of amino acids or ammonia absorbed from the digestive tract,is excreted in part in the urine. The rest is excreted in milk or recycled into the digestive tract (NRC, 1985). Urea that escapes urinary excretion may pass to the rumen by way of the saliva and by diffusion across the ruminal wall. Transfer of urea from blood to the rumen wall is thought to be more important than transfer by saliva in re-cycling, because it has been found that 16 times more urea reaches the rumen via diffusion than via saliva (Fontenot, 1972).

Nitrogen that returns to the reticulo-rumen, supplements the diet and contributes to the amount of nitrogen available for microbial growth (Chalupa, 1984; NRC, 1985).

2.1.5 Metabolism of absorbed amino acids

All absorbed amino acids are transported by blood through the portal vein to the liver before being carried to other tissues as free amino acids, in red blood cells and as peptide (NRC, 1985).

Free amino acids are mostly used for synthesis of body protein but also as source of energy and as a carbon source in the synthesis of glucose. In early lactation, where both amino acids and glucose are in short supply, oxidation of both of them appears to be reduced while oxidation of fatty acids is increased (Chalupa, 1984; NRC, 1985).

Synthesis and degradation of body protein is continuous; in cattle, 30-40% of total synthesis occurs in the gastro-intestinal tract, 10-20% in skin, 15-20% in skeletal muscle and 4-8% in the liver. Increasing amino acid intake above requirements increases oxidation that occurs almost totally in the liver. This is the major irreversible loss of essential amino acids from the body that may occur (NRC, 1985).

2.2 Role of energy in protein metabolism

A sufficient supply of energy is an essential ruminal nutritive requirement for an animal to meet its maintenance and productivity needs (Smith, 1972).

The amount of protein that may be synthesised by the rumen and by the mammary gland depends primarily on the amount of dietary energy available for microbial growth (Chalupa, 1984). An asynchronous supply of nitrogen and energy could reduce the efficiency of nutrient utilization for microbial growth (Newbold and Rust, 1992). Thus, requirements for intake protein dry matter (IPDM) are determined by changes that may occur in energy intake and milk production throughout lactation (Ferguson and Chalupa, 1989).

2.2.1 Energy metabolism

Energy is made available as the result of various metabolic processes such as the metabolism of carbohydrates, proteins and lipids (McDonald *et al*, 1988). In ruminants the efficiency of metabolizable energy (ME) utilization, for growth and fattening, is dependant upon the relative amounts of volatile fatty acids (VFA) produced in rumen (Van der Walt and Linington, 1989 and 1990).

Diet has the greatest effect on the pattern of VFA production by fermentation. Diet therefore plays a major role in controlling the supply of VFA to the animal. About 75% of VFA produced in the rumen are absorbed by free diffusion from the rumen, 20% from the omasum and abomasum and the rest from the small intestine (Van der Walt and Linington, 1989).

In the ruminant animal's ration, carbohydrates make up at least 75% of the ration's weight (Lloyd *et al*, 1978). On the other hand, fat usually does not exceed 5%. It has been reported that only 3 to 5% fat added to common feeds appears to be tolerated by ruminal microorganisms (Chalupa *et al*, 1986).

Crude fibre represents the principal source of carbohydrates in the diet of dairy cattle. Cellulose and hemicellulose are the major crude fibre carbohydrates. Their presence in the diet, as a potential energy source, is of primary importance (Lloyd *et al*, 1978).

The major nutrients absorbed from the dietary carbohydrates broken down in the rumen are acetic, propionic and butyric acids (Oldham, 1982). Butyric acid is extensively metabolized in the ruminal epithelium and about half of the amount passes into the portal blood as ß-hydroxybutyric acid (BOHBA) (McDonald *et al*, 1988, Van der Walt and Linington, 1989).

Acetic acid and propionic acid pass almost unchanged across the rumen wall into the portal blood and are carried, together with the ß-hydroxybutyric acid, to the liver (McDonald *et al*, 1988). The liver extracts from the portal blood virtually all of the propionate, acetate and any butyrate which escapes transformation at the rumen wall (Kronfeld, 1964). Some propionate is metabolized during transport through the epithelium but only about 5% of that absorbed appears as lactate in portal blood (Van der Walt and Linington, 1989).

The ruminant liver has a net output of glucose,lactose, acetate,formate and ß-OH-butyrate (Kronfeld, 1964). Acetic acid and BOHBA pass from the liver, via the systemic blood, to various organs and tissues where they are used as sources of energy and fatty acids. Propionic acid is converted to glucose in the liver and joins the liver glucose pool. This may be converted partly into glycogen and stored, or to fatty acids, reduced coenzymes and L-glycerol-3-phosphate. The remainder of the glucose enters the systemic blood supply and is transported to various body tissues where it may be used as an energy source, as a source of reduced coenzymes in fatty acid synthesis, and for glycogen synthesis (McDonald *et al*, 1988). In figure 2 the major metabolic pathways in the liver are shown.

Even though little carbohydrate bypasses the rumen in roughage-fed ruminants, considerable amounts may pass into the duodenum of animals fed diets containing a high proportion of grain.

A dairy cow ingesting 15 kg of forage will take in about 1 kg of lipids. Lipids are hydrolysed in the rumen to free fatty acids, largely by means of plant lipase activity (Van der Walt and Linington, 1989).

2.3 Normal protein requirements

It is important to maintain an adequate nutrient supply in dairy cows in order to achieve high production and good health.

The major determinants of total milk yield for a complete lactation are peak milk yield and persistency of production. Both of these are influenced by nutrient intake and body nutrient reserves (Wohlt and Clark, 1978).

Since milk is high in protein content (on a dry matter basis), milk production results in a substantial increase in protein requirements (Fontenot, 1971). Protein can affect milk production by providing more amino acids, increasing available energy and altering the efficiency of use of absorbed nitrogen (Chalupa, 1984).

An increase in the intake of CP, that is very palatable and promotes higher intake of dry matter, increases milk production in early lactation (Swanson, 1989); protein

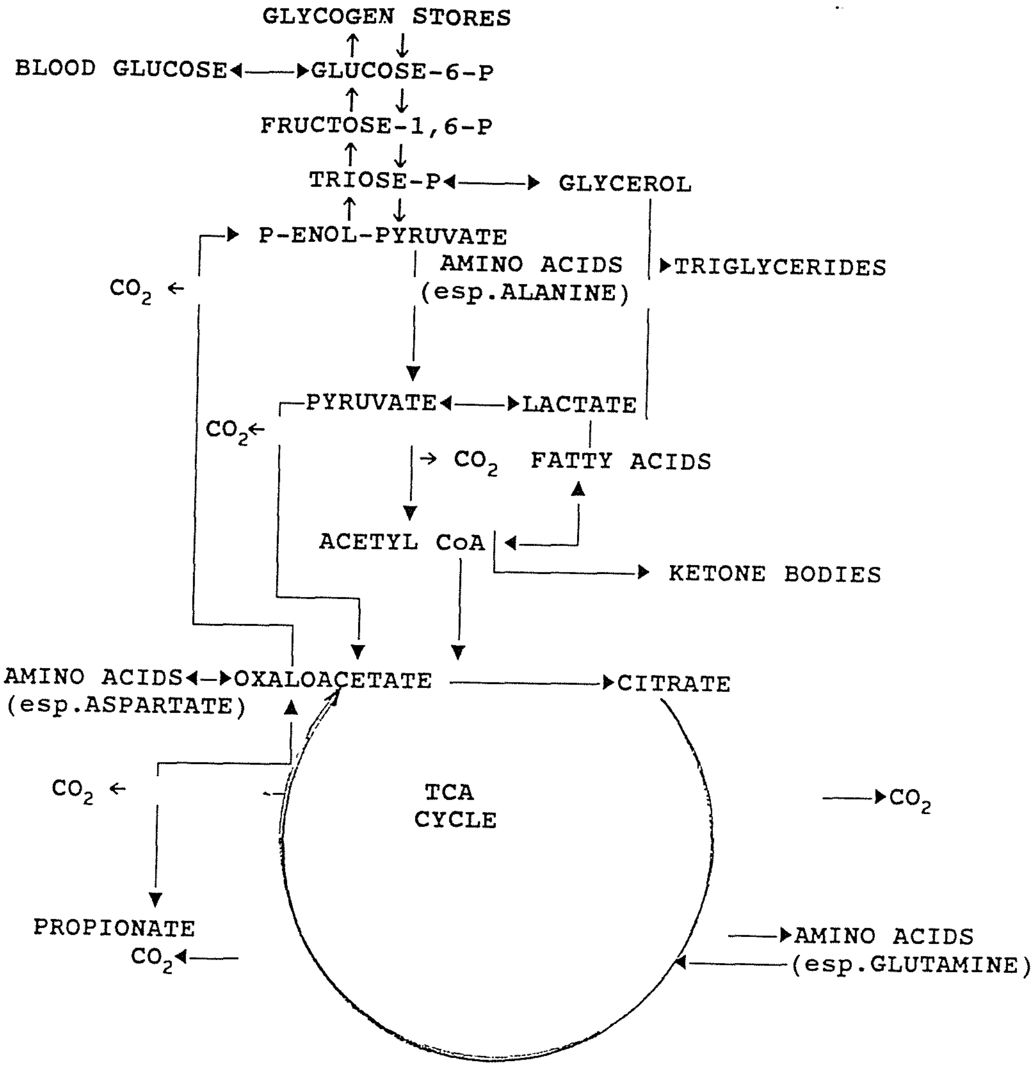

Figure 2. Major metabolic pathways in the ruminant liver (Gluconeogenesis)

From: Van der Walt and Linington, 1989a.

supplementation favours partition of available nutrients towards mammary secretion (Oldham, 1984; Carroll *et al*,1987). However, intake DCP is not solely directed towards use in milk production. Therefore, it is necessary to define the levels of dietary protein that should be fed to the dairy cow to cover all its maintenance needs as well.

According to the National Research Council (NRC), DCP requirements for high producing cows range from 14 to 22% of dietary dry matter (Howard *et al*, 1987). A diet of approximately 18% CP supports requirements for a 650 kg cow that produces 9000 kg of 3.5% fat milk in 305 days of lactation (about 30 kg of milk per day) and that consumes 3% of her body weight in dry matter (Blauwiekel *et al*, 1986; Ferguson and Chalupa, 1989).

Feeding rations containing less than 12% protein dry matter has been seen to result in less milk production than when rations with higher proportions of protein are fed, but consuming rations higher in protein may mean an increase in dry matter intake and a decrease in efficiency of protein use (Edwards *et al*, 1980).

Edwards *et al* (1980), in a study performed using diets containing 13, 15 and 17% of protein, observed that 15% protein, as recommended by the NRC, appeared to be the most adequate for cows producing up to 29 kg milk/day while 13% was insufficient for an entire lactation and 17% was the least efficient for converting feed protein to milk protein.

Likewise, Chalupa (1984) reports that increasing CP above 14% results in smaller and declining rates of increase in milk yields when using diets based on corn and soybean meal. Danfaer *et al* (1980) showed a reduction in milk output equivalent to 4.5 MJ net energy/day when DCP content increased from 190 to more than 230 g/kg DM of feed intake.

However, Wohlt and Clark (1978) observed that soybean meal could be a beneficial supplement. When 13.5 to 14.5% CP was fed to high producing cows during early lactation, with supplemental nitrogen supplied as soybean meal (18% CP and 0.36% soluble nitrogen), a greater milk production occurred when compared to those cows fed the same amount of CP but with a urea supplement or a supplement containing 50% urea-50% soybean. This they thought was probably due to the fact that more protein escaped degradation in the rumen, as soybean meal protein is protected, resulting in a greater quantity of amino acids being absorbed from the small intestine and used in synthesis of milk protein.

The concentration of protein to be included in the diet of dairy cows and its effect on milk production may also depend on the stage of lactation and age of the cow. Roffler and Thacker (1983), found that reducing DCP from 17 to 13.5% during early lactation (5 weeks post-partum), decreased subsequent milk production and dry matter intake of multiparous but not first-calf heifers which showed to be less responsive to increased concentrations of DCP during this period. Likewise, Claypool *et al* (1980) reported similar findings in their studies.

Some studies have concluded that the amount of protein recommended to meet requirements could be above that actually needed. Jordan and Swanson (1979a) found that decreasing protein to 80% of NRC recommendations for cows producing more than 30 kg of milk per day increased fertility by decreasing services per conception and days open.

Treacher *et al* (1976) did not observe any improvement on fertility but neither did they see any adverse effect nor reduction in milk yield when feeding 75% (12.75% CP) vs 100% (16.5% CP) of protein requirements to dairy cows during the first 14 weeks of lactation. This contrasts with the views of Roffler and Thacker (1983) as these authors propose that low intake protein during the first 8 weeks of lactation has detrimental effects on milk yield as was mentioned above in this review.

In the work by Treacher *et al* (1976), cows fed a high protein ration had a mean interval between calving and conception of 27.5 weeks while those fed low protein had a mean of 20 weeks. Although this difference was not significant, the low protein group had a numerically lower calving to conception interval.

2.4 Protein and fertility relationship

Feeding CP dry matter at levels in excess of the recommended amounts to fulfil requirements, is a practice sometimes seen in high producing North American commercial herds as a means of increasing milk production (Swanson, 1989). Some authors see no harm in this practice. Blauwiekel and Kincaid (1986) concluded that dairy cows in the first ten weeks of lactation can use higher DCP than levels currently recommended by the NRC without problems being produced. Furthermore, Thatcher (1986) cites an NRC publication where excess protein intake is not considered harmful to cows.

However, Gould (1969) suggested that an excess of CP intake could be associated with infertility. King (1971) hypothesised that excess CP in the diet could make food unpalatable limiting voluntary intake. As a result, energy intake would also be limited to the point where the cows could suffer from hypoglycaemia and finally develop ketosis, a syndrome associated with infertility (Eldon *et al*, 1988; Anderson and Emanuelson, 1985; McClure and Payne, 1978).

There is a growing list of published research work that strongly supports the hypothesis of the influence of protein on fertility. These studies suggest that feeding diets with high concentrations of CP dry matter may depress fertility; while milk production may increase. There seems to be a negative impact on conception rates so animals require more services per conception (Sonderegger and Schurch, 1977; Ferguson *et al*, 1986; Ferguson and Chalupa, 1989). Levels of 19.3, 20 and 23% of DCP have been considered as excessive in early lactation and detrimental to post-partum fertility (Jordan and Swanson, 1979a; Swanson, 1989).

There is some inconsistency in the literature about the effects of CP on fertility. Some authors, like Howard *et al* (1987), report no effect on reproductive traits of animals fed 20% CP, considered by some authors as excessive, and concluded that there was no conflict in their experiment, between the amount of dietary protein provided for lactation and reproductive performance. Blowey *et al* (1973) measured urea and albumin in the blood of cows as an assessment of dietary protein, but they did not analyze for any effect on reproduction, despite studying the effect of changes in blood glucose level on reproduction.

Thatcher (1986), reported that cows receiving complete silage based rations and protein levels exceeding requirements by 10-15% needed more services per conception and had longer intervals to conception, despite being observed in heat sooner than cows fed 90% of their protein requirements.

There are also studies where, although no statistically significant relationship has been found between reproductive performance and protein, it has been observed that an increase in services per conception or days open occurs when protein concentration in the diet is increased. Edwards *et al* (1980), reported that although differences in services per conception or days open were not statistically related to protein concentration ($P>.05$) in their study, they tended to increase as protein concentration increased.

Carroll *et al* (1988) also observed that days to first ovulation were longer (22 vs 17) in cows fed 20% CP rations versus those fed 13% although they did not see any significant difference in reproductive efficiency between the two groups.

Recently published research studies have further investigated the relationship between CP and fertility and have concluded that the amount of degradable and undegradable protein in the diet may better describe this relationship and in some way answer some of the previous inconsistencies in findings relating protein intake to fertility seen in the literature (Ferguson and Chalupa, 1989; Simons,1990).

Some amount of degradable protein is necessary to meet the requirements of ruminal micro-organisms for microbial growth, replication and metabolism. Levels below the minimum amount needed for these purposes will lead to reduced voluntary intake and reduced digestibility (Holmes and Wilson, 1987). Any excess of degradable protein increases the waste of nitrogen in the rumen, leading to the provision of insufficient absorbable protein and increased ammonia concentration (Ferguson *et al*, 1986).

Jordan *et al* (1983) suggest that an increased circulating ammonia concentration occurs either because the enzymes of the urea cycle do not convert all the excess ruminal ammonia into urea-N, possibly because uptake into hepatic cells is insufficient, or because excess ammonia in the alimentary tract can pass across the peritoneal cavity to the general circulation and other tissues by simple diffusion without passing through the liver.

This means that when high quantities of degradable intake protein are consumed, there is an increase of levels of urea nitrogen and/or ammonia in biological fluids (Ferguson and Chalupa, 1989; Cody *et al*, 1990) such as blood (Jordan *et al*, 1983), vaginal mucus (Carroll *et al*, 1987; Holtz *et al*, 1986), and uterine secretions (Jordan *et al*, 1983).

In an experiment where the degradability of protein in the diet was not specified but where the relationship between urea-N in plasma and DCP was studied, Macleod *et al* (1984), reported that plasma urea nitrogen increased linearly as DCP was increased. In the experiment, 64 Holstein cows in their first lactation were assigned randomly 28 days postpartum to receive ad libitum, one of 9 rations with CP levels of 12, 15 or 18% in each of 3 energy densities in a 3x3 factorial design. The 3 energy densities were from ratios of forage: concentrate 75:25, 55:45, and 35:65. These authors reported that feed intake, milk fat yield and fat-corrected milk also increased linearly as dietary protein increased. As energy density increased, feed intake, milk yield, milk protein, and lactose increased curvilinearly while plasma urea-N decreased linearly.

Jordan *et al* (1983), studying urea nitrogen concentrations in uterine secretion and plasma, found 2.7 times higher levels of urea nitrogen in uterine secretions and 3.5 times higher levels in plasma, of cows fed excess CP (23% CP) than in those fed 12% CP. Johnson *et al* (1986) on the other hand, studied the effects of DCP on levels of urea-N and ammonia in cervical mucus in 2 groups of Holsteins fed a diet either high in CP (21.5%) or low in CP (11.2%). Both diets contained 90% silage and 10% grain but the low diet was adjusted with a 16% concentrate and the high diet was formulated using soybean meal. At oestrus, blood and cervical mucus were collected and analyzed. Blood and mucus urea levels were both significantly increased on high protein diets while ammonia was not. Again, in both studies, the proportions of degradable and undegradable protein that were included in the diet were not documented.

Aside from blood serum, vaginal and uterine secretions, the measurement of milk urea concentration can also be a useful method of assessing the protein content of the diet. Oltner and Sjaunja (1982) and later, Oltner (1983) used a simple and quick method for the determination of urea in milk and found that urea concentration is not only influenced by the amount of protein ingested but also the relationship between protein and energy in the ration. Milk urea concentrations were only slightly affected by the variations in the total amount of ingested protein when the ratio between protein and energy in the feed was held constant. If the ration composition was altered in a way that the protein/energy ratio was affected, a rapid change in milk urea concentration would occur as well.

Roseler *et al* (1990) studied the effect of dietary protein degradability and undegradability on milk urea-N, milk NPN and plasma urea-N in 10 mid-lactation dairy cows. These cows were fed isocaloric diets varying in degradable (DIP) and undegradable (UIP) protein (80:80, 100:100, 120:100, 100:120 and 120:120% proportions respectively). They observed that excessive DIP and/or UIP were associated with increases in milk urea-N, PUN and milk NPN but concluded that gross deficiencies of total protein intake can be monitored by PUN or milk urea-N while subtle deficiencies or excesses of DIP and UIP are masked by total protein intake.

Not only have the increased levels of urea nitrogen and/or ammonia in biological fluids been reported to reflect increases of CP in the diet, but furthermore, they have also been linked with reduced conception rates (Ferguson and Chalupa, 1989). Hewett (1974), for example, found that serum urea nitrogen was significantly inversely related to fertility in a study he did on the blood profiles of 650 and 130 Swedish Red and White cows and heifers.

Ferguson *et al* (1988) conclude from their observations of a herd with infertility problems, that serum urea-N (SUN) concentrations above 20 mg/dl, when the urea-N content in blood serum of cows fed adequate amounts of protein range from 10-20 mg/dl according to Thatcher (1986), may have indicated an excessive amount of CP in the ration the cows were fed and/or that an excessive amount of dietary protein was being degraded in the rumen. This excessive ruminal degradation of protein was diagnosed as being the cause of poor conception.

Eldon *et al* (1988), monitored during a period of 3 years the onset of post-partum ovarian activity, the number of artificial inseminations and the time of conception in 412 Icelandic dairy cows. When analyzing the levels of urea in blood, they found that urea values correlated significantly with the time of first post-partum ovulation. This correlation was negative for samples taken the first 20 days post-partum but positive after that.

High intakes of protein can be reflected in blood, not only as high concentrations of urea-N but also as hyperalbuminemia and this too has been related to fertility problems. Concentrations of albumin in serum have been observed to have a positive relationship with the number of services required per conception (Manston *et al*, 1975; Jordan and Swanson, 1979a).

Holtz *et al* (1986) did not find differences in reproductive performance (days to first ovulation, days to first observed heat or days open) in a total of 18 Holstein cows feeding on different isocaloric diets containing low and high levels of ration total protein and percentage of degradability. These diets contained: low protein/low degradability (15% total protein, 54% degradability); low protein/high degradability (15% total protein, 64% degradability); high protein/low degradability (20% total protein, 54% degradability); and high protein/high degradability (20% total protein, 64% degradability). However, cows consuming the high protein diets exhibited increased body weight loss (14.7% vs 7.9%), BUN (18.1 vs 10.6 mg%) and serum albumin (3.29 vs 3.16 g/dl). High degradability rations elevated BUN (16.1 vs 12.9 mg%). Cows receiving the low protein diets began to return towards positive energy balance 3 weeks sooner than high protein cows. The authors concluded that, in light of the metabolic differences, further research was needed to properly assess reproductive parameters utilizing greater animal numbers.

An association between urea concentration in bulk milk and fertility was observed by Ropstad and Refsdal (1987) in a study they made on 256 dairy herds in four districts of Southern Norway. They observed that the mean urea level in bulk milk was significantly higher (more than 5.2 mmol/l) in one district (Hedmark) than in the others and that it also had the lowest fertility. Herds in this district had a higher percentage of silage and concentrates in their diet compared to the rest. From this work, the authors concluded that there was a significant inverse correlation between mean urea level and fertility. Schulz (1992) also observed that an increased concentration of urea in milk above 5 mmol/l was associated with lowered conception rates on a herd basis in a study he did on 224 cows.

The positive association of fertility with the amount of UIP in the diet may explain why in some experiments, even though high amounts of protein were given, no fertility problems were observed and even some improvements in conception rate reported. Armstrong *et al* (1990) saw that the inclusion of fish meal (614 g/kg DM CP) significantly improved conception rates and reduced intervals from calving to conception, but it is necessary to point out that fish meal protein is among the proteins that are more resistant to ruminal degradation (NRC, 1985). Similarly, Wilson (1989) reported positive effects on submission rates associated with a high protein intake when feeding concentrate supplements that included protein ingredients with low protein degradability (eg brewers grain or soyabean).

From recent studies it can be concluded that levels of rumen degradable protein above 64% of total DCP, limit fertility. Ferguson *et al* (1986) observed that when cows were fed a diet that met NRC recommendations for protein and energy but 72% of protein was rumen degradable, the probability of pregnancy was lower than in those fed a diet with 62% of rumen degradable protein. In their study, they stratified each treatment by age. Group 1 was less than 28, group 2 was 28 to 56 and group 3 was more than 56 months old at calving. Probability of pregnancy for groups 1, 2 and 3 for the 72 and 62% RDP were .94 and .67 ($P<.08$), .67 and .67 ($P<.5$), .53 and .75 ($P<.01$) respectively.

In another paper, Ferguson *et al* (1988) also report the case of a herd of 60 Holstein cows where the first service conception rate decreased from 50% to less than 20%. After studying all probable causes, the diet was analyzed and it was found that the high producing cows were being fed more than 18% CP in the total ration dry matter (more than 10% above the recommended amount by the NRC, 1978) and from this amount, 67% corresponded to degradable protein. After decreasing the amount of CP to 16.0% and the DIP to 62%, fertility was significantly improved.

Furthermore, in later controlled studies, Blanchard *et al* (1990) also found that fertility rates were significantly lower in cows fed a diet containing 16% CP with 73% of DIP than in those fed the same amount of protein but with only 64% of it being rumen degradable protein.

In a study by Canfield *et al* (1990), 33 cows and 32 heifers were randomly assigned to two different diets (16 and 19% CP) which met undegradable requirements but differed in rumen degradable protein. The high CP diet exceeded the amount of degradable protein required. Both diets were isocaloric. They observed that first service conception rate was lower (31% vs. 48%) and plasma urea higher in animals fed the high protein diet. It was also seen that energy balance seemed to be affected by protein intake in excess of that required and especially if that protein was highly degradable. Energy balance status is thought to play an important role in determining the post-partum return of cyclic ovarian activity. When excess degradable protein is fed, large amounts of ammonia are produced and energy is required for detoxification by the liver. The energy used for this purpose could potentially alter the animal's energy balance status and subsequent fertility.

Diets high in protein contain, in many instances, excessive amounts of rumen degradable protein and this has been reported by many authors studying herds with fertility problems. Studies suggest that to avoid these problems, a diet with a moderate amount of protein of about 16% and levels of 64% or less of degradable protein should be fed. To decrease protein degradation, the simplest method would be to reduce degradable protein in the diet by formulating it with ingredients containing proteins having a natural resistance to ruminal breakdown.

Artificial methods of processing protein can also be used to protect it against degradation, such as grinding, heat treatments or treatments with chemical agents. One of the most used is the treatment of feedstuff with formaldehyde (Tamminga, 1979).

2.4.1 Mechanisms by which protein influences reproduction

Crude protein and more specifically degradable intake protein has been associated by various authors (Jordan and Swanson, 1979a; Jordan *et al*,1983; Visek, 1984; Ferguson and Chalupa, 1989) with negative effects on days open, production, the number of services per conception and calving intervals despite the observation that days to first ovulation and first service are reduced.

High intakes of highly degradable protein have also been reported as being the suspected cause of abortion in a few instances (Rupel et al 1943) and as being associated with a high incidence of post-parturient endometritis and anoestrum (Hibbitt, 1984).

Jordan and Swanson (1979a) performed an experiment in which three groups of 15 high producing dairy cows were fed from 4 days post partum for 91 days, on 3 isocaloric rations with CP contents of 12.7, 16.3 and 19.3%. The 19.3% group had fewer days to first observed oestrus (27 days) than the 12.7 and 16.3% (41 days), while these two groups had fewer services per conception (1.67) than the 19.3% group (2.47). Also the 12.7% group had fewer days open than the 16.3 and 19.3% group (69, 96 and 106 respectively). There was a linear relationship between days open, DCP intake (CP) and services per conception in the three groups combined.

In another experiment Jordan and Swanson, (1979b), studied how the intake of CP reflected itself on blood metabolites and the CP effect on fertility. They found that serum progesterone was significantly higher in cows fed 12.7% CP than in those fed 16.3 and 19.3% CP on day 14 of the first observed cycle following parturition and the cycle when conception occurred.

Folman *et al* (1981) fed isocaloric diets with different contents of protein to 3 groups, each of 20 multiparous cows, during the first 122 days after calving. They found that conception rates were 69, 56 and 44% and days open were 84, 98 and 102 for diets containing 16% protected protein and 16 and 20% unprotected CP respectively. Animals fed a diet containing protected protein had higher conception rates than those fed diets with the same amount of unprotected protein

(69 vs 56 and 44% CR respectively). The authors proposed that animals fed a protected protein diet had a higher proportion of dietary protein which escaped degradation in the reticulo-rumen therefore reducing factors detrimental to fertility.

In another trial, Folman *et al* (1983) reported that cows fed a 20% CP diet were less fertile and had a lower plasma progesterone concentration during the oestrus cycle preceding the first insemination than cows fed a 15% CP diet. This phenomenon was especially pronounced in cows fed a diet containing 85% concentrates and 15% hay, probably due to the greater amount of degradable protein found in the diet.

In a detailed study on the influence of protein on reproduction, a comparison of the effect of 2 isonitrogenous rations on the luteal function of dairy cattle was performed by Garverick *et al* (1971). They found that a soyabean meal fed group (Gp I) of 10 cows had significantly heavier corpora lutea ($P<0.005$) than a urea fed group (Gp II) of 12 cows (6.4 ± 0.6g versus 4.3 ± 0.3g respectively). They also found that the corpora lutea from Gp I, in comparison with those from Gp II, synthesized more progesterone ($P<0.05$) during an in vitro incubation in blood plasma, and contained more progesterone.

In a recent study, Blanchard *et al* (1990) observed fertilization failures or early degeneration of embryos in cows fed excessive rumen degradable protein levels. Mechanisms of fertilization failure or early embryonic degeneration in cows are unknown. Few studies have been done to correlate nutrition and embryonic mortality in dairy cattle (Blanchard *et al,* 1990), however in rats and mice, where more research has been done, there are reports that increasing dietary amounts of CP decrease embryonic survival (Knapka *et al*, 1977).

Studies have been performed on other animals such as chickens where reproductive problems have also been reported. One such study showed that diets containing 32% protein were associated with significantly lower hatchability than 16% protein diets (Visek, 1984).

Ferguson and Chalupa (1989) proposed the following mechanisms for the negative effect of protein on fertility:

a) Toxic by-products of nitrogen metabolism from the rumen may impair sperm, ova or early embryo survival.
b) Imbalances in protein and energy supply may affect the efficiency of protein metabolism and energy status.
c) Nitrogenous by-products or efficiency of energy utilization may alter the function of the hypophyseal-pituitary-ovarian axis.

All of these factors may act alone or together.

Toxic by-products of rumen metabolism, as mentioned above, have been postulated to reduce fertility. Ammonia, urea and other unidentified nitrogenous compounds in body tissues could increase when excess degradable intake protein is fed (Ferguson and Chalupa, 1989). This excess may cause cellular damage throughout the body, resulting in a tolerable, but sub-optimal uterine or ovarian environment which could reduce reproductive performance (Jordan and Swanson, 1979a). Randel (1990) proposes that perhaps high concentrations of certain metabolites or catabolites inhibit post-partum rebreeding performance in cattle.

Urea-N and ammonia increase pH in the reproductive tract, reducing fertility as sperm are more active and survive longest at a neutral pH (Jordan and Swanson, 1979a). Research on levels of urea-N in vaginal mucus related to fertility has been performed by Carroll *et al* (1987) where they concluded that cows did not conceive when they had vaginal mucus urea-N levels higher than 40 mg/100 ml. Impaired fertility can also result from toxic effects of ammonia and urea-N on ova, sperm and early embryos as it has been shown that urea is toxic to sperm and ova and can cause abortion when injected intra-amniotically (Jordan and Swanson, 1979a; Ferguson et al, 1988; Ferguson and Chalupa, 1989).

Elevation of tissue ammonia associated with high intakes of CPDM has been thought to delay clearance of uterine contaminants by reducing immunologic function of macrophages and white blood cells (Ferguson and Chalupa, 1989).

However results from Carroll *et al* (1988) do not support these theories. On studying the impact of DCP concentration (13 vs 20%) and feeding strategy (total mixed ration vs separate feeding of the forage and concentrate) on reproductive performance of 57 early lactation dairy cows, they reported that cows fed a 20% ration had a higher CP intake, higher ruminal ammonia and higher urea-N in plasma and vaginal mucus. Although there was no difference between low and high CP groups in days to first oestrus, days to first service or services per conception, days to first estimated ovulation were longer in the high CP group. They also observed that even though urea-N increased when high CP was consumed, no differences occurred between vaginal urea-N concentrations of cows that did and did not conceive (15.3 vs 16.3 mg/100ml urea-N). However, there is a contradictory point in this work. They point out that no cow conceived at any of 4 services that occurred when vaginal mucus urea-N concentrations exceeded 100 mg/100ml but consider that these high levels are due to sources other than CP intake like, for example, bacterial infection, urine or faeces. Although the authors do not clearly explain this observation, it can be speculated that these last observations were probably of individual animals and did not show a statistical significance.

The effect of excess protein on fertility can be expressed through the levels of reproductive hormones (Swanson, 1989). Nutrient effects on the hypothalamic-hypophyseal-ovarian axis ultimately seem to have an impact on progesterone production by the corpus luteum. It has been hypothesized that high systemic or local urea-N concentrations may reduce LH binding to ovarian receptors on luteal cells, leading to a decrease in progesterone concentrations and therefore a lowering of fertility (Carroll *et al*, 1988).

In many studies on the effect of excess DCP on fertility, progesterone has been used as a measure of luteal function as it has important influences on follicular maturation, the passage of fertilized embryos through the oviduct to the uterus, secretion of uterine milk by endometrial glands and maintenance of an environment in the uterus conducive to pregnancy. Mid-luteal serum progesterone concentrations in the cycle prior to breeding have been correlated with conception rate (Ferguson and Chalupa, 1989).

Low serum progesterone has been reported, in various studies, when concentrations of crude protein dry matter (CPDM) exceeded rumen requirements for DIP.

Jordan and Swanson (1979b) found that the serum progesterone concentration was significantly higher in cows fed 12.7% CP when compared to those fed 16.3% and 19.3% CP. This occurred when measurements were made on day 14 of the first observed cycle and the cycle in which conception occurred. Also, LH tended to be lower on days 2 and 14 of the first post-partum oestrous cycle, the preconception cycle and the conception cycle in those fed 12% CP compared to the other two groups.

However, Erb *et al* (1976) found that when increasing the amount of dietary urea, as a substitute for plant nitrogen, progesterone in blood plasma also increased significantly during three consecutive lactation periods. On the other hand, Blauwiekel *et al* (1986) demonstrated that CP did not have a primary effect on the pituitary or hypothalamo-hypophyseal regulation of LH or progesterone in both intact and ovariectomized dry cows fed rations containing 15 or 25% CP.

Garcia-Bojalil *et al* (1991) performed a study using superovulated non-lactating Holstein cows fed diets with high and low levels of protein (27.4 vs. 12.3%). They observed that these widely different protein dietary treatments failed to alter ovarian follicular development and subsequent embryo yields or quality associated with superovulation. The animals used in this experiment were not under the stress of lactation so it could be assumed that although one group of animals was fed a diet high in protein, the amount of energy in the diet, though not specified in the paper, might have been sufficient to maintain a balance between ingestion and metabolism of protein and thus result in no adverse effects on fertility.

Another hypothesis relating a high intake of DCP to fertility problems that has been suggested is that excessive protein intake may lower fertility by suppression of the immune system through high concentrations of circulating ammonia reducing the immuno-responsiveness of animals against disease (Carroll *et al*, 1983; Visek, 1984). Thus, cows fed high CPDM often had more health problems post-partum and during early periods of lactation (Visek, 1984).

Jordan *et al* (1983), observed a significant difference between plasma urea concentrations (6.3 vs. 8 μg/ml) of cows fed 12 and 23% CP rations. First lactation cows with uterine infections in the high protein group had a prolonged period to first estimated ovulation compared with first lactation cows with uterine infections in the low protein group. Results of in vitro trials show that elevated systemic ammonia reduces the immuno-responsiveness of animals against infectious diseases (Carroll *et al*, 1988).

2.5 Energy-Protein balance and its effect on reproduction

Many studies have demonstrated conjoint effects of energy and protein on reproduction. Energy is the primary determinant of microbial growth. It is also needed for degradable protein metabolism. Ruminal ammonia not used for microbial growth is absorbed into the blood stream and converted to urea in the liver. If dietary energy is deficient, the feeding of excess rumen degradable protein can have the effect of exaggerating a negative energy balance (Ferguson and Chalupa, 1989) and thus affect reproductive efficiency.

Excess ammonia from ruminal degradation of protein, or non-protein nitrogen from amino-acid in excess of the production or growth needs of a cow are converted to urea. High concentrations of ammonia in the rumen of animals fed on high protein diets, may require excessive expenditure of energy for its detoxification. Energetically this is an expensive process (Martin and Blaxter, 1965) and a reduced efficiency of ME utilization at excessive CP intakes would be expected (Oldham, 1984).

Energy needs may vary according to the animal's status. In lactating cows, maintenance needs for energy are approximately 10% higher than for dry, non-pregnant cows. It has also been seen that energy needs are about 10% higher in cows grazing lush pastures and as much as 20% more for those grazing sparse pastures (Miller, 1979).

Energy deficient diets or negative energy states, as reflected by weight loss and low blood glucose, have been reported as having adverse effects on fertility (Blanchard *et al*, 1990). However some degree of energy deficiency in early lactation is inevitable in dairy cows of potentially high yield. But, provided it is kept to a minimum, satisfactory production, health and fertility can still be maintained (Whitaker *et al*, 1983).

Energy balance is an important regulator of days to first ovulation in cows (Ferguson and Chalupa,1989). A low energy status due to dietary restrictions, during the late prepartum period, results in weight loss and decreased body fat at calving lowering the number of cows and first lactation heifers that return to oestrus early in a defined breeding season (Randel, 1990).

The first effect expected to be observed in lactating cows when there is energy deficiency would be a reduction of milk production, accompanied by loss of body condition (Miller, 1979). Factors associated with negative energy balance, weight loss and milk yield have been implicated as causes of reproductive failure (Weaver, 1987).

A low energy status may manifest itself either in the form of a subclinical ketosis or, in the case of extreme cases of energy deficiency, as a clinical ketosis (Whitaker *et al*, 1983, Hibbit *et al*, 1969). Energy deficiencies are most likely to occur in early lactation during the periods of uterine involution, reinitiation of oestrous activity, and first inseminations (Weaver, 1987).

In early lactation, cows must mobilize body fat and protein to supply energy and amino acids for maximum milk production. However, body stores can only supply limited amounts of nutrients. Either milk production suffers or metabolic disturbances such as fatty liver and ketosis can occur if energy deficits are acute or severe. Hibbit *et al* (1969) observed that the amount of CP in the diet had a marked effect on the severity of induced ketosis, the most severe clinical signs being seen in the cows on the high protein ration. Cows that are too thin at calving have reduced milk yield and reproductive performance (Weaver, 1987). Hibbitt (1984) cites an experiment done by Reid (1983) involving 100 dairy cows in 3 herds, where he demonstrated that cows with fatty livers after calving had a longer calving interval than normal cows in the same herd.

Butler *et al* (1981) and later, Butler and Smith (1987) found that during early lactation, increasing dietary intake fails to keep up with requirements for rising milk production. The negative energy balance that results from this can be directly related to the post-partum interval to first ovulation, lower conception rates and delayed ovarian activity through decreased LH pulsatile secretion. Dunn *et al* (1969), observed that beef cows can similarly suffer from reproductive failure due to low levels of energy intake.

Using intact cows and heifers and spayed cows, Beal *et al* (1978) measured the effect of dietary energy on pituitary and luteal function by administering gonadotropin releasing hormone (GnRH) following a prolonged period of either energy restriction or adequate energy intake to determine if energy intake influenced the pituitary responsiveness to GnRH. They concluded that dietary energy restriction may influence LH release directly at the pituitary level as well as indirectly through effects on ovarian steroid production. Randel (1990) also reported that post-partum cows receiving low energy diets had been observed to have decreased mean serum concentrations of LH. It has also been proposed that restricting feed intake can reduce the response to LH by the corpora lutea, possibly due to a lack of substrate reserves with which to respond to LH, rather than to a sensitivity to LH (Apgar *et al*, 1975).

Sonderegger and Schurch (1977) observed that there was a significant influence of energy and protein supply on fertility. A deficient intake of energy and protein resulted in declining fertility. When animals were supplied sufficient energy during the first four months of lactation, the interval from first service to conception and from parturition to conception was reduced.

Lucy (1990) in a 2 year study, with post-partum holstein dairy cows, aiming to observe the interaction of diet, energy and reproductive performance, found that ovulation occurred earlier in cows consuming more dry matter (19.8±.5 kg/d vs 18.2±.5 kg/d; P<.01) and pregnancy occurred in cows with higher energy balance (EB). Fat feeding however, reduced post-partum reproductive efficiency. In another study, Lucy *et al* (1991), observed that there was an influence of energy balance on follicle populations at different times after calving.

Ropstad and Refsdal (1987) conclude that a negative energy balance, together with high CP, will tend to increase the frequency of ovarian cysts and postpone the onset of ovarian activity after calving.

2.5.1 Blood metabolites as indicators of nutritional energy status

A cow's energy status can be measured in the form of non-esterified fatty acids (NEFA), glucose and ketone bodies such as aceto-acetate, acetone and ß-hydroxybutyrate (BOHB).

NEFA levels in blood have been proposed as useful indicators of carbohydrate utilization. NEFA are released in the blood plasma when adipose tissue is mobilized to meet the energy demands of the animal and thus are considered sensitive indicators of fat mobilization during energy insufficiency (Holmes and Lambourne, 1970).

Nevertheless,there have been several reports where variations have been observed in their concentration unrelated to energy intake (Holmes and Lambourne, 1970; Bowden, 1971). For example, NEFA have been observed to increase with the excitement provoked by blood sampling and handling of animals (Parker and Blowey, 1976).

Blood NEFA levels can also show a strong variation related to time of feeding, with the highest levels being found before feeding and the lowest, 2 to 3 hours after feeding. Holmes and Lambourne (1970) observed that in Hereford heifers fed 6 times per day, NEFA levels fell after each meal.

Levels of feed intake can markedly influence the levels of NEFA. If feed intake is restricted, NEFA increase. Increased energy requirements of pregnancy and lactation, sometimes combined with a decrease in dietary intake of energy sources usually produces an increase in plasma NEFA levels (Bowden, 1971).

BOHB is a ketone body which is present in the greatest quantity in the blood of normal, well fed animals. Blood BOHB concentrations have been observed to be inversely correlated with estimated energy level of the ration during the first weeks of lactation, when more energy is required. However, the correlations between energy balance and BOHB are weak and therefore some authors do not think BOHB is an adequate indicator of energy balance (Herdt *et al*, 1981) while other authors see this measurement as one of the best measurements of energy changes in the diet (Ducker *et al*, 1985b).

Kelly (1977), measured BOHB serum concentrations at regular intervals throughout a lactation in groups of cows from 3 commercial herds. He found that BOHB levels changed significantly only when the dietary intake of the cows was altered in quantity or in quality. In grazing animals he found that serum BOHB concentrations were significantly higher as the quality of pastures deteriorated.

Aside from feed intake, other factors such as blood collection techniques, stage of lactation, pregnancy and time of the day can also influence BOHB concentrations (Herdt *et al*, 1981).

A very commonly and more frequently used measurement to aid monitoring of the nutritional status in animals is blood glucose concentration.

However, some authors do not consider blood glucose a valid indicator of energy balance. Erfle *et al* (1974) found that blood glucose levels have a limited correlation with energy balance. Herdt *et al* (1981) studying the effect of ration composition and energy balance on BOHB and plasma glucose concentrations of Holstein cows over the first 6 weeks of lactation, assessed the correlation of these metabolites with estimated energy balance and the effects of the ration variables (concentrate, CP, and methionine hydroxyanalog content) on this correlation. He concluded that although there was a correlation between plasma glucose and energy balance, it was weak and therefore subject to the influence of variations in ration composition.

Blood metabolites such as glucose, NEFA and BOHB, not only reflect energy status but can also be related to dietary protein intake. A study by Nachtomi *et al* (1991) concluded that plasma parameters such as NEFA, 3-hydroxybutyrates (3-OHB), glucose and total alpha-amino nitrogen reflected the dietary protein and energy status of 46 multiparous dairy cows in early lactation fed 3 dietary concentrate levels (50, 65 and 80%) and 2 CP levels (17 and 21%) of dry matter.

Plasma NEFA and 3-OHB were lowered and glucose levels raised as energy intake increased when concentrate levels were raised. On the other hand, the high CP diet elevated the levels of 3-OHB while plasma levels of NEFA and glucose remained unchanged.

2.5.2 Blood metabolites as predictors of fertility problems

Ducker *et al* (1985b), evaluating the diagnostic role of blood measures as predictors of the reproductive performance of dairy cattle, analyzed serum and plasma samples for BOHB and for NEFA as measures of energy status. They found that heifers with serum BOHB levels above or equal to 0.7 mmol/l had significantly higher pregnancy rates to first AI than with those with levels lower than 0.7 mmol/l. Furthermore, Blauwiekel and Kincaid (1986), report that NEFA have been shown to be negatively correlated with energy balance.

Miettinen (1991), studied the relationship of serum glucose, aspartate amino transferase (ASAT) and urea to reproductive performance in 45 Finnish dairy cows from 8 different herds. He found no correlation between blood metabolites and fertility parameters before calving, but 2 weeks after calving he found a significant negative correlation between glucose levels and the interval from calving to first insemination.

On the other hand, Eldon *et al* (1988) observed a significant positive correlation between the level of glucose and the time of conception. Their results also suggest that low blood glucose levels early post-partum, due to negative energy balance, are probably the cause of subclinical and clinical ketosis which leads to delayed onset of ovarian activity.

McClure and Payne (1978), studied the metabolic profile of 12 herds. The first service non-return rate of each herd was calculated from breeding records of 10 cows mated closest to the date of each profile test. These rates were found to be positively related to the mean blood-glucose concentrations of lactating cows.

However, findings by Blowey *et al* (1973), do not agree with these results. They concluded that there was no relationship between blood glucose and fertility in a study on 12 dairy herds that were bled at 4 weekly intervals and tested for levels of glucose, urea and albumin.

Aside from blood metabolites, milk acetone as a measurement of energy status has been used by Andersson and Emanuelson (1985). In an epidemiological study of hyperketonaemia in Swedish dairy cows, they measured the milk acetone of 3078 animals from 126 herds at the first 3 monthly production tests after calving. Milk acetone concentrations above 0.4 mmol/l were considered as indicators of hyperketonaemia. Incidence of clinical ketosis and ovarian cysts was greater with increasing milk acetone concentration. A significant correlation was found between the prevalence of hyperketonaemia in milk and herd means of the intervals from calving to first and last service.

2.6 Other factors that may affect protein-fertility relationships

Protein and fertility relationships may depend on pre-existing conditions like metritis (animals in poor health) and on factors such as age (animals in first lactation or older cows), or on body condition, dietary concentration of energy and other factors not yet identified that may or may not interact with each other. All of these factors may be exacerbated by high protein intake (Ferguson *et al*, 1989; Carroll *et al*, 1988).

Age has been related to fertility problems in dairy cattle in studies such as the one performed by Ball (1978). He measured milk progesterone concentrations twice weekly in approximately 200 autumn calving cows during two successive years. From this study it was concluded that embryo death, later than the fourteenth day after AI, occurred in about 10% of cows in each year. He observed that the incidence of embryo deaths increased with age of the cow and that most deaths occurred at 35 days.

In New Zealand seasonal dairy herds, first calving heifers and cows more than 7 years old have lower conception rates than cows aged 3 to 7 years. In heifers this seems to be due to a longer period of post-partum anoestrus and is only a minimal difference. Older cows on the other hand experience a higher incidence of repeat breeding and early embryonic death (Macmillan, 1985a).

Experiments reviewed by Otterby and Linn (1983), indicate that animals losing weight had lower conception rates than those that were gaining it. Various authors (Swanson, 1989; Otterby and Linn, 1983; Thatcher, 1986) point out the importance of condition scoring animals in late lactation or when dry and feeding them according to the need to increase weight or at least minimize weight losses in body weight for ovulation and conception to occur.

Kaim *et al* (1983) observed that cows in their fourth and later lactations lost significantly more weight than cows in their second and third lactations and fertility was seriously affected when they were fed high protein diets. From these results they concluded that increased weight loss in older cows combined with higher milk yield, interacts with the increased energy cost of detoxification of ammonia and the excretion of urea in high CPDM diets to impair fertility. Excessive protein intake may depress post-partum rebreeding performance, especially in older cows (Randel, 1989).

Howard *et al* (1987), on the other hand, in their experiment in which 146 cows were fed either a 15% or 20% CP diet of 45% sorghum silage and 55% concentrate from 10±3.5 days after parturition to 149±3.5 days of lactation, observed that older cows (fourth and greater lactation) tended to have fewer services per conception (1.2 vs. 1.5) than younger cows in their second and third lactation. Conception rate was not affected negatively in this study probably due to the considerably smaller body weight losses (0.4 and 2%) in animals fed on a high protein diet. Therefore these cows responded in a very different manner to those observed by

Kaim *et al* (1983). An explanation for the different outcomes is that whereas energy was limiting in this latter study, it was probably in ample supply in the study of Howard *et al* (1987).

Urea consumption is believed to affect reproductive efficiency in dairy cattle and is also associated with higher incidence of retained placenta in cows at second calving.

Erb *et al* (1976) reported that 191-290 g/day of urea reduced reproductive efficiency, while Otterby and Linn (1983) note that cows fed urea at 1.8% of total ration dry matter, had a 44% incidence of retained placenta. Norton (1989) cites an experiment performed by Rupel et al. in 1943, using holstein cows to evaluate the use of a urea diet versus a vegetable protein diet. An abortion from one of 6 cows receiving an 18% CP concentrate supplement equal to about 10% of their diet was reported; this diet contained 3% urea.

Other researchers report the occurrence of abortion with the inclusion of urea in the diet. Norton (1989) mentions a study performed by Ryley (1961), where a comparison of the value of sorghum silage fed ad libitum with or without urea, to pregnant Hereford heifers was done. In this study, he recorded 2 abortions in a group being fed 42g of urea per day. Heifers that had been on the experiment for 23 and 74 days, aborted at 205 and 246 days of gestation respectively. No abortions were reported in the control group. An analysis of the ration of the group being fed 42g of urea/day, indicated that the rumen degradable protein (RDP) was several times in excess of that required and that ME was deficient.

In a study by Oltjen (1967), one of each of two sets of twins was given a urea purified diet while the other was given a natural diet. The two heifers fed with urea produced 2 calves and had 3 abortions while the other two had no abortions and produced 4 calves. Also it was observed that urea-fed heifers appeared to have shorter oestrus cycles, a longer gestation period and required more services per conception.

Neither infertility nor abortion were reported in other experiments using urea feeding and conducted by authors such as Archibald (1943), Bond and Oltjen (1973) or Wohlt and Clark (1978).

It has been noted that the use of nitrogenous fertilizers seems also to have been related to infertility. Stables and Bounds (1969), early observed that the increased use of artificial nitrogenous fertilizer seemed to have a negative effect on fertility. In a survey performed by these authors, records of land fertilization and reproductive data (number of services, non-returns, number of services per conception) from 100 herds from the years 1965, 1966 and 1967 were collected. From these data they observed that when there was an increase in nitrogen application, at 100 + units of nitrogen per acre, a parallel fall in fertility occurred.

3.0 STUDIES ON PROTEIN DONE IN NEW ZEALAND

3.1 Use of pastures in dairy cattle feeding

New Zealand dairy farms, for economic and climatic reasons, rely heavily on grazed pastures as the main source of feed for cows. Thus the supply of feed varies during the year because of the changes in growth rate of pasture. The average size of a seasonal dairy herd in New Zealand is around 134 cows that are intensively stocked in about 30 paddocks of one to two hectares (Macmillan,1985a).

Feed requirements of cows will vary according to their reproductive and lactational status. On the seasonal supply New Zealand dairy farm, cows calve in spring, during which their feed requirements increase due to lactation. Calving is timed so that increasing feed requirements coincide with the increase in pasture growth rates in spring (Holmes and Wilson, 1987).

Most of the pastures on dairy farms contain several different species and varieties of grasses and legumes. Perennial ryegrass forms the basis of the majority of pastures on intensive dairy farms. The Nui cultivar is the most productive in summer and autumn amongst the other cultivars found here. Other grasses used are Cocksfoot, Timothy, Prairie grass and Paspalum.

Clover is commonly found as a component of pasture mixtures with white clover being most widely used. Red clover is also used but although it grows more rapidly in summer, it is slower in winter and less persistent. Lucerne is used but usually it is sown as a pure sward.

Application of fertilizers has been done routinely every year on the vast majority of intensive dairy farms in New Zealand in the past. Fertilizers used contain phosphorus and often potassium. Nitrogenous fertilizers are also applied on many dairy farms particularly in autumn or early spring in order to provide extra feed at these times. Other farmers do not apply them at all and rely only on clover fixation of atmospheric nitrogen.

Nitrogen is normally applied at rates of 20 to 40 kg N per Ha. at each application, with individual paddocks receiving only one dressing per year on the majority of farms.

Pasture growth often surpasses feed requirements in late spring and early summer. This excess is cut and conserved in many cases as silage in spring and hay in summer.

Aside from pastures, annual greenfeed crops are also grown to be fed at various times of the year. Among those used are maize, sorghum, rape, kale, swedes, turnips, oats and Italian ryegrass.

A variety of meals or concentrates, based mainly on barley or maize meals, are available to be fed to cattle but at high prices so they are not commonly used unless there is a severe shortage of feed.

Urea is rarely used to replace protein in diets where the rest of the ration is deficient in rumen degradable protein.

Rotational grazing is the most common practice here because it simplifies the controlled grazing and conservation of pastures, minimizing pasture wastage.

3.2 Nutritional content of pasture based diets (Holmes and Wilson, 1987)

The feeding value of pastures depends primarily on their species composition, their maturity and stage of growth. Immature pastures have a higher feeding value than mature ones. They contain relatively large amounts of digestible cell contents and voluntary intake is high. In mature pastures, ME decreases and there is a low proportion of cell constituents with a high content of indigestible lignin. This makes them less palatable and therefore decreases voluntary intakes.

The CP concentration of immature pastures normally ranges between 200-300 g/kgDM and as pasture matures it can fall to 100-200 g/kgDM. In young pastures, 85-90% of the total nitrogen is in the form of true plant protein and the rest as non-protein nitrogen. Non-protein nitrogen concentrations are highest in immature rapidly growing grasses. The degradability of pasture protein is still unknown but it is likely to be high. The amounts of RDP and undegradable dietary protein (UDP) likely to be supplied by some typical feedstuffs can be observed in table 1. A summary of the nutrient composition of most commonly used feeds is shown in table 2.

3.3 Seasonal variations in pastures

Grasses generally grow well during the spring and autumn while legumes grow better in the late spring and summer. Digestible energy in mixed ryegrass-white clover pastures appears to decline from a maximum of 80% in late October to a minimum of 60% in mid February and then rise again to about 75% in mid April (Holmes and Wilson, 1987). The chemical composition of pasture components can follow a seasonal pattern which may be more evident in some components than in others (Metson and Saunders, 1978).

Autumn pastures are thought to have a lower nutritional content than spring pastures (table 3) although both can have similar digestibilities. The efficiency with which ME is used is also low in these pastures and this could be associated with high protein and non-protein nitrogen concentrations and low soluble sugar levels (Holmes and Wilson, 1987).

Metson and Saunders (1978) found that even though trends in total nitrogen (or "crude protein" = N_u x 6.25) were similar in grass and clover components of pastures in the lower North Island of New Zealand, levels in clover were generally higher. They also saw that the highest concentrations occurred in late autumn to early spring and the lowest in summer. Small seasonal variations were seen in total sulphur. Ross et al (1978) reported similar findings but in their study they also noted the influence of fertiliser nitrogen on nitrogen and carbohydrates in grass-clover pastures. The highest level of fertiliser N was reflected in higher total nitrogen/total water soluble carbohydrate ratios and higher herbage nitrate N contents especially in early spring.

Table 1. Rumen degradable and undegradable protein concentrations in some typical feedstuffs for dairy cattle

	ME (MJ/kgDM)	CP (g/kgDM)	dg	RDP		UDP (g/kgDM)
				(g/kg)	(g/MJME)	
Leafy pasture	11.0	240	0.8	192	17.5	48
Maize silage	10.3	80	0.8	64	6.2	16
Hay (good quality)	9.7	170	0.8	136	14.0	34
Pasture silage	10.0	220	0.8	176	17.6	44
Pasture silage (+Formaldehyde)	10.0	220	0.4	88	8.8	132
Green feed maize	10.3	90	0.8	72	7.0	18
Stemmy pasture	8.0	100	0.8	80	10.0	20
Barley meal	13.0	110	0.8	88	6.8	22
Barley straw	6.5	40	0.8	32	4.9	8
Protein supplements						
Soya bean meal (untreated)	12.9	500	0.8	400	31.0	100
Linseed meal	12.0	350	0.6	210	17.5	140
Urea	--	2875	1.0	2875	--	--

From: Holmes and Wilson, 1987

Table 2. Summary of nutrient composition of commonly used feeds for dairy cattle

Feedstuff	Dry matter (%)	Crude protein (g/kg DM)	Metabolisable energy (MJ/kg DM)	Mineral conc. (g/kg DM)			
				Ca	P	Mg	Na
GREEN FEEDS							
Grass/clover mixtures							
Spring, leafy	14	240	11.8	6.0	4.5	1.5	1.5
Summer, leafy	20	150	10.0	8.5	4.0	2.0	2.0
dry & stalky	25	100	8.0	7.0	3.0	2.0	1.0
Winter, autumn saved	17	200	10.0	7.0	4.0	1.8	1.5
leafy	14	260	11.2	7.0	4.5	1.5	1.5
Kikuyu grass, summer	22	140	8.5	6.0	3.9	1.8	0.6
Lucerne, leafy	18	280	12.0	16.0	3.0	2.5	0.6
10-20% flower	23	220	10.0	13.0	2.8	2.4	0.5
Maize, 1.3-1.6m	22	90	10.3	4.0	2.5	1.5	0.2
Oats, leafy	18	180	12.3	6.0	3.0	1.5	4.0
Paspalum, leafy	18	180	10.5	7.5	4.0	2.5	0.6
flowering	23	100	9.3	5.6	3.0	2.5	0.4
Red clover, spring	17	280	11.5	11.0	3.5	3.0	0.8
Sorghum, Sudax (1m)	20	180	10.0	4.7	2.3	2.0	0.2
Tama ryegrass	12	240	12.0	4.0	4.0	1.5	2.5
White clover	15	280	12.2	12.0	4.0	3.0	3.0
SILAGES							
Grass/Clover mixtures							
Good quality	23	200	10.0	7.0	4.3	1.7	1.7
Poor quality	28	150	8.0	5.5	2.8	1.4	1.6
Lucerne	20	200	9.5	10.0	2.6	2.0	0.5
Maize, early dent	30	80	10.3	3.0	2.0	1.2	0.1
HAYS							
Grass/Clover mixtures							
Good quality	85	170	9.7	8.0	4.0	2.0	2.0
Medium	85	110	8.5	6.0	3.5	1.9	1.7
Poor	85	70	7.[illegible]	4.0	3.0	1.8	1.5
STRAWS							
Barley	85	40	6.5	3.0	0.8	1.7	1.1
Maize stover	85	50	7.5	6.0	1.0	4.5	0.7
Pea	85	80	7.0	16.0	1.2		
Ryegrass	85	60	7.5	4.0	3.0	1.5	1.5
CROPS							
Choumoellier	15	145	11.5	15.0	2.4	2.7	3.3
Fodder beet (whole plant)	18	100	11.5	1.2	1.7		
Maize (see green feeds)							
Mangolds (roots)	10	100	11.5	1.5	1.8	2.0	6.0
Potatoes	24	90	12.0	0.3	2.5	1.0	1.0
Rape	17	160	12.0	15.0	4.0	0.7	0.5
Swedes, bulbs	10	120	12.4	1.3	2.0	2.0	1.0
tops	15	150	12.8	25.0	2.7	4.0	2.0
Turnips, bulbs	9	150	12.4	6.0	3.0	2.0	2.0
tops	13	180	12.8	35.0	3.4	4.0	3.0
CONCENTRATES							
Barley	86	110	13.0	0.6	4.4	1.8	0.3
Bran (wheat)	86	160	9.8	1.0	12.0	6.0	0.4
Linseed cake	87	300	12.0	4.4	8.0	6.0	0.7
Lucerne meal	87	200	11.0	16.0	3.0	3.0	1.5
Maize	86	80	13.6	0.03	4.2	2.0	0.03
Meat and bone meal	94	500	10.7	103	50	12.0	7.0
Oats	86	130	11.5	1.1	3.9	1.4	0.1
Peas	87	240	13.0	1.4	4.3	1.7	0.1
Skim milk powder	94	350	13.0	12.5	10.0	1.2	6.0
Soya beans	90	500	12.9	2.7	5.5	2.6	0.1
Wheat	86	130	12.6	0.6	4.0	1.6	0.1
MISCELLANEOUS							
Brewers grain	24	230	10.0	3.0	6.0	1.0	2.0
Molasses	75	40	12.0	12.0	1.0	4.3	1.5
Urea	99	2875	-	-	-	-	-

From: Holmes and Wilson, 1987

Time of the day also has an effect on levels of water soluble carbohydrates. Hott and Hilts (1969), when studying water soluble carbohydrate levels in alfalfa and blue grass, found that water soluble carbohydrate percentages in alfalfa followed a curvilinear trend from a low at 6:00 am to a maximum level at 12:00 noon with a slight decrease at 6:00 pm. Grasses underwent linear increases in water soluble carbohydrate percentage from 6:00 am to 6:00 pm. Likewise, the non-structural polysaccharide content followed an increasing linear trend in the day time. Variations in water soluble carbohydrate content accounted for almost all of the daily variation in total nonstructural carbohydrate content in blue grass.

Metson and Saunders (1978) observed that mean levels of total carbohydrates seemed to reach their maximum in winter and their minimum in late summer. Varta and Bailey (1974) also mention that water soluble carbohydrates in perennial ryegrass in Canterbury increase during winter to a peak value of 19% of herbage dry matter and occasionally they can exceed 30% of herbage dry matter. The same authors (Varta and Bailey, 1980) in a later study using rye grass, found that the highest concentration of water soluble carbohydrates (47% of DM) is in the stubble.

The mineral content in mixed pastures also exhibits seasonal variations (table 3).

3.4 Breeding patterns in New Zealand: Background

New Zealand dairy herds are mostly seasonal in their calving patterns. Approximately 90% of cows are artificially bred in spring (Macmillan, 1985b) while others will be bred in autumn.

Aside from seasonal dairy herds, there are also town supply dairy herds. Town supply dairies work on a quota basis. This

Table 3. Seasonal variations in organic matter (OM) digestibility, ME concentration and some minerals in grazed pasture

	July-Sept	Oct-Dec	Jan- March	Apr-June
OM digestibility (%)	80.1	76.5	69.2	75.1
ME concentration (MJ/kgDM)	11.8	11.2	9.8	10.9
N concentration (g/kgDM)	42.0	41.0	38.0	44.0
P concentration (g/kgDM)	4.4	4.0	3.2	4.1
Ca concentration (g/kgDM)	5.7	6.9	6.7	6.7
K concentration (g/kgDM)	30.5	31.1	27.1	32.3
Mg concentration (g/kgDM)	1.7	1.9	2.1	2.1
Na concentration (g/kgDM)	1.7	1.6	1.1	1.5

From: Holmes and Wilson, 1987

is, a nominated amount of milk must be produced each day for whole milk consumption and this is paid for at a higher rate than that received by a seasonal producer. Surpluses over the quota are generally transferred to a dairy company and processed into a variety of dairy milk products, and for this the dairy producer receives a payment similar to that received by the seasonal producer (Fielden *et al*, 1980).

Breeding in this type of herd, is not limited to only spring season, it can be done in any of 4 seasons. However, in studies comparing reproductive performance between the 4 seasons, it was reported that the best results ($P<0.01$) were obtained during the spring period (Fielden *et al*, 1980).

To be effective, oestrus detection had in the past been done by frequent observation of mounting activity. With adequate checks at each milking, an experienced eye could detect about 90% of all the heats (Foote, 1975). More recently heat detection accuracy levels can be equally high or even higher by observing for mounting activity at each milking with the added help of detection aids. Tail paint is one of these aids and is commonly used in effective oestrus detection with a rate of over 90% detection routinely achieved at first ovulation in well managed New Zealand herds (Macmillan, 1985a and b).

Cows that demonstrate clear signs of oestrous behaviour are inseminated. Oestrus manifests itself visually by the willingness of the cow to stand when mounted, and/or the presence of ruffled hair over the rump. Other signs can be restlessness, sensitivity to palpation of the rump, chin resting and rubbing,group activity, the cow standing up when others are lying down, raising her tail when contacted by others, having a pink and swollen vulva, clear mucus discharge from the vulva, nudging and sniffing other cows or demonstrating an appetite and milk production decrease (Williamson *et al*, 1972; Foote, 1975).

Once the breeding season begins in all herds, an intensive breeding programme commences during which it is expected that cows will conceive during the first 3 to 4 weeks irrespective of their post-partum interval. The breeding program in New Zealand may last in total 17 weeks or 4 calendar months that spans the interval from the date of the first cow being inseminated to the date the bull is finally removed from the herd (Macmillan, 1985b).

A common practice in New Zealand is to inseminate cows one occasion per day. Any cow seen in heat will be inseminated at the next occasion on which the inseminator visits, generally in the morning after milking. Thus, a cow seen on heat that morning will be inseminated, as will any cow seen after the inseminators visit on the previous day. Also, any cow that still is obviously in oestrus the morning after previous insemination is usually re-inseminated on the second day (Macmillan, 1985a).

Only 2-3% of dairy cows will not display oestrous behaviour where they are ridden at least 3 or 4 times by other herdmates if oestrus occurs during the first 6-7 weeks of the breeding programme (Macmillan, 1985c).

In some well managed herds, prostaglandin F_{3b} (PGF), intravaginal progesterone devices (CIDR) and gonadotrophin releasing hormone are used to reduce the intervals from planned start of breeding program date (PSB) to mean first insemination date (MFID) and mean conception date (MConD), thus concentrating calving patterns and potentially increasing average lactation length and reducing herd empty rates (Macmillan, 1985c).

The average conception rate (CR) to first insemination in New Zealand is around 67 to 69% but when subsequent abortions and cow deaths are taken into consideration, the final conception rate is only 60% (Macmillan, 1985a) which is still considered as high by international standards. According to studies by Macmillan and Moller (1977), the average proportion of empty cows by the end of the breeding season in their survey, was 5.4%.

The gestation length for Zealand dairy herds is relatively constant at 282 days but it can range from 272 to 293 days (Macmillan and Curnow, 1976; Fielden *et al*, 1980). Macmillan and Curnow (1976) report that a seasonal effect on gestation length can be observed sometimes and that it is usually reflected in longer gestation periods in cows that calve during the winter.

Macmillan and Moller (1977), studied records for over 28,000 cows from 316 herds serviced by the Bay of Plenty Livestock Improvement Association obtained from the Bay of Plenty Fertility Survey and observed that the estimated intervals from calving to first service varied from 57 to 76 days in seasonal herds while Fielden *et al* (1980) observed in their study, a mean calving-to-first-service interval of 85 days for town-supply dairy herds.

The average calving interval observed in New Zealand is 365 days (Macmillan and Moller, 1977). However, within herd variation can be large. To reduce this variation, oestrus synchronisation is sometimes used thus increasing the seasonal concentration of calving pattern and improving reproductive performance.

3.5 Studies in New Zealand on the effects of crude protein on reproduction

Fertility problems related to nutritional status in pasture based New Zealand dairy cattle have been observed and studied frequently. McClure (1959) performed a study of a random sample of 66 Waikato dairy herds covering 148 herd mating seasons. His goal was to determine the incidence of each of the known causes of herd infertility and to define the types of infertility of unknown causes. The majority of problems in artificially inseminated herds and 50% of problems in naturally mated herds were due to unknown causes. He proposed that these unknown factors could be environmental and that the problems are mainly related to female infertility rather than to that of the male.

In another study (McClure, 1961a), the same author proposed that the evidence indicated that some of these unknown causes may be provoked by a nutritional lactational stress that in its milder form causes infertility in adult cows but not in first lactation cows. However, in its severe form it can cause equally poor fertility in all ages of lactating cows. An inverse association between the fertility and total nitrogen content of the pasture was observed in herds feeding on short, rapid growing pasture, in which the clover content was low (McClure, 1961b).

The problem was seen to be temporary, lasting only 3 to 9 weeks and was associated with the failure of the cows to regain weight lost under the stress of increasing milk production during early lactation. McClure (1965 and 1970a) proposed that possible reasons for this marked loss of body weight would include gross underfeeding, either because of insufficient availability of pasture or because of high content of water (lushness) in pasture that restricted the amount of dry matter ingested; or the occurrence of specific nutrient deficiencies or excesses in the pasture.

The same author (McClure, 1970b), in further studies which attempted to reproduce the same fertility problem experimentally, found that immature grasses and green oats, especially when fed in limited amounts, contained sufficient protein but insufficient energy to provide for a high milk yield. This causes the cow to draw on her reserves of energy resulting in loss of weight.

Little research has been done in New Zealand on protein effects on reproduction. Wilson (1989), in a study performed on autumn calvers, found a positive effect of protein supplementation on fertility. He noted an association between high protein intake and an improved submission rate. However, the increase in the diet used in this experiment was based on protein ingredients such as brewers grain and soyabean with 18-19% CP content and which has a low protein percentage and low protein degradability and limited quantities (residual DM 800-1000 kg/ha) of irrigated pasture (CP 22%).

Wilson *et al* (1985) found that cows with low submission and conception rates, showed a marked improvement in conception rate when fed a concentrate supplement containing a relatively high concentration of protein (190 g/kg DM). The supplement was based on soyabean meal, linseed meal, barley meal, maize meal and brewer's grains which are reported to contain UIP protein.

The most recent work on this subject was reported by Moller (1991) on a preliminary study of nutritional balance and reproductive efficiency. He took blood samples from ten cows in 4 dairy herds, weekly for 17 weeks, starting at calving. Pasture samples were collected weekly as well as milk production data, cow fertility data and weather information. Two herds with the highest level of anoestrum also had the highest blood urea levels. These herds grazed pastures with higher protein and lower soluble carbohydrate content. It was seen that conception rate was lower when peak blood urea levels occurred during artificial breeding (AB) mating.

4.0 CONCLUSIONS

From the literature reviewed it is evident that a large number of the studies done overseas suggest that an increased intake of degradable protein, usually combined with a low or relatively inadequate intake of energy can be detrimental to post-partum fertility. However, there are also some studies that find no relationship between an excess intake of dietary protein and reproduction.

The use of blood metabolites such as BUN, BOHB, albumin and glucose, among others, as aids to indicate nutritional status, has been observed in a number of the studies reviewed. Some of these measurements were found to be useful in reflecting the levels of DCP and ME ingested by the animals and thus have been also included as an aid in studies on DCP and its relationship to fertility.

Many questions remain about the relationship between dietary protein intake and fertility. While a majority of works agree on the existence of a significant negative relationship between both, there are still a number that see a positive relationship or no relationship at all. Contradictions can only be cleared by means of more research adding to our understanding of all of the factors involved.

Areas of study, such as the effect of pasture CP levels on reproduction are of great interest in New Zealand, since no studies seem to exist on that area in the literature reviewed and New Zealand cattle are almost entirely fed from pasture. The majority of works have been based on combinations of concentrates and pastures or on concentrates alone.

In New Zealand, milk production is mainly based on pastures, and pastures are known to contain a high percentage of CP, a considerable amount of it being degradable intake protein; and a low percentage of ME. Therefore DCP intake is high.

However,the quoted average CR for New Zealand dairy herds is higher than that reported in most other countries.

The studies done overseas and their findings on the relationship between protein and fertility are of great interest in New Zealand given the conditions mentioned above. These overseas studies have raised the question of whether a relationship exists between pasture protein and energy levels and conception efficiency in the New Zealand context.

Until the time this study commenced, research had been done to investigate if a relationship between intake CP and conception exists in New Zealand dairy cows by providing protein supplementation but no survey studies had been done under normal circumstances on the average New Zealand dairy farm specifically to investigate this relationship. In New Zealand, CRs range from 60 to 65%. Though still very high by world standards, it would be of interest to clarify if a similar negative influence of pasture crude protein on fertility occurs here and if so, determine why the apparent influence of high dietary protein levels is not as harmful to herd fertility in New Zealand as it appears to be else where.

The following study was proposed with this purpose.

In order to investigate the influence of pasture protein and energy on conception efficiency, a study was designed with the following objectives:

1) To determine if relationship exists between dietary crude protein and conception rate in New Zealand dairy cattle grazing typical pastures.

2) To determine if a combined measurement of dietary energy and protein in grazed pastures can be related to conception rate.

3) To study if the age of cows interacts with the above to influence conception rate.

4) To investigate the relationship between urea-N levels in blood and/or vaginal mucus and conception rate.

5) To investigate if blood and vaginal mucus nitrogen are related to dietary protein and energy levels.

6) To study if energy levels in blood relate to dietary metabolizable energy and protein levels.

III

MATERIALS AND METHODS

1.0 DATA COLLECTION PLANNING

An on farm survey of cows selected for insemination was conducted in the Manawatu region during the 1990 spring breeding season from the 25th of October to the 26th of November.

Letters were sent to various farms in the area explaining the purpose of the project and inviting farmers to take part (Appendix 1). They were asked to return an acceptance slip included with the letter if they were willing to participate. From those farms returning the slip, twelve were selected. Location, known cooperativeness of the farmer and appropriate working facilities were points taken into consideration in selecting farms.

The number of cows to be sampled was decided based on a power calculation so as to achieve a 95% level of confidence. The decision on how many farms would be selected for the survey was based on the limitation in the number of people available for sampling. After consultations with the people to be involved in the trial and calculating the time each of them would need to gather samples from one farm and then go to the next one, it was decided that twelve farms was an appropriate number.

However, this number did not turn out to be the definitive one. As the survey took place, one of the farms was eliminated after two weeks of sampling since during all this time, only 4 samples were collected. The lack of animals to sample was due to the fact that the farmer was not using the services of the Livestock Improvement's inseminators. He performed the AI's himself, mostly at night, immediately after visually detecting the animals that were in heat during evening milking, rather than leaving them until the next morning. As collection of samples at night on this one farm would have introduced a time of day variable to the results, it was decided not to undertake night collection. Thus this farm was excluded, reducing the herds to eleven.

Later, when the survey entered the phase of pregnancy diagnosis, a second farm was excluded as they did not wish their cows to go through rectal palpation fearing that abortions might occur. This took the final number of farms to ten.

Although the number of herds was reduced below the number which this survey intended to study, this did not affect the final goal for the number of samples to be obtained. An appropriate number of samples was collected and a confidence level of 95% was reached.

The ten farms used in the trial were all located in the Manawatu area, near Palmerston North. Four were situated in Glen Oroua, one in Rangiotu, one in Mangawhata, one in Fitzherbert West, another one in Aokautere, one in Linton and one in Tokomaru (table 3 and appendix 2).

Herd size ranged from approximately 115 dairy cows and heifers listed in the animal records of the smallest herd to the largest herd that had approximately 389 dairy cows. Total number of animals per farm as well as location of the farm are presented in table 3.

The number of workers in the farms varied according to the size of the herds as can be observed in table 4. Small herds of less than 200 animals, as those found in farms 1, 3 and 5 were operated by the owners themselves and any extra help

Table 4. Summary of farms included in the study.

Farm	Location	Number of workers	Number of animals[1]	Breed[2]	Mean lactation number
1	Rangiotu	1[#]	131	1,2,3	3.589 (0.222)
2	Aokautere	2[*]	389	1,3	3.690 (0.187)
3	Glen Oroua	1[#]	162	1,3	2.949 (0.183)
4	Tokomaru	1[**]	212	1,3	3.000 (0.156)
5	Glen Oroua	1[#]	115	2	2.774 (0.228)
6	Glen Oroua	2[*]	234	1,3	3.560 (0.148)
7	Glen Oroua	1[**]	171	1,3	2.906 (0.166)
8	Mangawata	2[**]	256	1,2,3	3.743 (0.163)
9	Fitzherbert West	2[**]	322	1,3	2.908 (0.112)
10	Linton	2[*]	285	1,3	3.063 (0.125)

[#] Owner in charge of farm with help of family members, especially the wife.
[*] Owner in charge of farm with help of one or two hired workers.
[**] Manager hired by the owner to be in charge of the farm. They either work alone or have a helper.
[1] Includes cows and heifers.
[2] 1=New Zealand Friesian, 2=Jersey and 3=Cross breeds.
Standard error ofthe mean lactation number is shown in parenthesis.

needed came from family members. Farms with larger herds such as farms 2, 6 and 10, were operated by the owners but had one hired employee. While farms 8 and 9 had hired managers in charge and one employee under their charge. Farms 4 and 7, were managed by share milkers.

Cow breeds were typical of the area. Only one of the farms surveyed had an all Pedigree Jersey breed herd while the rest of the herds mainly consisted of New Zealand Friesian, some Jersey and Jersey cross cows.

Animal records, from which all the information about the history of the animals used in this survey was extracted, were kept by farmers using the computerized services of the Livestock Improvement Corporation which aside from performing herd tests and artificial breeding services, also kept updated records of all client farms.

The use of nitrogen fertilizers was one of the points for consideration in selecting farms. However, the great majority of the farms that were willing to participate in the survey applied nitrogen regularly which made it impossible to obtain an equal number of farms that applied and did not apply nitrogen fertilizers.

1.1 Selection of parameters to be measured in the animals

Blood samples and vaginal mucus were used to measure urea-N levels in this study. Since urea is one of the major end products of protein metabolism and is found in biological fluids, the concentration of urea nitrogen was measured as BUN (Blood urea nitrogen) in blood and MUN (Vaginal mucus urea nitrogen) in vaginal mucus.

Protein was measured by determination of blood albumin levels. Albumin is one of the most prominent of the serum proteins, constituting between 40 and 60% of the total serum proteins. It is a major storage reservoir of proteins and a transporter of amino acids. Changes in the nitrogen balance can be reflected in changes in albumin concentration (Coles, 1980).

Levels of non-esterified fatty acids (NEFA), glucose and ß-hydroxybutyrate (BOHB), were used as indicators of a cow's energy status.

All of these measurements were selected as the parameters to be measured in the study cows based on other studies conducted overseas where they have been seen to have a statistically significant relationship with dietary crude protein and energy.

Studies, such as that performed by Ducker (1984), have included all these blood measures as a means of indicating the energy status of dairy cattle while other studies have used one or two blood parameters and studied their relationship with DCP intake and energy balance.

For example, Manston *et al* (1975), in a study performed to observe the influence of dietary protein on blood composition in dairy cows, measured levels of urea in serum and concluded that the concentration of urea in the serum of dairy cows can be a sensitive indicator of current intake of DCP especially when cows are in mid-lactation. Similarly, Macleod *et al* (1984), reported that plasma urea nitrogen increased linearly as DCP was increased in an experiment where sixty four Holstein cows in their first lactation were assigned randomly 28 days post-partum to receive ad libitum one of 9 rations with CP levels of 12, 15 or 18%.

Likewise, Jordan *et al* (1983), observed that levels of BUN increased as the intake of CP increased from 12% to 23% while Howard *et al* (1987) observed that concentrations of urea nitrogen increased rapidly in cows on a 20% protein diet during the first 4 weeks of their study. Ferguson *et al* (1988) have used serum urea nitrogen levels as a diagnostic tool to reflect the intake of DCP and degradable protein in a herd with poor conception.

On the other hand, the measurement of vaginal mucus urea nitrogen (MUN) has been used by Carroll *et al* (1987, 1988) who observed that MUN levels show a positive relationship with CP intake, this means that an increase in DCP was accompanied by an increase of urea nitrogen in vaginal mucus. Similar findings were observed by Holtz *et al* (1986). They found that urea nitrogen levels were elevated in vaginal mucus (17.04 vs 7.03 mg%) from oestrous cows on high protein diets (20% vs 15% ration total protein).

Plasma albumin concentration is known to reflect the blood tissue protein content of animals and can rise slowly in response to the increment in levels of DCP (Wilson *et al*, 1985). There is evidence that cows with low protein intake have hypoalbuminemia while those on high intakes show hyperalbuminemia (Visek, 1984). Jordan and Swanson (1978) found that increasing the amount of CP in the diet of dairy cows from 12.7 to 19.3% during a period of 14 weeks, was associated with a simultaneous quadratic increase in serum albumin.

Glucose concentrations in plasma from cows has been used extensively as an indicator of energy status (Oltner and Wiktorsson, 1983). An increase of the energy intake in the diet is reflected in a significant rise in the blood glucose levels of dairy cows in early lactation (Nachtomi *et al*, 1991).

Levels of BOHB in blood have also been seen to be affected by energy balance on the diet (Ducker *et al*, 1985b). Erfle *et al* (1974), found a correlation, though low, between both blood glucose and BOHB, and energy balance when studying the use of blood metabolites as criteria of energy status of cows in early lactation. To understand better the variations in relationships, two groups were used in their study, one of stressed (ketotic) animals and one of non-stressed (control) animals.

Serum NEFA have been shown to be negatively correlated with energy balance (Blauwiekel and Kincaid, 1986; Erfle *et al*, 1974). Plasma NEFA levels were used by Nachtomi *et al* (1991) to measure of the effect of three dietary concentrate levels on 46 multiparous Holstein cows in early lactation. They found that increasing energy intake by raising the concentrate level from 50% to 65% and 80% in the diet, significantly lowered ($P<0.05$) the plasma NEFA in the 80% concentrate group.

Blood serum was also used to assess progesterone levels. Progesterone concentrations in blood are widely used to monitor the reproductive status of cows as well as mares (Foote, 1988).

In this experiment, progesterone concentrations were measured to eliminate the possibility that non-conception of experimental cows was due to erroneous heat detection rather than to high protein levels. Progesterone levels are low during the oestrous phase (Perera and Abeyratne 1979) as can be observed in figure 3. Cows with low levels of progesterone (<1.00 ng/ml) were considered to be in oestrus (Jordan and Swanson, 1979; Chenault *et al*, 1975).

1.2 Parameters to be measured in the farms

Animal records were collected to obtain information on identification number that was later used to identify those

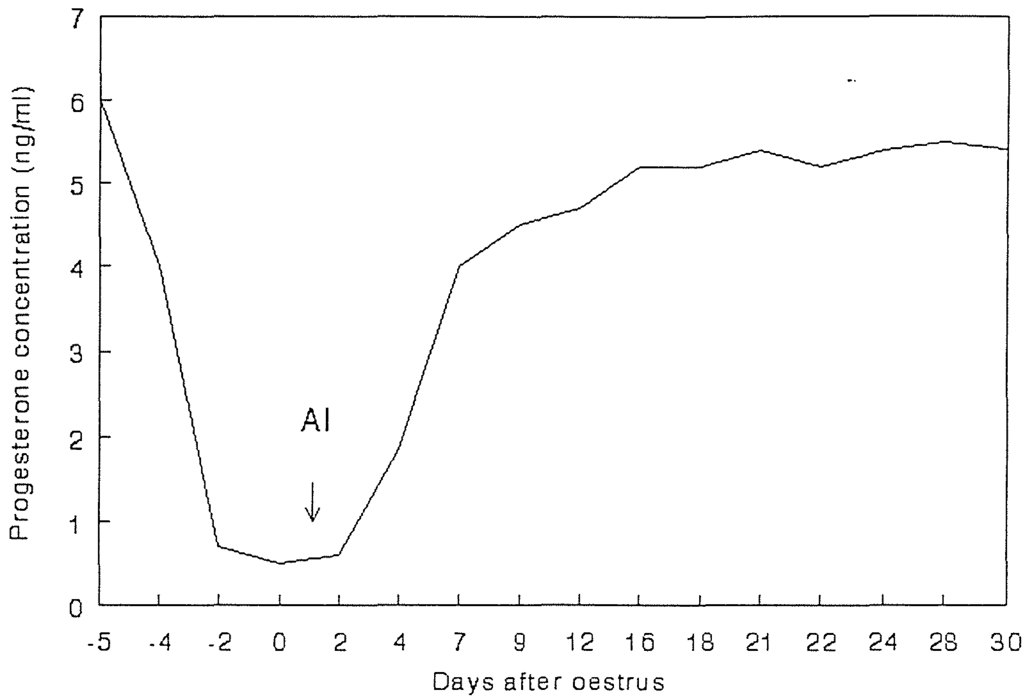

Figure 3. Progesterone concentration in the peripheral blood of a cow after insemination.

From: Perera and Abeyrante, 1979.

animals sampled, and lactation number that was used for the statistical analyses to determine the effect of age. Also current reproductive history, including all breeding dates and reproductive status were collected.

As blood metabolites were being collected to measure the nutritional status of animals and see how the intake of DCP and energy balance influenced them, it was necessary to know the nutritional content of the pastures. Thus, pasture samples were collected once a week and analyzed for CP and ME content.

2.0 Working procedures

2.1 Animal sample collection

Herds were visited by veterinarians who collected blood and vaginal mucus from cows which farmers visually detected in heat, and held back to be artificially inseminated on that day. Sampling started after the morning milking and within 2 hours before artificial insemination, thus precluding semen contamination of the sample obtained. In total, 745 samples were collected. However, only 702 were used in the analysis.

Forty three samples were not used. More than half of these samples (29) belonged to farm 2. Among the reasons for not including these samples in the study were that some of the cows the samples had been collected from did not exist in the animal records. Although in some cases the owner "remembered" the cows birth date, this information could not be considered reliable, especially in such a large herd. Animals with no birth date or lactation number could not be entered in to the DairyCHAMP program that was used in this study to keep records.

Likewise, during pregnancy diagnosis in one farm, it was observed that there were cows that had duplicate identification numbers and it was impossible to know which was the one that had been sampled, especially if neither of the cows had conception dates that corresponded to the sampling date nor to any of the previous or subsequent insemination dates. Thus, it was decided that all animals found with duplicate ID numbers in a farm would be eliminated.

Some animals that had been sampled but were no longer found in the herd either because they had been culled, had died or had just simply "gone missing" by the time of pregnancy diagnosis were excluded from the study.

Procedures used for the collection of vaginal mucus were consistent. The area around the vulva was thoroughly cleaned with water from a squeeze bottle then dried with paper towels, eliminating as much faeces as possible around the perineal area and inside the entrance of the vestibule and vagina (figure 5).

Once the area was thoroughly clean, the mucus was collected. For this purpose, a modified preputial smegma collector was used. This collector consists of a long and slender PVC tube and a rubber bulb (figure 4). The end of the tube was smoothed to avoid causing trauma to the vaginal walls during insertion and collection.

Mucus was collected from the anterior vaginal secretions following a method used by Carroll *et al.* (1988). The collector was inserted into the vagina and a sample taken from the pool of mucus located caudal and ventral to the external os of the cervix (figure 6 and 7). Once it was procured, the mucus was transferred into a sterile container and labelled.

Blood was obtained from the coccygeal vein and collected into two vacuum sealed tubes; an heparinized one and a plain one. All procedures were conducted for every cow.

It was not always possible to collect a complete set of samples of blood and vaginal mucus from all animals that were included in the survey. Fifty two vaginal mucus samples and three blood samples could not be obtained during the survey. The 52 mucus samples were not collected in some cases because

Figure 4. Vaginal mucus collector

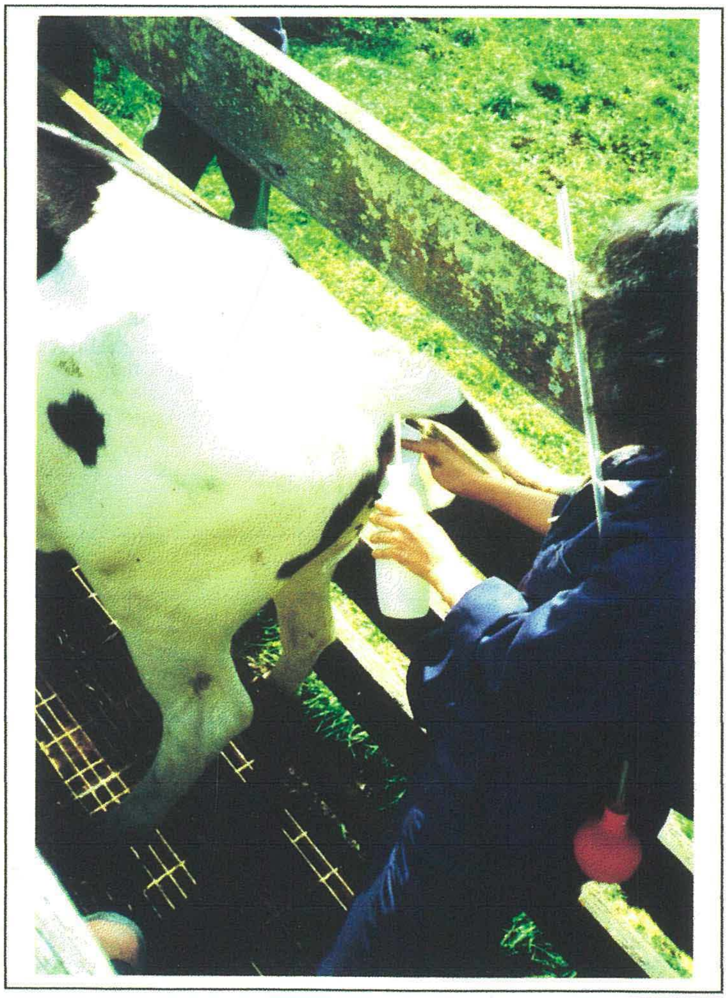

Figure 5. Cleaning process

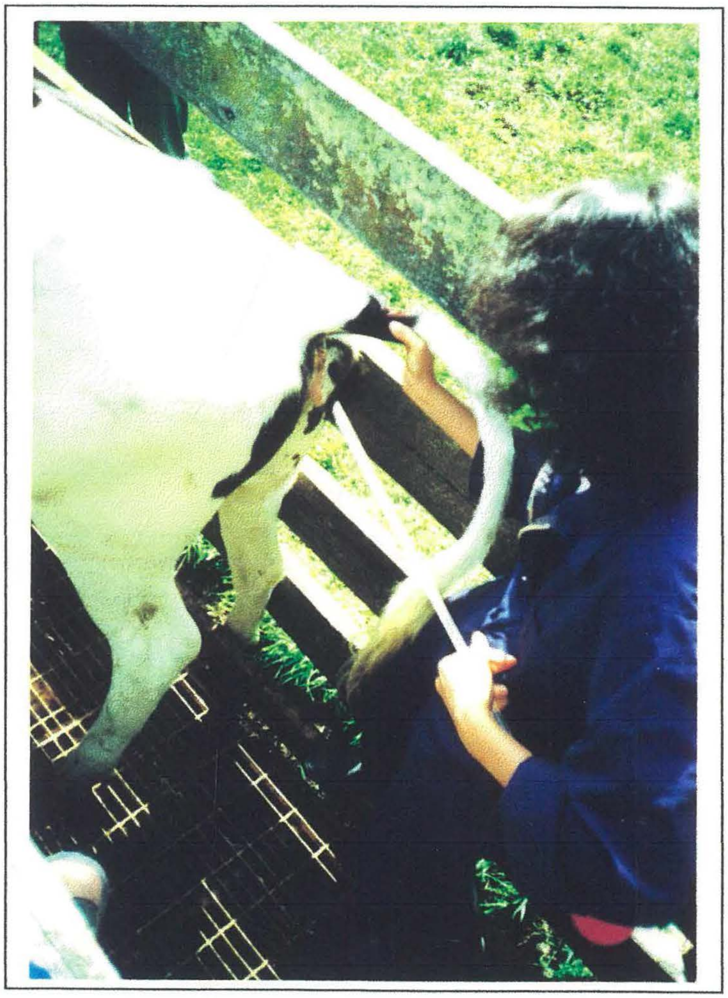

Figure 6. Sampling procedure

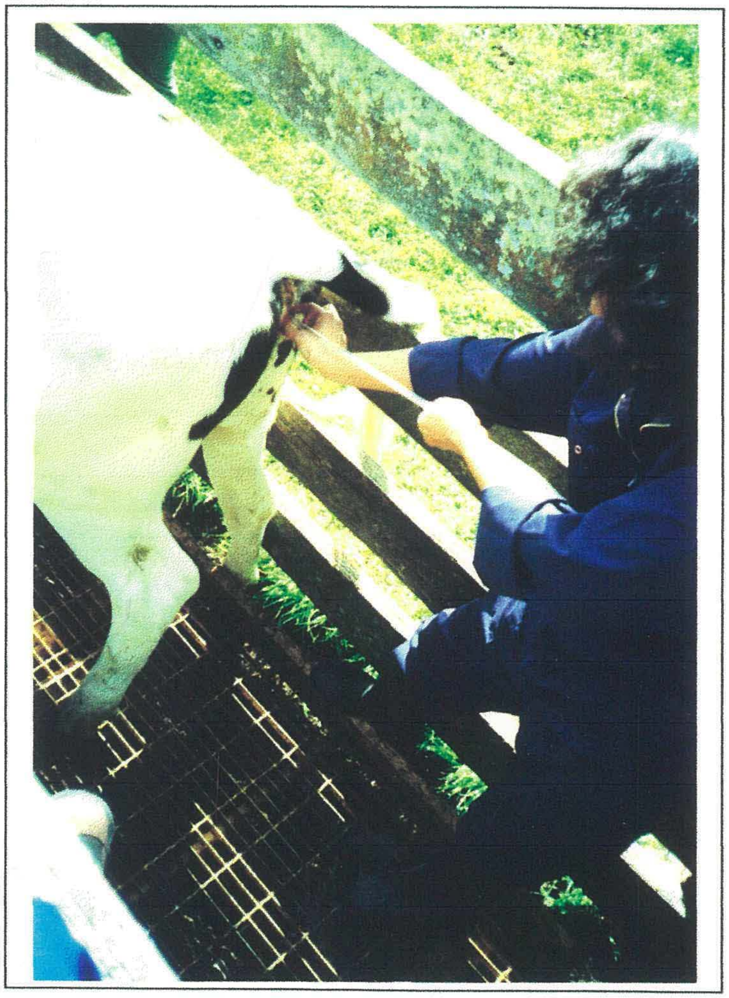

Figure 7. Sampling procedure

it was not possible to introduce the mucus collector due to the dryness of the vagina and since work was against the clock, as the inseminator would be working along side the veterinarian in most of the cases, it was decided not to continue trying to get the tube through. In other cases, the mucus collector could penetrate into the vagina easily but when positioned in the area of the mucus pool, no mucus would be found.

Blood and vaginal mucus samples that could not be taken to the laboratory for analysis within 4 hours of collection were stored in a portable cooler box containing a frozen block until delivery was possible. This was most necessary on days when pasture samples were collected.

Animal samples were collected every two days and where possible every day, depending on the number of people and animals available for sampling.

2.2 Farm data collection

Pasture was collected to determine CP and ME content. Samples were collected from the paddocks where animals were to be taken after insemination.

Pasture samples were collected weekly from each farm, following recommendations given by the Ministry of Agriculture and Fisheries (MAF). Sampling was done prior to grazing, in a grid pattern taking mixed pasture samples of clover and grass from at least 15 points in the paddock until the plant sampling bag MAF provides for this purpose was full (approximately 500g). Samples were cut using scissors at grazing height and areas which were not typical, like fence lines, hedges, gates, water troughs, dung or urine patches, were avoided.

One veterinarian was in charge of collection so that a standard procedure in the sampling would be followed. That is, all samples would always be cut at the same level while if more than one person did this, there would be differences in the cuts and in the sampling pattern.

Animal records were collected from all herds, including information on the age of cows, their lactation number, previous reproductive history (last calving date) and current mating information that recorded natural mating as well as all artificial insemination dates. Records of each farm were entered into the DairyCHAMP program (Udomprasert and Williamson, 1990) as was the resulting data from the laboratory analysis of all samples.

Pregnancy diagnosis by rectal palpation was performed on all herds 10 to 11 weeks after the last samples were taken. The time of conception was estimated according to the structures found at the examination. With the information obtained, conceptions and failures of conception to insemination on the sampling date were determined.

3.0 LABORATORY ANALYSIS

3.1 Blood samples

Serum was separated by centrifugation from blood collected into plain evacuated tubes and stored at -20^{P}C until analysis. This serum was used for determination of protein levels in the form of albumin, and of energy by analyzing levels of glucose, NEFA and BOHB.

All of these quantitative analyses were performed in the Physiology laboratory of the Department of Animal Science by in vitro colorimetric methods using a Roche Cobas Fara II autoanalyzer (Hoffman-La Roche, Basel, Switzerland).

Colorimetry is a simple and sensitive method. Blood constituents for analysis are converted into a coloured solution by the addition of various chemical reagents and then the quantity of those constituents can be estimated by the intensity of the colour. Spectrophotometry is the most widely used technique in the clinical chemistry laboratory (Coles, 1980).

All of the assays were conducted following the same steps:

- Serum samples were thawed and placed in the sample portion of a cuvette.
- At the same time reagent mixture was pipetted to the reagent portion of a cuvette. The nature of the reagent mixture depended on which of the assays was being done at that moment.
- After centrifugal mixing, absorbency measurements were taken. Changes in absorbency for a sample were compared to that of the standard curve, and results were produced to give mM values for the glucose and BOHB assays, in mEq/l for NEFA and in g/l for albumin (Scott, 1989a, 1989b).

Heparinized blood was also centrifuged and the plasma separated. This plasma was used for the BUN assay. The assay was done in the Veterinary Faculty Clinical Pathology Laboratory, also by colorimetry in a Cobas MIRA blood biochemical autoanalyzer (Hoffman-La Roche, Basel, Switzerland).

Progesterone was determined by means of a Radioimmuno-assay performed in the Veterinary Physiology and Anatomy laboratory.

The progesterone assay responds by competitive binding with labelled hormone and the level of this hormone present in a sample of blood or milk. Radioimmunoassays (RIA) use a radioactive label and can provide highly accurate results (Lynch and Abbott, 1988).

The assay consisted of the following steps as indicated by Scott (1989):

- Plasma progesterone was extracted from the samples and standard sera by a Toluene:Hexane solution. After shaking horizontally for ten minutes and then centrifuging for another 5 minutes at 1000 rpm, samples were stood to freeze for at least 3/4 of an hour in an upright position. After this period of time, the solvent was decanted off into tubes and dried under air flow.

- After drying, the tubes were washed with Ethanol then vortexed. From each of these tubes, 100 μg were placed into labelled plastic tubes in duplicate. At the same time, 100 μg of standards and zeros were also prepared in duplicate. Standards, standard sera, and samples were then redried.

- Once dried, 600 μg of a tracer cocktail -containing Tritium- was added to all the tubes. Duplicates of total count tubes containing 600 μg of the tracer cocktail plus 600 μg phosphate buffer solution in EDTA and gelatine buffer (PBSEG) and duplicate blank tubes containing 100 μg of tracer working solution plus 500 μg PBSEG were prepared as well. All tubes were incubated overnight at 4^{P}C.

- The next morning, 600 μg charcoal in PBSEG buffer was added to precipitate out the antiserum-tracer complex. Tubes were centrifuged and the supernatant decanted into scintillation vials. Finally, 5 ml scintillation fluid was added and vials were placed on a Beckman LS 7500 Microprocessor Liquid Scintillation System beta counter

(Beckman Instruments, Inc., Fullerton, CA.). Counts per minute results were analyzed by LKB Wallac, RIACALC program to give ng/ml values.

Samples with values lower than 1.00 ng/ml were considered as having been taken from cows that were truly not in dioestrus.

3.2 Vaginal mucus

Mucus was analyzed in the Veterinary Faculty Clinical Pathology laboratory with the same reagents and method used for BUN assays. Samples were thawed, diluted with saline solution in a 1:1 proportion and then used for performing the assay.

3.3 Pasture Samples

Pasture samples collected weekly were analyzed to assess the percentage dry matter, percentage CP, ME (measured in MJ/kg DM) and percentage digestibility. Analyses were done at the Batchelar Animal Health Laboratories of The Ministry of Agriculture and Fisheries, Palmerston North.

4.0 STATISTICAL ANALYSIS

To study if differences existed between farms in the levels of each metabolite, a SAS GLM or general linear model procedure for comparison of means was performed (SAS,1988). This procedure, that performs an analysis of variance, was used to compare the means of each metabolite per farm. In this procedure, when there are more than two means to compare, the GLM procedure tells if the means are significantly different from each other by multiple comparison.

The analysis of variance used can be explained by the following model

$$X - \mu = t + \varepsilon$$

This equation says that any given measurement (X) differs from the population mean (μ) by an amount that is due to the combined effects of the treatment (t) to which the measurement is subjected, and random variation or error (ε). If the treatment has no effect then t will be zero, and the only source of difference from the population mean is random variation.

For this model to be true, it is assumed that the variable in question is normally distributed in the population. However, the reasonable departures from normality do not seriously affect the reliability of the results. It is also assumed that the treatment groups are homogeneous, that is, they should not differ from one another by an amount greater than one would expect by chance alone.

The SAS model used for these calculations was the following:

$$X = \mu + F + \varepsilon$$

where:

X = Mean level of blood and vaginal mucus metabolites per farm.

μ = General mean.

F = Design variable that corresponds to farms.

ε = Error term.

A multiple linear regression analysis was performed to investigate if blood and vaginal mucus nitrogen levels and blood energy levels were related to dietary protein and energy. Using regression analysis, this relationship can be expressed as an equation that predicts a response or dependent variable from a function of regression variables, or independent variables, and parameters. The parameters are adjusted so that a measure of fit is optimized. In regression analysis, an estimating equation is developed to describe the pattern or functional nature of the relationship that exists between the variables. The regression equation used is

$$Y = a + b_2X_2 + b_3X_3,$$

where a is the Y intercept, X_2 and X_3 are dependent variables and b_2 and b_3 are called partial regression coefficients.

To examine the degree of co-relationship between variables, the following model was used

$$Y = a + b_2CP + b_3ME + b_4ME2 + b_5(CP*ME)$$

where

Y = response or dependent variables that in this case were each of the blood and vaginal mucus metabolites
the independent variables:
a = unknown parameter (Y intercept)
CP = crude protein
ME = metabolizable energy
ME2 = quadratic term for ME
CP*ME = interaction term

Data were analyzed using the SAS regression procedure for analysis of variance (SAS, 1988).

A SAS general linear models procedure for least square means was used where lactation number was included to determine if any improvements in the fit of the model were observed when this variable was in the model.

Linear and quadratic calculations were performed and the best fit and final model for each metabolite chosen, eliminating those that did not show any level of significance. Results were seen in terms of the dependent variable Y (metabolites) for various levels of X (CP and ME).

At farm level, the difference in conception efficiency among farms was studied. This study was done in both sampled animals and in those that were not sampled but that had been submitted to AB while the survey was taking place. This was performed in the same SAS GLM or general linear model procedure used for blood and vaginal mucus metabolite (SAS,1988). In this case X represented mean conception values.

Also the existence of differences in conception efficiency between sampled and non-sampled animals was studied, this time regardless of farm. These analyses were done by comparing both groups without considering lactation number, and comparing conception efficiency between age groups for all animals.

To compare conceptions in sampled and non-sampled animals without looking at age group, a new variable was created. This variable, called Type, consisted of two values, 0 and 1. If the breeding event had not been sampled, it was considered as a type 0 whereas, if the breeding event had been sampled it was given a type 1.

For the comparison between lactations, three groups were formed. The first one encompassed all first lactation heifers, the second one all second and third lactation cows and the third one included cows in their fourth or greater lactation.

To perform all these calculations, a CATMOD procedure (SAS, 1988) was used. This model fits linear models to functions of categorical data, in this case, facilitating a logistic regression analysis. For the first and second analysis, the response variables were type and age group respectively while the population profile was conception. This variable was treated in a quantitative rather than in a qualitative way.

A forward and a stepwise logistic regression, using the SAS Logistic procedure (SAS, 1990) was performed to examine the relationship between conception and the concentration of the blood metabolites, MUN and lactation number.

Conception was the dichotomous dependent variable because either it occurred or it did not. Blood metabolites and MUN were considered exposure variables as they were of primary importance while lactation number was classified as a confounding variable.

The probability of conception was calculated from the logistic regression describing the relationship between the dependent variable (conception) and the independent variables (blood and vaginal mucus metabolites and lactation number) and is represented in the following equation

$$\mathrm{logit}(p) = \log(p/1-p) = a+\beta'x$$

where a is the intercept parameter, β' is the vector of the slope parameters and x is the independent variable that is, the blood metabolites, vaginal mucus urea nitrogen and lactation number.

As only those variables that met the 0.05 significance level for entry to the model were included, the model for probability of conception was as follows

$$\mathrm{logit}(p) = 1/[1+(0.8382)+(-0.1813*BUN)+(0.0219*MUN) +(-0.074*LACTNO)]$$

Six hundred forty-nine samples were used for the logistic regression analysis. Fifty four observations were excluded by the Logistic SAS procedure due to missing values for the response or explanatory variables. No interaction terms met the 0.05 significance level for entry to the model.

To determine if a combined measurement of dietary energy and protein can be related to conception rate, a Chi square test was performed using the Statistix[2] program. For this purpose, the different pasture CP values obtained each week were classified into low, medium and high protein categories regardless of which farm they belonged to. Values below 16.5% CP were considered as low protein, those from 16.5 to 20.4% CP were classified as medium levels of protein and 20.5% CP or more were considered as high protein levels.

Breeding events for cows were grouped into each of these categories according to the date of pasture sampling ±4 days to the day of blood and vaginal mucus sampling. One hundred and fifty nine breeding events for cows that did not fall into this period of time were excluded. Observed conception rate values were compared to the expected ones in the remaining 490 breeding events.

To study if an interaction existed between lactation number and the amount of protein in the diet on conception, the three groups formed were used again. Chi square tests were done stratifying for the different lactations. Three groups were formed. The first included all breeding events for cows in their first parity (first calf heifers); the second included breeding events for cows in their second and third lactations and the last group included all in their fourth or higher lactations. Chi square values were calculated for each of these groups to see if any relationship could be found between conception and CP intake.

Chi-square calculations were also performed for the non-sampled animals for each lactation group and regardless of age. Chi-square analysis was done as well, for sampled and non-sampled animals together, as one group.

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[2] Statistix version 3.5.$^{)d*}$ Analytical software. Available from CHAMP Informatics Ltd., R.D. 4, Palmerston North.

IV

RESULTS

1.0 THE NUTRITIONAL CONTENT OF PASTURE

The results from the weekly pasture nutritional content assessments for each of the 10 farms used in this survey can be seen in table 5.

In table 5, as well as in figure 8, it can be observed that pasture CP levels ranged from 13% to 28%. Farm 5 had the highest documented weekly variation in CP among the 10 farms surveyed with values ranging from 13 to 23.1% followed by farm 7 that had values that ranged from 18.5 to 26.1% CP.

Metabolizable energy values showed little variation between weeks (table 5 and figure 9). When observing CP levels in association with those of ME, it can be seen that both fluctuate with a similar pattern, although the latter in a much less marked manner.

No reliable statistical evaluations could be performed to determine if there was any significant difference in pasture CP or ME between weeks within farms. Not all farms started their breeding programs at the same time. This meant that, even though this study was scheduled to begin on the first week of the breeding season, this was not possible. Some farms,observing that their cows were already in oestrus, had started their AB earlier than the start of this study, while others started 1 or 2 weeks after sampling was begun. Thus, as the number of weeks for which herds were included in the study varied, so did the number of pasture samples, since it would have been of no direct use to collect pasture when blood and mucus samples were not being collected.

Table 5. Weekly nutritional content of pastures per farm and percentage conceptions obtained during those weeks for all animals.

Farm	Nitrogen fertilizer[1]	Week	CP (%)	ME (MJ/Kg DM)	DM (%)	Conception % (n)[2]
1	Yes	1	26.4	10.7	26.4	6.8 (44)
		2	28.0	10.5	28.0	13.6 (44)
		3	25.5	9.6	12.3	25.0 (16)
2	Yes	2	17.2	9.8	20.7	13.6 (103)
		3	24.5	10.2	18.1	14.3 (42)
3	Yes	1	18.5	10.6	23.8	24.4 (45)
		2	20.2	9.8	19.4	29.4 (34)
		3	20.8	10.1	19.0	71.4 (14)
		4	17.8	8.9	19.8	46.6 (15)
4	Yes	3	23.7	9.8	22.5	51.4 (35)
		4	21.4	9.3	27.7	12.5 (8)
5	No	1	16.2	10.1	19.7	88.8 (9)
		2	23.1	10.3	17.6	60.0 (5)
		3	13.0	9.2	17.0	100 (2)
		4	20.9	9.6	22.1	50.0 (4)
6	Yes	1	19.9	10.3	21.5	50.8 (63)
		2	25.4	10.4	20.5	61.5 (39)
		3	20.7	10.0	21.9	42.8 (28)
		4	22.7	9.9	26.9	52.9 (17)
7	Yes	1	26.1	10.9	12.5	28.6 (56)
		2	18.5	9.6	20.0	42.3 (26)
		3	24.6	10.6	17.8	41.6 (12)
8	Yes	1	20.4	10.4	18.8	61.5 (65)
		2	23.5	10.6	19.3	63.8 (72)
		3	18.8	9.6	17.0	50.0 (46)
		4	20.5	8.7	25.0	60.5 (38)
9	Yes	1	14.0	9.7	18.4	58.1 (93)
		2	15.2	9.6	19.7	57.5 (80)
		3	19.2	9.3	20.1	48.3 (58)
		4	15.6	8.5	29.7	44.4 (18)**
10	Yes	1	19.5	10.3	15.5	53.2 (62)
		2	15.3	9.0	21.4	33.7 (77)
		3	16.5	8.7	21.8	48.6 (35)

[1] The **Yes** answer was used to indicate that a farm applied nitrogen fertilizer while a **No** answer was used for farms that did not apply nitrogen fertilizer.

[2] Total number of sampled and non-sampled breeding events that happened within ± 4 days of pasture sampling.

** Only non-sampled breeding events were found.

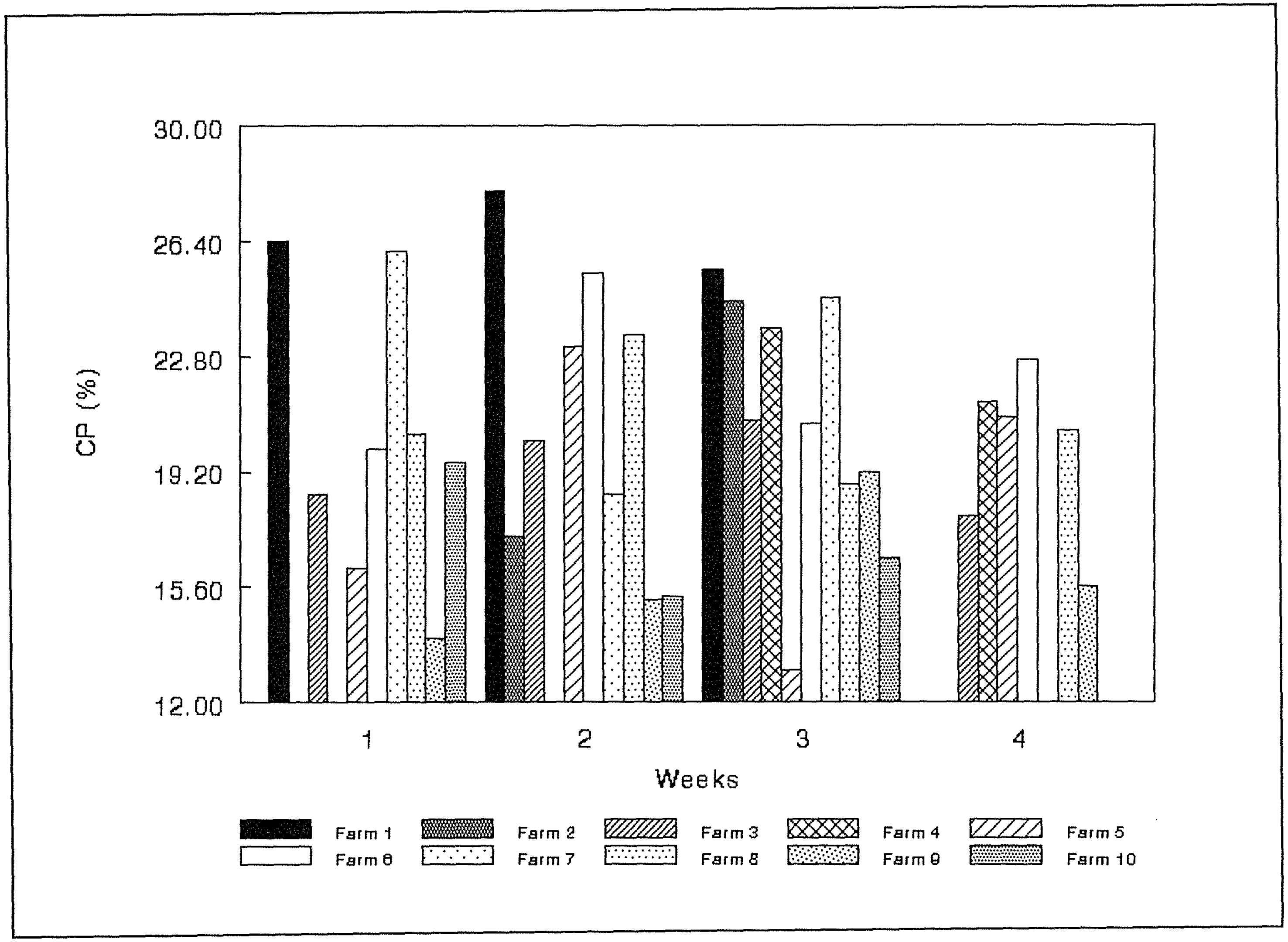

Figure 8. Weekly pasture crude protein content per farm

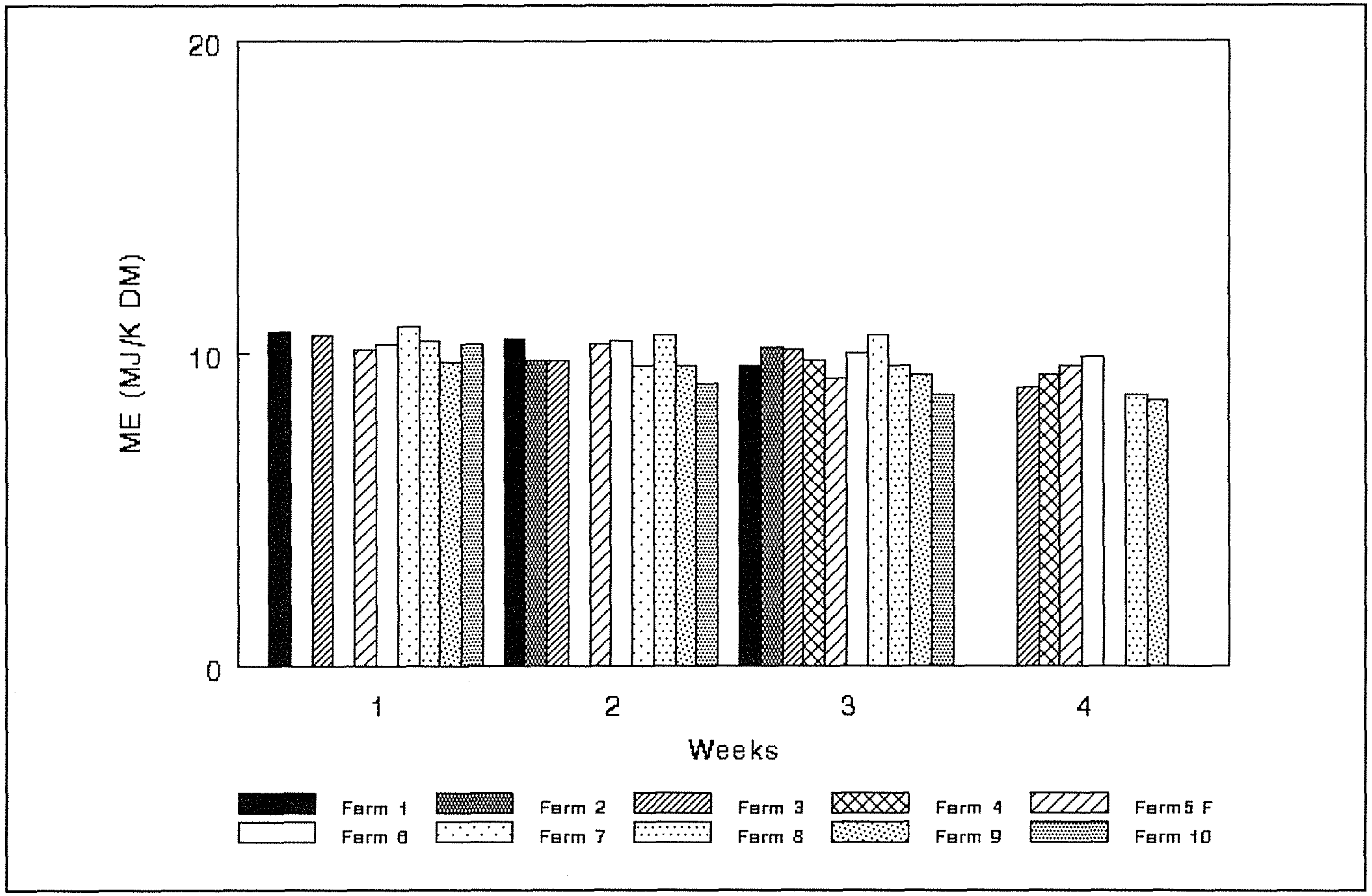

Figure 9. Weekly pasture metabolizable energy content per farm

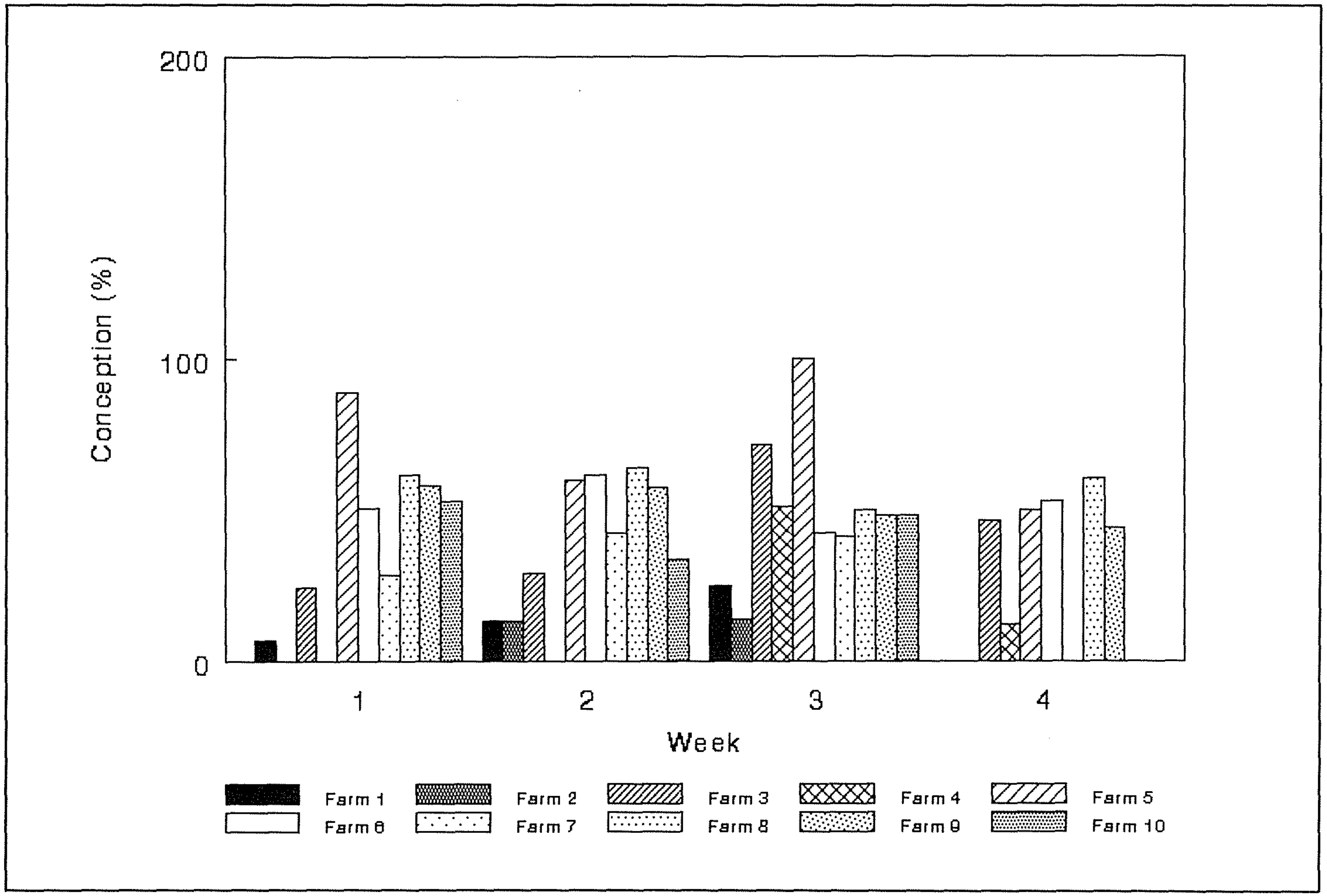

Figure 10. Weekly conception rate for each farm

Table 5 also shows that nine of ten farms surveyed, applied nitrogenous fertilisers except farm 5. This farm, as mentioned above, showed the greatest variation in pasture CP levels between weeks. However, it can not be assumed that the fact that no nitrogenous fertilizer was applied had any influence on the variability of the pasture CP levels.

Conception efficiency per week and per farm for sampled and non-sampled animals is presented in the same table and in figure 10. Neither the figure nor the table show a clear trend in variability in the percentage of conceptions with the exception of farm 5 where a slight tendency to an increase on conception efficiency was observed as pasture CP levels decreased. However, this pattern was not observed in all farms. The relationship between pasture CP levels and conception, from a statistical point of view, will be presented later in this chapter.

2.0 BLOOD AND VAGINAL MUCUS MEASURES

Descriptive statistics were performed for all the metabolites analyzed for the 702 breeding events as a total and by farm. Mean values for BUN, MUN, albumin (ALB), glucose (GLUC), NEFA and BOHB for the 702 breeding events are shown in table 6 while a summary of the descriptive statistics by farm is recorded in table 7.

When studying the mean values for blood and vaginal mucus metabolites for all breeding events regardless of farm, MUN levels are observed to range from 0.02 to 175.8 with a standard error of 0.42. Albumin and BUN also have large standard errors (0.19 and 0.05) but to a lesser extent than MUN. While BOHB, NEFA, and GLUC have the lowest SE of the mean and therefore the lowest observed variability among the metabolites.

Table 6. Mean values for levels of blood and vaginal mucus metabolites in samples and mean lactation number obtained during the 1990 spring breeding season.

Variable	**Mean**	**SE (Mean)**	**Minimum**	**Maximum**
BUN (mM/l) (n)	5.58 (701)	0.05	2.45	11.73
BOHB (mMl/l) (n)	1.62 (699)	0.015	0.72	3.99
MUN (mM/l) (n)	3.96 (654)	0.42	0.02	175.80
ALB (g/l) (n)	34.63 (700)	0.19	20.70	49.70
GLUC (g/l) (n)	2.86 (700)	0.02	1.00	6.60
NEFA (Meq/l) (n)	0.62 (700)	0.01	0.04	1.84
LACTNO[1] (n)	3.315 (702)	0.095	1.00	14.00

(n) corresponds to total number of samples used to perform descriptive statistics for each metabolite.

[1] Lactation number

Table 7

Descriptive statistics per farm for each of the variables studied.

Farm		Lactation[b]	BUN (mM/l)	MUN (mM/l)	ALB (g/l)	GLUC (mM/l)	BOHB (mM/l)	NEFA (MEq/l)
1	Cases[a]	40	40	39	40	40	40	40
	Mean	3.825	6.357	5.039	33.87	2.600	1.782	0.674
	SE(Mean)	0.347	0.197	1.552	0.880	0.076	0.0628	0.0504
	Minimum	1.000	3.810	0.380	20.80	1.300	1.034	0.0932
	Maximum	9.000	9.440	55.80	44.50	3.500	3.274	1.300
2	Cases	104	104	100	104	104	104	104
	Mean	4.423	5.808	3.150	32.97	2.940	1.573	0.722
	SE(Mean)	0.324	0.112	0.402	0.523	0.053	0.0402	0.0353
	Minimum	1.000	2.950	0.3	20.90	1.600	0.772	0.138
	Maximum	14.00	9.350	24.36	49.10	4.100	2.899	1.813
3	Cases	40	40	36	40	40	40	40
	Mean	3.100	5.777	2.826	33.26	2.800	1.725	0.567
	SE(Mean)	0.395	0.126	0.738	0.853	0.0975	0.069	0.0482
	Minimum	1.000	4.330	0.2	21.30	1.500	1.071	0.170
	Maximum	12.00	7.590	26.68	43.60	4.800	3.208	1.291
4	Cases	33	33	33	33	33	32	33
	Mean	3.485	6.273	3.557	33.32	3.070	1.669	0.650
	SE(Mean)	0.305	0.138	1.554	0.962	0.0915	0.059	0.051
	Minimum	1.000	4.890	0.2	21.10	1.800	1.201	0.102
	Maximum	7.000	8.310	52.38	45.90	4.100	2.468	1.454
5	Cases	12	12	11	12	12	12	12
	Mean	2.500	6.164	1.607	31.19	2.950	1.907	0.684
	SE(Mean)	0.645	0.269	0.366	1.887	0.228	0.0881	0.123
	Minimum	1.000	4.840	0.640	21.80	1.800	1.488	0.253
	Maximum	9.000	7.840	4.820	46.20	4.000	2.395	1.523

Descriptive statistics per farm... (Continued)

Farm		Lactation[b]	BUN (mM/l)	MUN (mM/l)	ALB (g/l)	GLUC (mM/l)	BOHB (mM/l)	NEFA (MEq/l)
6	Cases[a]	53	53	50	53	53	53	53
	Mean	3.717	5.734	4.980	34.24	2.566	1.584	0.611
	SE(Mean)	0.294	0.121	1.474	0.702	0.0967	0.044	0.044
	Minimum	1.000	3.890	0.460	22.40	1.100	1.023	0.118
	Maximum	10.00	7.380	68.20	46.10	3.700	2.413	1.441
7	Cases	49	49	45	49	49	49	49
	Mean	2.837	5.973	3.747	35.28	2.637	1.599	0.533
	SE(Mean)	0.320	0.185	0.664	0.630	0.087	0.043	0.036
	Minimum	1.000	3.630	0.220	27.00	1.100	1.115	0.163
	Maximum	7.000	8.630	22.20	45.10	4.500	2.557	1.286
8	Cases	74	74	71	74	74	74	74
	Mean	3.243	5.698	5.742	34.14	3.049	1.504	0.885
	SE(Mean)	0.281	0.106	1.468	0.641	0.055	0.035	0.040
	Minimum	1.000	4.090	0.02	20.70	2.100	0.832	0.212
	Maximum	9.000	8.060	64.22	49.70	4.300	2.249	1.667
9	Cases	173	173	165	172	172	172	172
	Mean	2.827	5.011	5.159	36.20	2.973	1.614	0.591
	SE(Mean)	0.187	0.103	1.323	0.332	0.043	0.033	0.031
	Minimum	1.000	2.450	0.220	24.50	1.000	0.720	0.043
	Maximum	12.00	11.73	175.8	48.40	6.600	3.988	1.840
10	Cases	125	125	105	125	125	125	125
	Mean	3.008	5.181	3.292	35.47	2.758	1.641	0.433
	SE(Mean)	0.203	0.104	0.574	0.401	0.032	0.032	0.020
	Minimum	1.000	2.970	0.240	25.60	1.500	1.079	0.081
	Maximum	9.000	8.380	36.00	48.40	4.100	2.670	1.237

[1] Number of animals sampled for each of the variables.
[2] Lactation number at the moment of sampling.

Variability of each of the metabolites per farm was also observed. The descriptive statistics per farm for each of these values can be seen in table 7. From the numerical point of view, MUN and ALB show similar variability in their standard error values among the farms (figures 11 and 12).

Analysis of variance showed that the differences in MUN levels between farms were not statistically significant. ALB, on the contrary, proved to have a statistically significantly different value ($P<0.0001$) among farms, as can be seen in table 8. BUN (figure 13), GLUC (figure 14), NEFA (figure 15) and BOHB (figure 16), also showed significant differences between farms with P values less than 0.0001 (table 8).

Table 8 shows which farms presented the highest difference between means for each of the metabolites analyzed.

Table 8. Comparison of means among farms for each of the metabolites analyzed during the study.

Variable	Model F value (P value)	Farm comparison[1]
BUN (mM/l)	12.76 (P<0.001)**	1 ↔ 9
MUN (mM/l)	0.83	____
ALB (g/l)	5.13 (P<0.001)**	9 ↔ 5
GLUC (g/l)	6.93 (P<0.001)**	4 ↔ 6
BOHB (mM/l)	3.21 (P<0.001)**	5 ↔ 8
NEFA (MEq/l)	11.34 (P<0.001)**	8 ↔ 10

[1] Farms that present the highest significant difference between their means at the 0.05 level.

** Means differ significantly

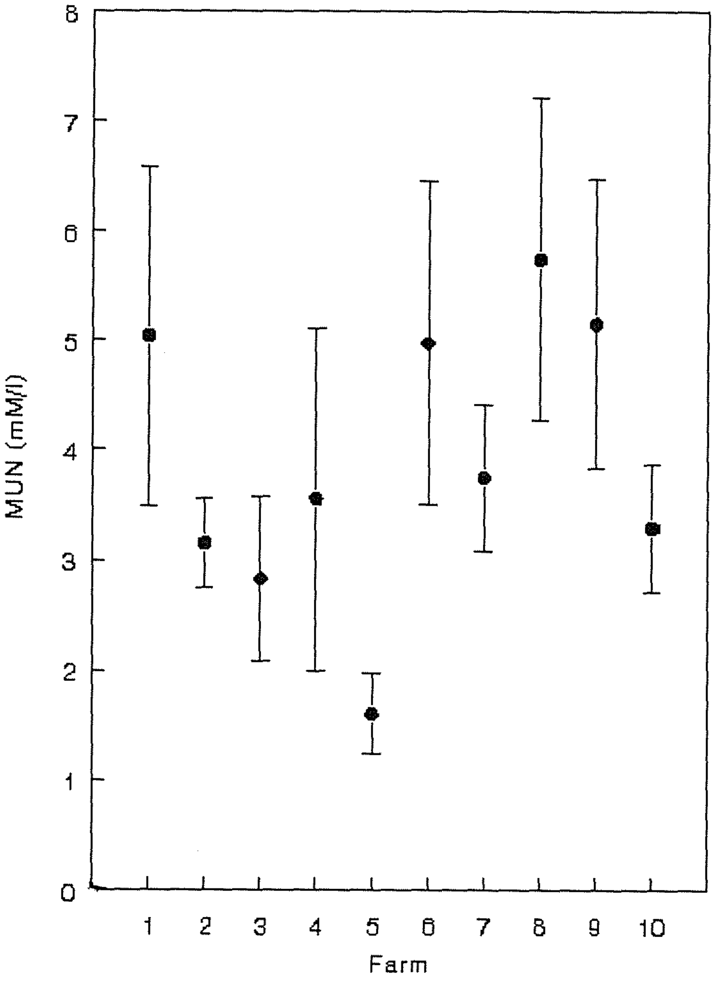

Figure 11. Mean MUN levels for each farm

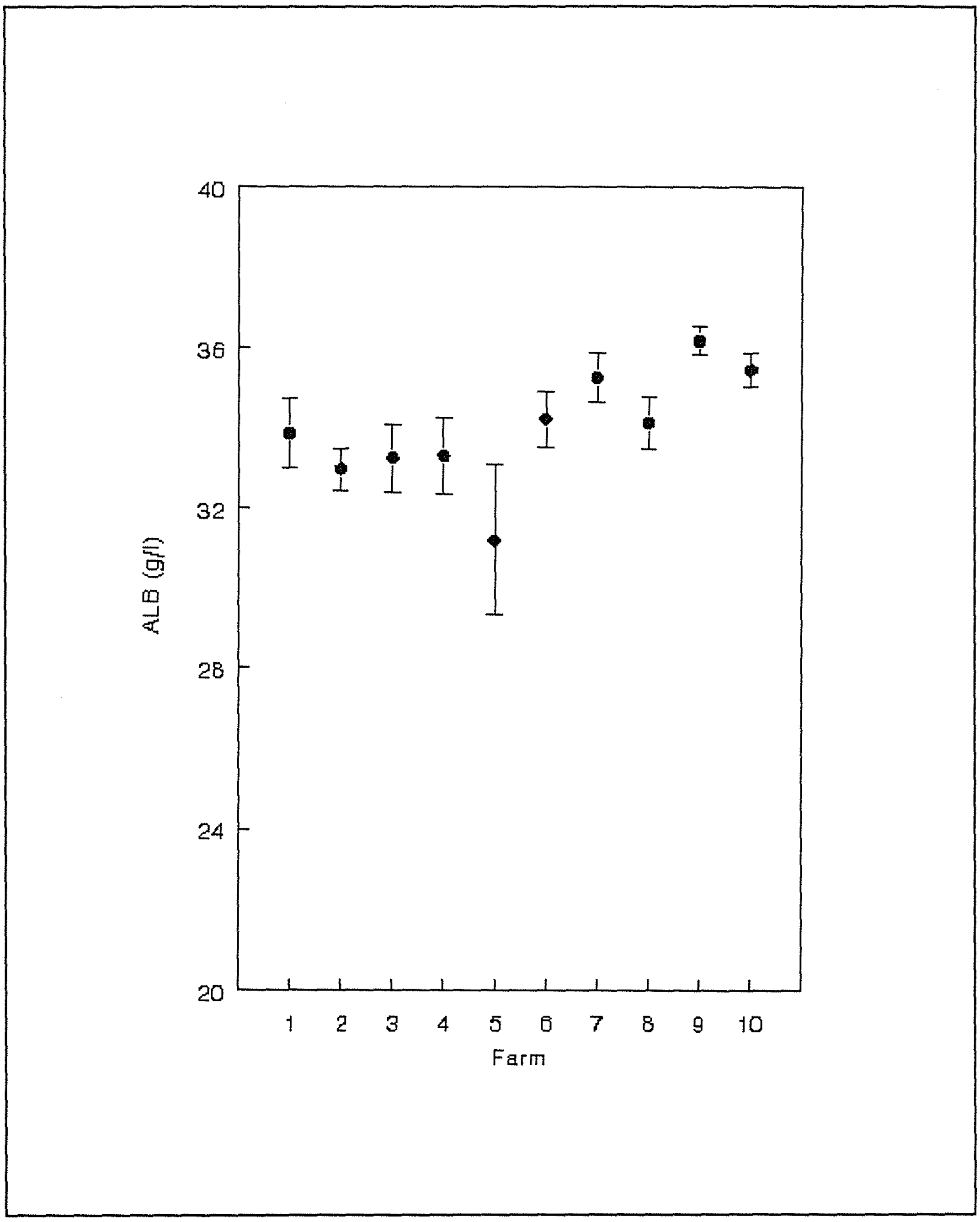

Figure 12. Mean Albumin levels for each farm

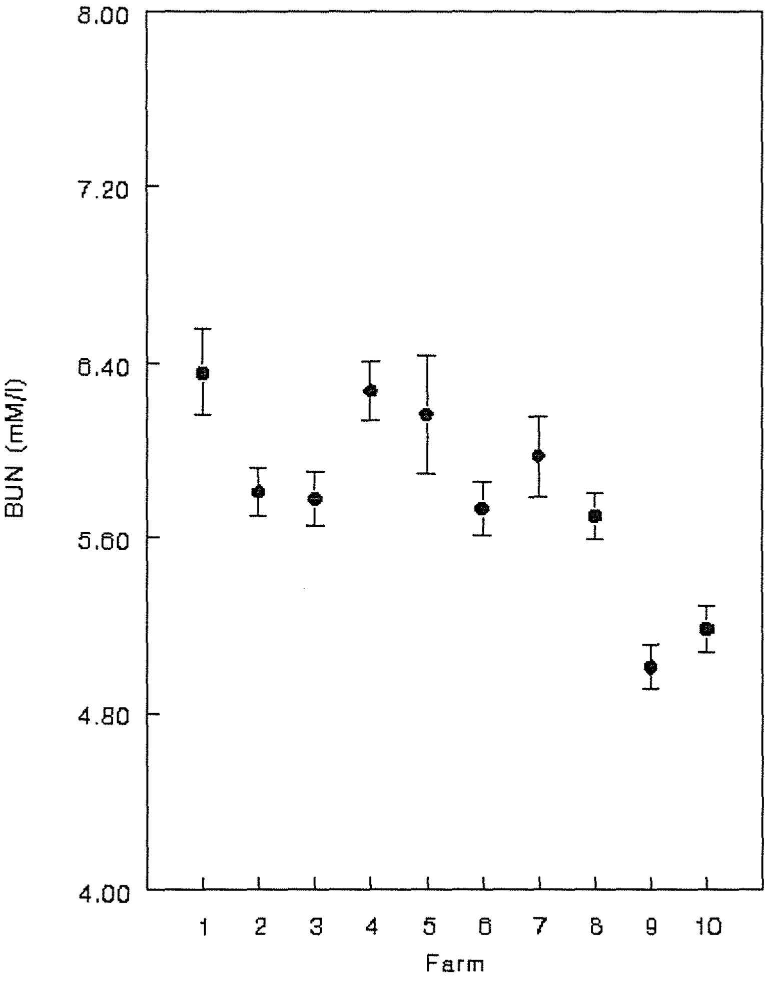

Figure 13. Mean BUN levels for every farm

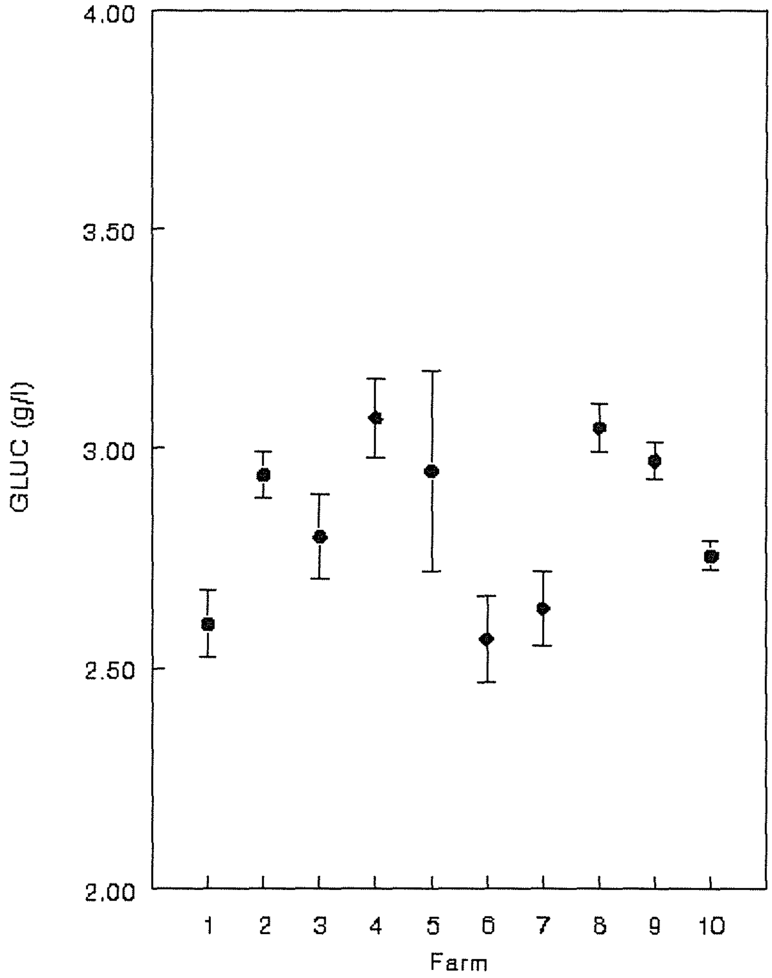

Figure 14. Mean Glucose levels for each farm

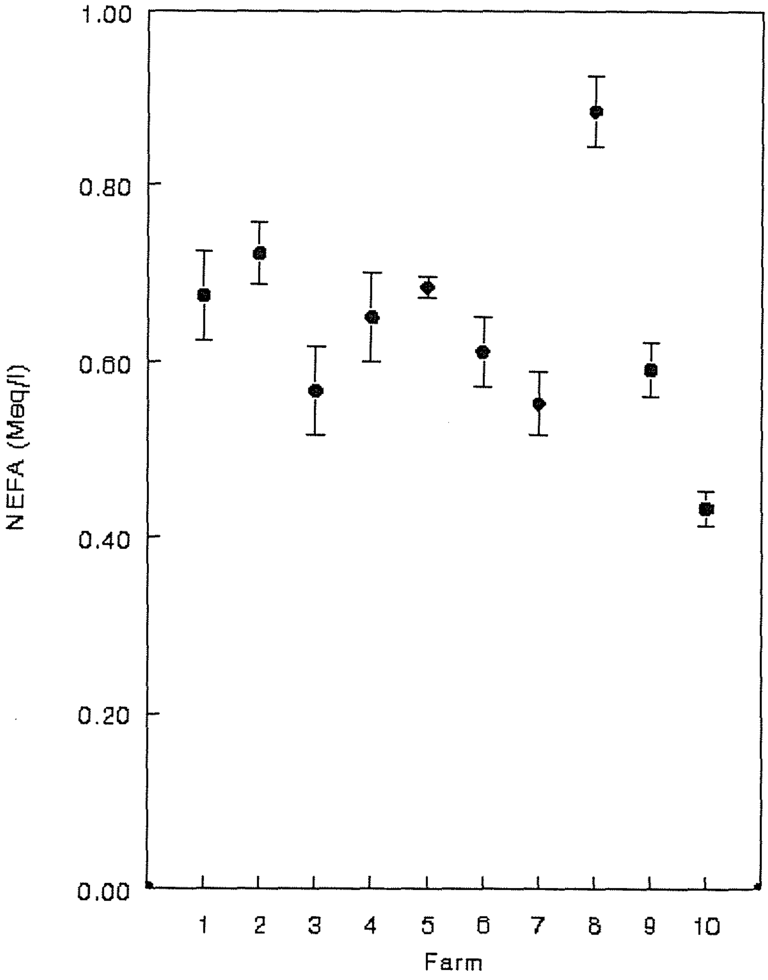

Figure 15. Mean NEFA levels per farm

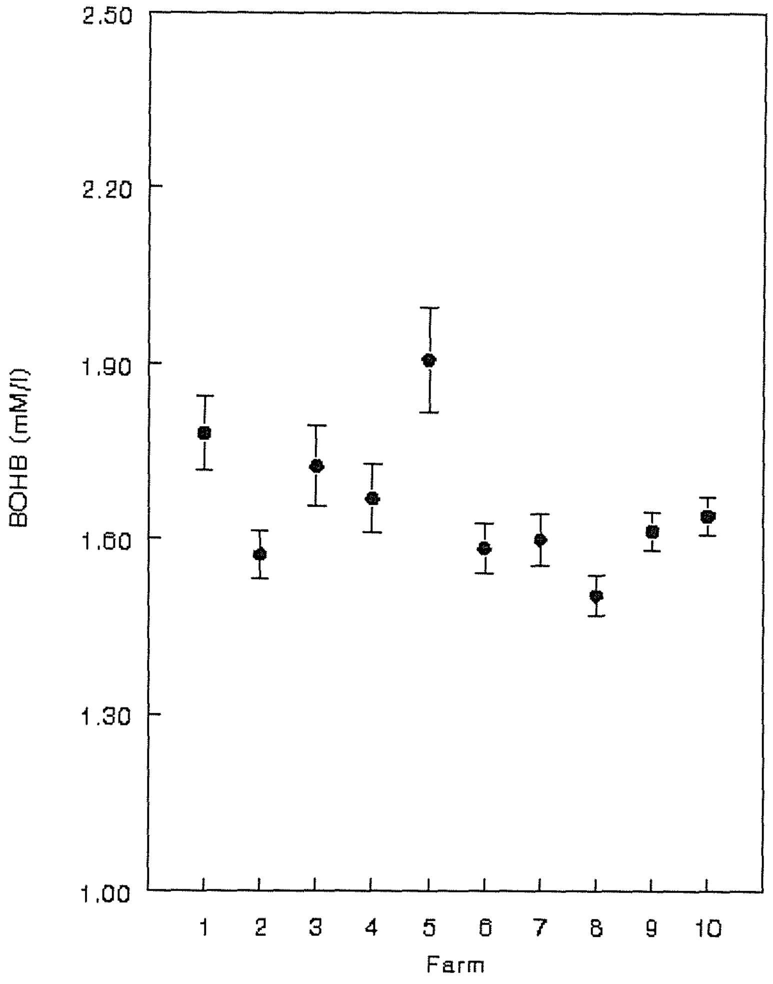

Figure 16. Mean BOHB levels for each farm

3.0 THE RELATIONSHIP BETWEEN BLOOD AND VAGINAL MUCUS METABOLITES AND DIETARY CRUDE PROTEIN

Pasture CP values obtained each week were classified into low (less than 16.5% CP), medium (16.5% to 20.5% CP), and high protein (20.5% or more CP) categories regardless of which farm they belonged to. Means for each of these groups were 14.95%, 18.90% and 24.38% CP respectively and are shown in table 9. Pasture ME means for the same groups are included in this table as is a summary of mean levels for the metabolites analyzed in this study.

The relationship of blood and vaginal mucus metabolite levels with CP and ME was investigated by multiple linear regression. Figures 17, 18, 19, 20, 21, 22, 23 and 24 show the linear regression between CP and ME with each of the metabolites which showed a statistically significant relationship between levels of CP and their levels. A clear linear trend is not observed in the plots but the results obtained from the analysis of variance show a significant relationship between CP and BUN, ALB, GLUC and NEFA (Table 10).

BUN is related in a highly significant positive way with CP ($P<0.0001$) and has an R-square of 0.089. Even though it is not a high R^3, it is the highest among all of the models that do not include an interaction term. This value means that almost 9% of the variation in BUN can be accounted for by CP levels. Even though it is a small percentage, that explained proportion of variation in BUN levels is highly significant.

ALB and GLUC had significant negative relationships with CP. Their R-squares were 0.028 and 0.019 respectively. On the other hand, NEFA showed a significant positive relationship with CP (R-square = 0.01).

The levels of blood metabolites versus levels of ME and the

Table 9. Descriptive statistics for blood and vaginal mucus metabolites levels in breeding events of cows fed low, medium and high levels of pasture crude protein.

Variable	Low crude protein[1]	Medium crude protein[2]	High crude protein [3]
Cases[a]	141	158	190
Mean BUN (mM/l)	5.016	5.660	5.982
(SE Mean)	0.102	0.084	0.078
Minimum	2.450	2.950	3.630
Maximum	8.130	8.380	9.350
Mean MUN (mM/l)	4.447	4.100	4.270
(SE Mean)	1.001	0.648	0.659
Minimum	0.24	0.02	0.22
Maximum	80.60	64.22	68.20
Mean ALB (g/l)	35.67	34.69	33.01
(SE Mean)	0.417	0.386	0.413
Minimum	24.50	21.30	20.70
Maximum	48.40	49.10	49.70
Mean GLUC (g/l)	3.036	2.925	2.845
(SE Mean)	0.0373	0.039	0.044
Minimum	1.500	1.500	1.200
Maximum	4.100	4.800	4.500
Mean BOHB (mM/l)	1.616	1.570	1.604
(SE Mean)	0.034	0.028	0.025
Minimum	0.72	0.783	0.832
Maximum	3.988	2.653	2.899

Table 9. Descriptive statistics for blood and vaginal mucus metabolite levels...(continued)

Variable	Low crude protein[1]	Medium crude protein[2]	High crude protein[3]
Mean NEFA (MEq/l)	0.599	0.624	0.686
(SE Mean)	0.035	0.029	0.025
Minimum	0.043	0.070	0.102
Maximum	1.840	1.667	1.567
Mean CP (%)	14.80	18.60	24.12
Mean ME (MJ/kgDM)	9.458	9.775	10.24

[a] Number of samples included in each CP group.
[1] Low = <16.5% dietary crude protein.
[2] Medium = 16.5% to 20.5% dietary crude protein.
[3] High = 20.5% dietary crude protein or more.

Table 10. Analysis of variance for blood metabolites vs. crude protein and metabolizable energy.

SAS Model	Parameter estimate	F value for model	P value for model	R-square
BUN=CP	0.08	43.94	0.0001^{++}	0.089
ALB=-CP	-0.21	13.16	0.0003^{++}	0.028
GLUC=CP	-0.018	8.99	0.0029^{++}	0.019
NEFA=CP	0.009	4.89	0.027^{+}	0.01
BUN=ME	0.39	19.67	0.0001^{++}	0.04
ALB=ME ME2	-1.32	2.88	0.05^{+}	0.01
GLUC=ME ME2	-0.28	11.70	0.0001^{++}	0.04
NEFA=ME ME2	-0.098	8.28	0.0003^{++}	0.03
ALB=CP ME	-0.47 2.67	19.24	0.0001^{++}	0.078
BUN=CP ME CPME	-0.11	20.40	0.0001^{++}	0.12
GLUC=CP ME CPME	-0.033	5.18	0.002^{+}	0.03
NEFA=CP ME CPME	-0.03	7.79	0.0001^{++}	0.049

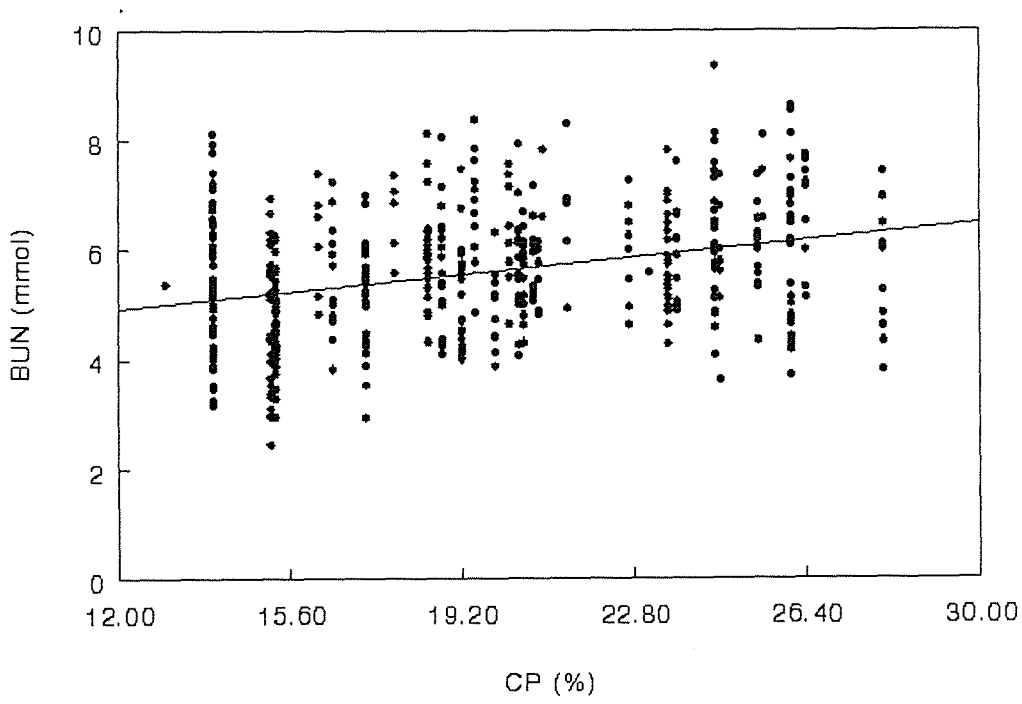

Figure 17. BUN-Pasture crude protein relationship

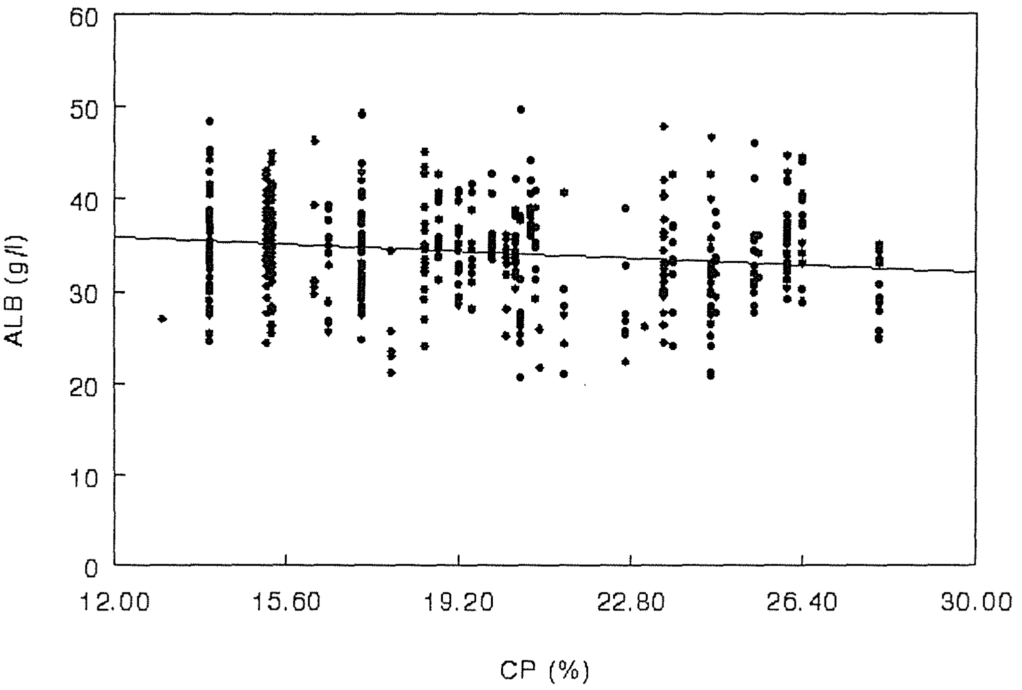

Figure 18. Albumin-Pasture crude protein relationship

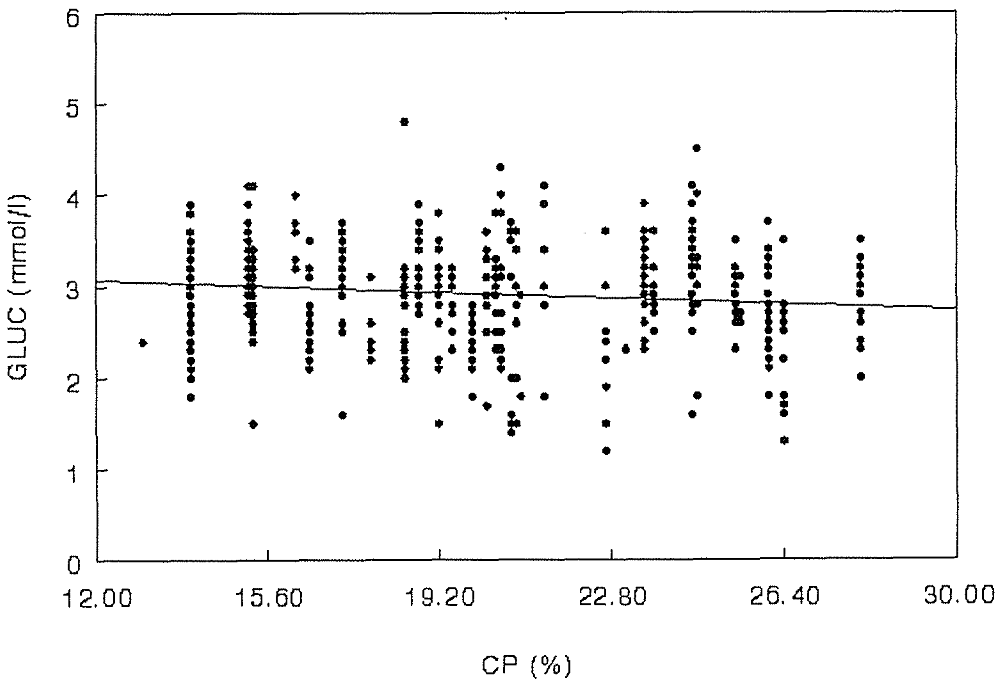

Figure 19. Glucose-Pasture crude protein relationship

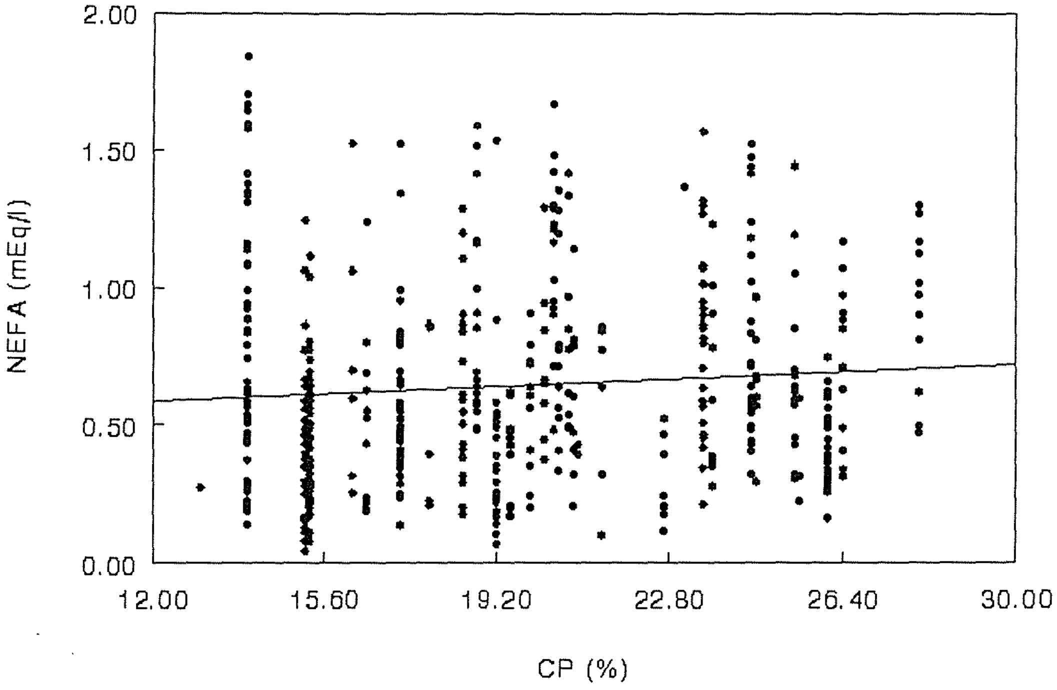

Figure 20. NEFA-Pasture crude protein relationship

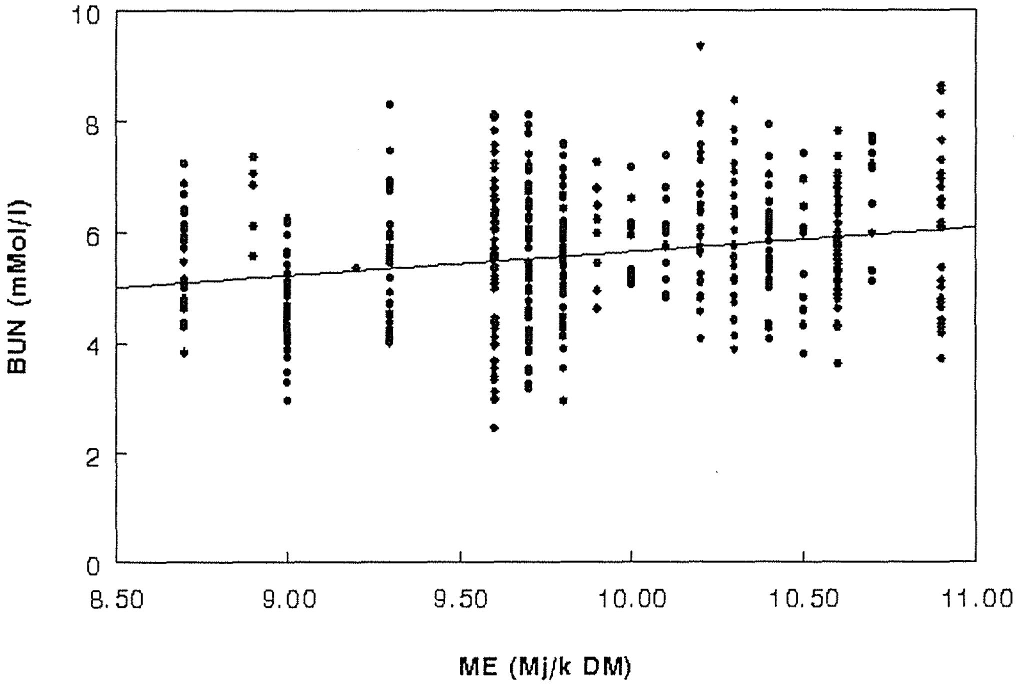

Figure 21. BUN-Pasture metabolizable energy relationship

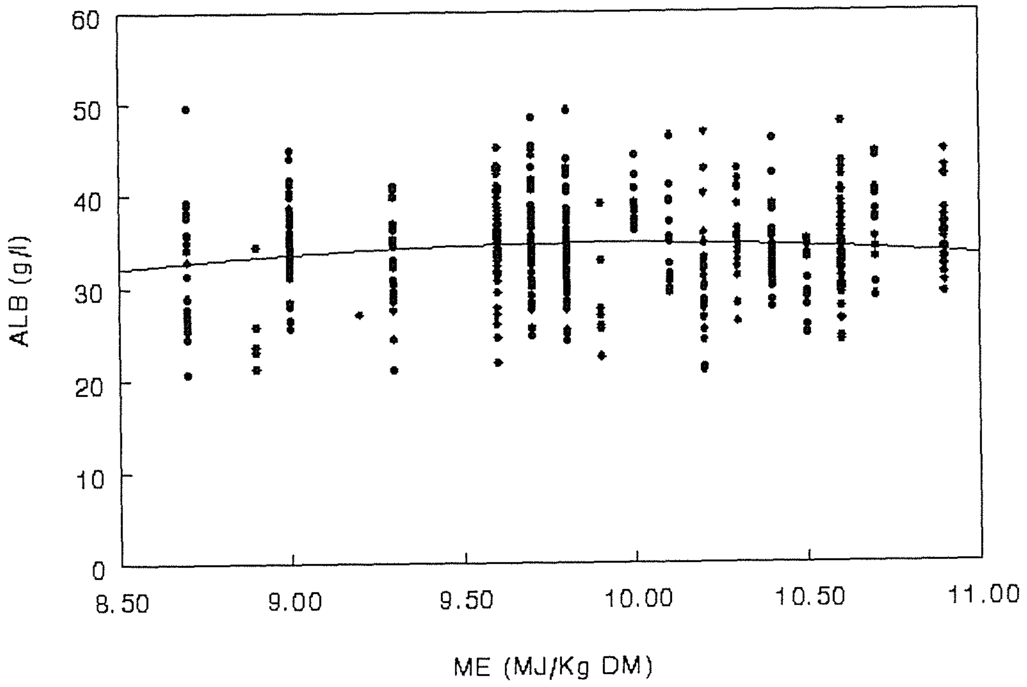

Figure 22. Albumin - Pasture metabolizable energy relationship

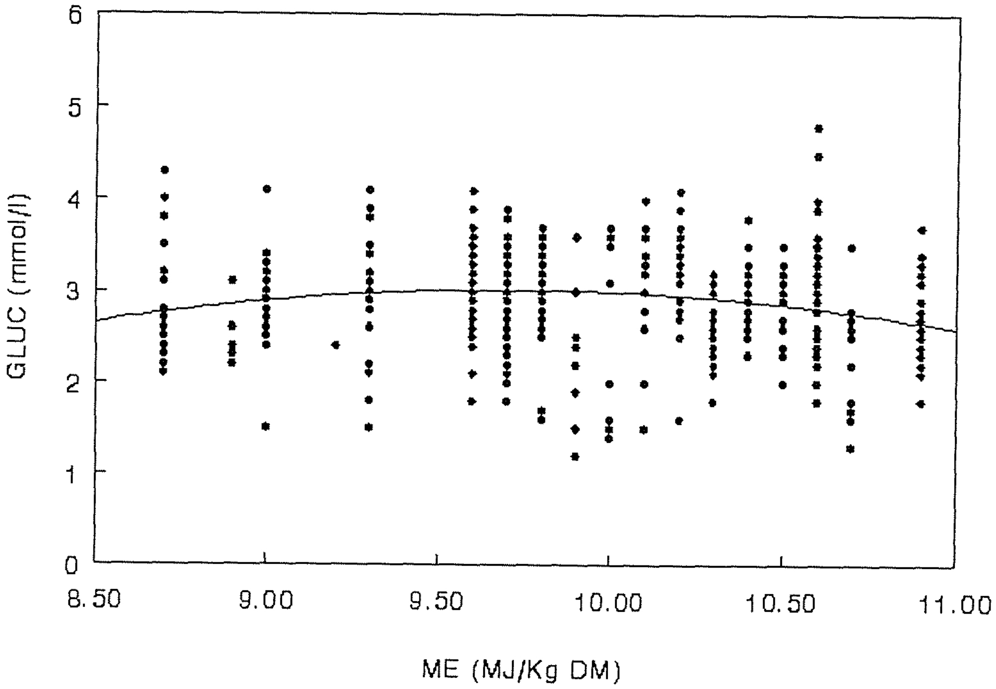

Figure 23. Glucose-Pasture metabolizable energy relationship

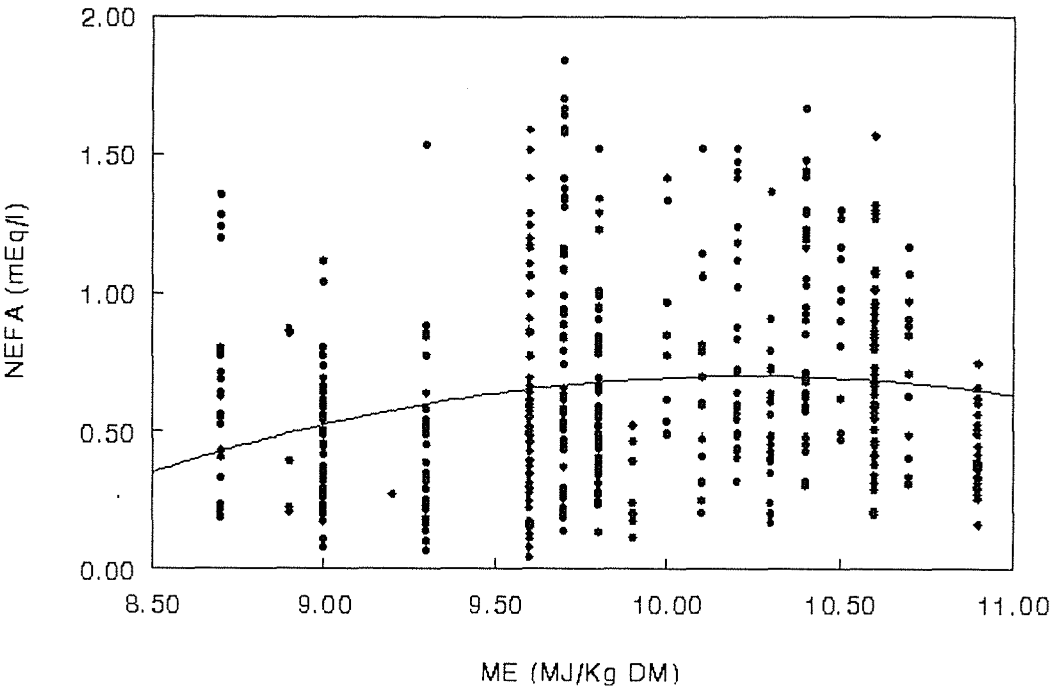

Figure 24. NEFA-Pasture metabolizable energy relationship

statistical significance of the relationship can also be observed in the same table. Here BUN had the only significant positive linear relationship ($P<0.0001$) with ME. No other values met the significance level as linear relationships but, when using the independent variable ME in a quadratic model, ALB and GLUC showed significant negative relationships with ME.

When an interaction term (CP*ME) was added to the model, a significant negative relationship with BUN was observed. The R-square value increased to 0.12 which meant that 12% of the variation in BUN could be accounted for the new model which included an interaction between CP and ME levels.

Glucose and NEFA levels also have a significant relationship when the interaction term was included. Glucose had an R-square equal to 0.027 while NEFA had an R-square of 0.049.

Lactation number had no significant effect over any of the metabolites.

4.0 CONCEPTION

In table 11 and figure 25, conception rate per farm for all 702 sampled and 1025 non-sampled breeding events are presented separately. This data is not related to the CP levels but to the total conception rate per farm regardless of what pasture CP levels the animals were fed.

Table 11. Percentage conceptions for sampled and non-sampled breeding events that took place during the period from the 25th of October to the 26th of November, 1990

Farm	Sampled Breeding events	Non-sampled breeding events
1	22.5 (40)	8.6 (81)
2	14.4 (104)	12.6 (135)
3	37.5 (40)	32.1 (84)
4	21.2 (33)	64.3 (28)
5	66.7 (12)	63.6 (11)
6	54.7 (53)	51.3 (117)
7	28.6 (49)	36.5 (63)
8	56.8 (74)	61.0 (156)
9	53.8 (173)	47.3 (207)
10	44.0 (125)	43.3 (143)

Total number of animals per farm are included inside parenthesis.

A large variation between farms was observed in figure 25 and table 11 and this was confirmed by performing a SAS general linear model procedure that yielded significantly different results in conception efficiency (P<0.005) between farms for sampled and non-sampled breeding events (table 12). The farms that presented the most significant differences between means were farms 5 and 2 for the sampled events and farms 4 and 1 for the non-sampled events.

Table 12. Comparison of conception efficiency among farms for sampled and non-sampled breeding events.

Variable	Model F value	Farm comparison[1]
Conception sampled	11.91 P>0.0001**	5 ⇔ 2
Conception non-sampled	15.92 P>0.0001**	4 ⇔ 1

** Highly significant

[1] Farms that present the highest significant difference between their means at the 0.05 level.

However when looking at these differences in conception efficiency between farms, it was observed that it was necessary to consider period of sampling as a factor that might influence conception efficiency. As mentioned above, not all farms had started their AB in the same week. Some had already been inseminating for one or two weeks before this study began which meant that in these farms, some of the cows would be receiving their second AB at the time of sampling. While, in farms that started their AB on the same week of sampling, animals would be submitted to their first insemination.

Hypothetically, this would improve the chances of more conceptions in those farms that started artificial breeding earlier. Thus, it was necessary to see if any significant difference existed in conception efficiency between animals sampled in the later stages of AB and those that were sampled during the beginning of the breeding season.

Two groups were formed. Group one included animals that were sampled during the first two weeks of insemination; and group two included all animals that were sampled after the second week of insemination. With these two groups, a Chi-square

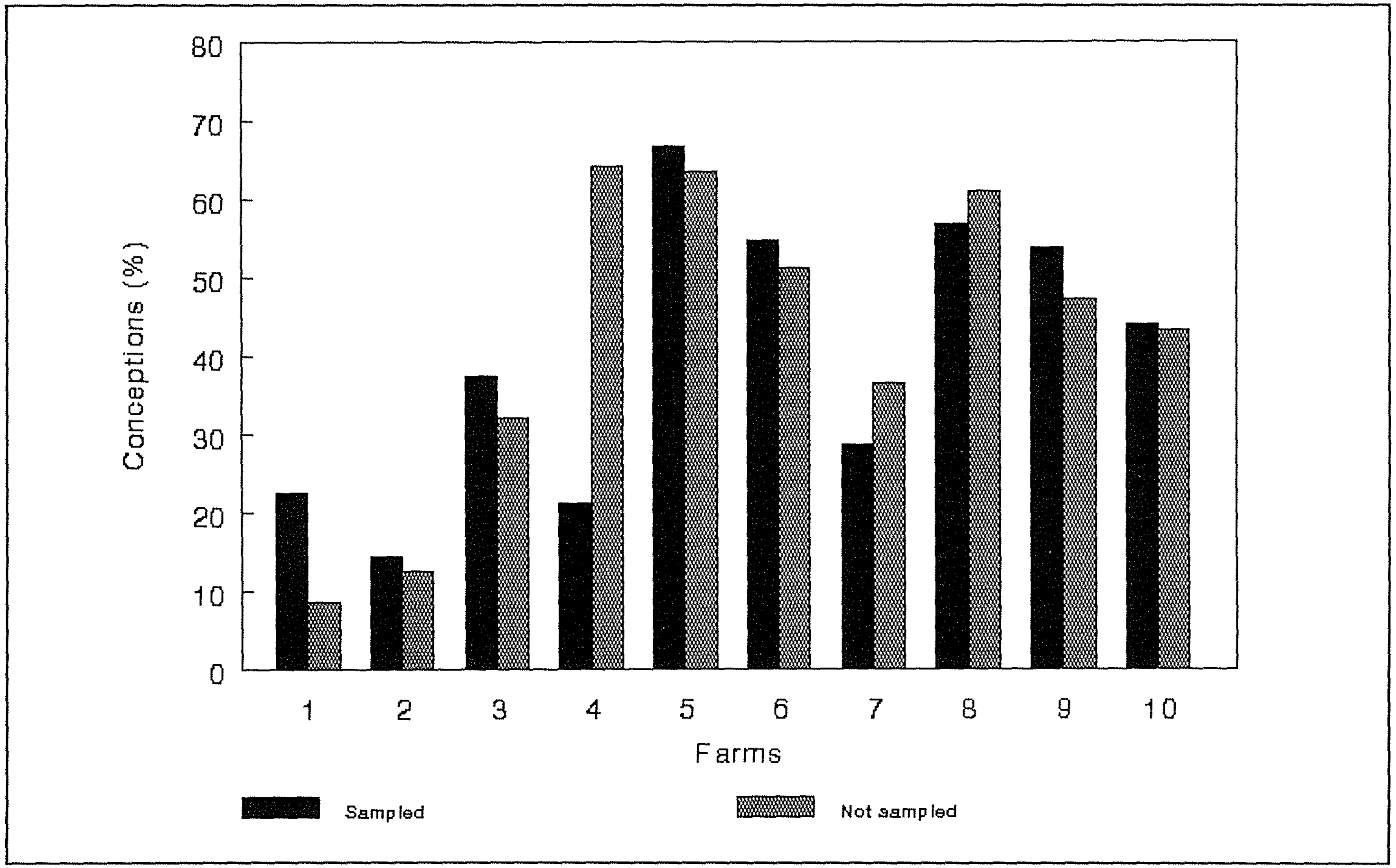

Figure 25. Conception rate per farm for sampled and non-sampled breeding events

analysis was performed. Results obtained were highly significant ($P<0.005$). This showed that there was a difference in conception efficiency between breeding events that happened during the early stages of AB and those that happened during the late stages.

Conception efficiency within farm for sampled and non-sampled events was similar for most of the farms. However, in farm 1 sampled events had a conception rate of 22% while non-sampled events had a lower conception rate of 8.6%. Likewise, in farm 4 conception in sampled breeding events was of 21.2% while in non-sampled it was of 64.3%. These differences were shown to be of no statistical significance ($P>0.05$).

Furthermore, a logistic regression analysis was performed to see if conception efficiency in sampled animals was comparable or different from that observed in non-sampled animals, regardless of what farm they belonged to. For this analysis, a new variable called type, with a binary response, was created. Non-sampled animals were considered as 0 while all sampled animals were 1. Results obtained with this procedure showed no significant difference between sampled and non-sampled breeding events in conception efficiency ($P>0.05$).

Lactation number was a variable that was also taken into account in this study as it has been reported as a factor that can influence conception efficiency (Kaim *et al*, 1983; Ferguson and Chalupa, 1989). As mentioned above, three age groups were formed; one that included all first lactation heifers, another one that included cows in their second or third lactation and finally, a third group that consisted of all cows in their fourth or greater lactation. The three lactation groups included all animals that had been sampled and those that had not been sampled but that had been inseminated during the same period that sampling took place. Percentage conception within the three lactation groups for

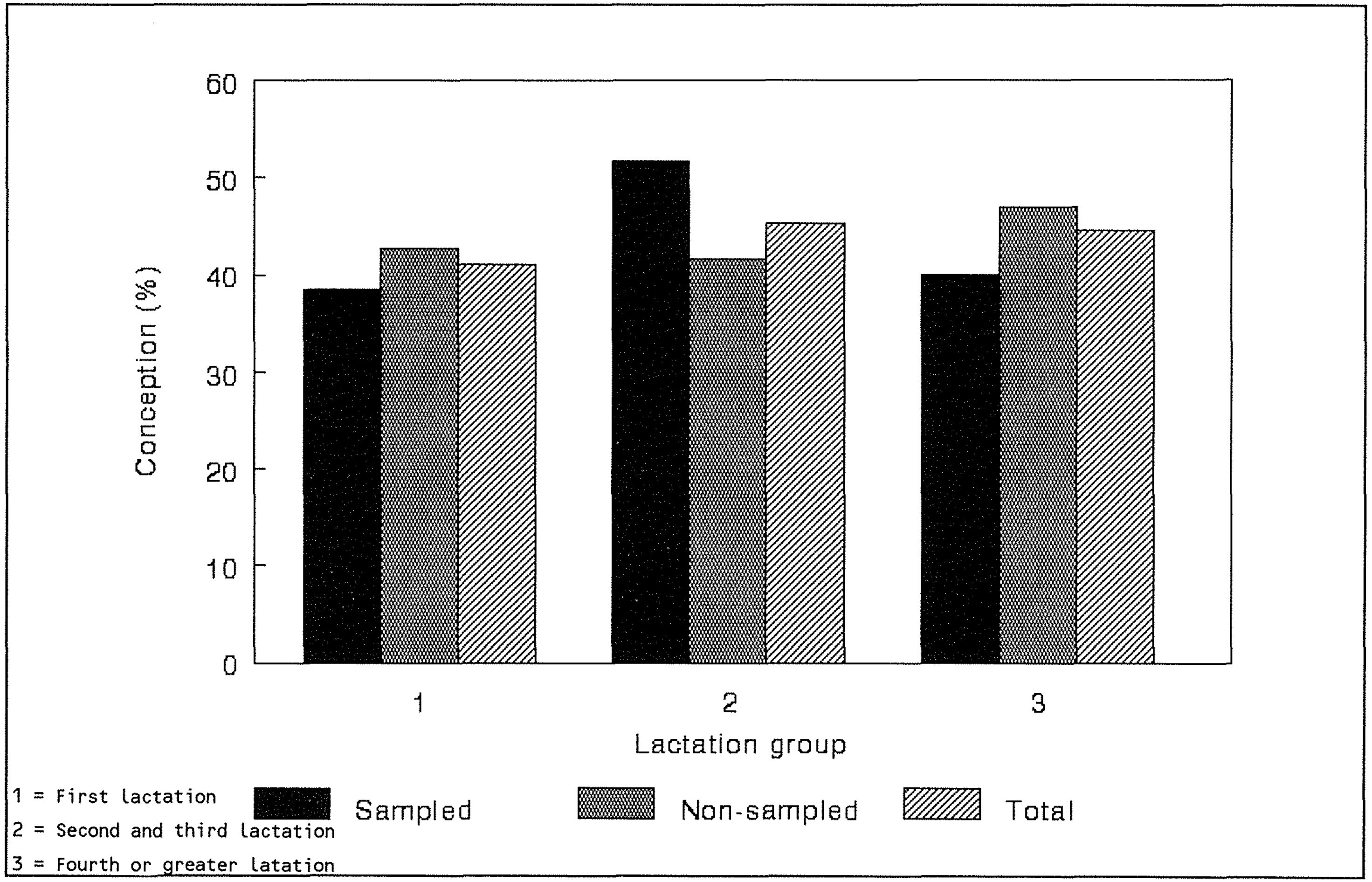

Figure 26. Conception rate of sampled, non-sampled and total breeding events for the different lactation groups

sampled, non-sampled and total breeding events can be observed in figure 26.

The overall conception rate within each lactation number group, without considering CP intake, was then calculated (table 13). Unexpectedly, it was observed that the highest conception rate occurred in those cows in their fourth and greater lactation, while the second and third lactation group had a similar conception rates and the first lactation group had the lowest (41.8, 41.1 and 38.2 respectively).

However, these observed differences in conception rate among the three lactation groups, proved to be non-significant (P>0.05) when the CATMOD procedure was performed.

Table 13. Overall conception rate within each lactation number group for total number of breeding events, sampled and non-sampled, that took place during the study.

Lactation group	Conception (%)
First lactation	38.2 (521)
Second and third lactation	41.1 (501)
Fourth and greater lactation	41.8 (706)

Number of total breeding events per lactation group are in parenthesis.

5.0 DETERMINATION OF THE RELATIONSHIP BETWEEN BLOOD AND/OR VAGINAL MUCUS METABOLITE LEVELS AND CONCEPTION

Table 14 shows the mean values for each of the metabolites tested during the collection period grouped according to

Table 14. Descriptive statistics for all conceptions and non-conceptions.

	Conceptions	Non-conceptions
Cases[1]	286	416
Mean Lactno[2]	3.105	3.445
SE(Mean)	0.136	0.132
Minimum	1.000	1.000
Maximum	12.00	14.00
Cases	286	415
Mean BUN (mM/l)	5.433	5.660
SE(Mean)	0.071	0.061
Minimum	2.450	2.730
Maximum	9.350	11.73
Cases	273	381
Mean MUN (mM/l)	4.747	3.852
SE(Mean)	0.638	0.568
Minimum	0.1	0.02
Maximum	80.60	175.8
Cases	285	415
Mean albumin (g/l)	34.77	34.53
SE(Mean)	0.297	0.252
Minimum	20.70	20.80
Maximum	49.70	47.90
Cases	285	415
Mean glucose (mM/l)	2.886	2.830
SE(Mean)	0.033	0.028
Minimum	1.100	1.000
Maximum	6.600	4.800
Cases	285	414
Mean BOHB (mM/l)	1.627	1.619
SE(Mean)	0.021	0.020
Minimum	0.842	0.720
Maximum	3.988	3.519
Cases	285	415
Mean NEFA (MEq/l)	0.636	0.608
SE(Mean)	0.021	0.017
Minimum	0.081	0.043
Maximum	1.840	1.813

[1] Total number of animals sampled for each of the variables.

[2] lactation number.

whether or not conception took place at the mating. It can be observed that when conceptions occurred, BUN levels were slightly lower than when non-conception occurred. Whereas, contrary to what would have been expected, the opposite happened with MUN levels. It can also be observed that there was a tendency towards non-conception in breeding events that had a slightly higher lactation number.

It was necessary to determine if these observations were significant, so a logistic regression analysis was conducted to determine if the association of the probability of conception with blood and vaginal mucus urea-nitrogen, and blood levels of ALB, GLUC, BOHB and NEFA were significant.

Only those independent variables that met the 0.05 significance level for entry in to the model were included. BUN, MUN and lactation number (LACTNO) met this significance level, which meant that these variables significantly influenced the probability of conception.

In table 15, a significant negative association between BUN and conception ($P<.005$) can be observed. While MUN was positively associated with conception at a lower level of probability ($P<.05$). The increase in the likelihood of pregnancy associated with the decrease in the levels of BUN by one unit was equal to 1.2. As BUN levels were lower, the probability of conception increased. On the other hand, conception was 1.02 times more likely to occur when the levels of MUN were high. The odds ratio for lactation number was of only 1.08. This meant that as lactation number increased, the probability of conception decreased and those breeding events with smaller lactation numbers were more likely to conceive than those with greater lactation numbers.

Table 15. Occurrence of conception related to BUN and MUN levels and lactation number.

Variable	Parameter estimate	Wald Chi-square	Pr> Chi-square
Intercept	0.838	4.458	0.034
BUN	-0.181	7.066	0.005^{++}
MUN	0.0219	4.801	0.028^{+}
LACTNO	-0.074	5.136	0.023^{+}

$^{+}$ $P<0.05$
$^{++}$ $P=0.005$

6.0 DETERMINATION OF THE RELATIONSHIP BETWEEN DIETARY CRUDE PROTEIN AND CONCEPTION RATE

Pasture CP values obtained each week were classified for the purpose of statistical analysis into low, medium and high protein categories regardless of which farm they belonged to.

The low CP group included all those breeding events that had been sampled when the pasture CP levels cows were fed were less than 16.5%; while the medium CP group included all breeding events where cows had been fed pastures with CP levels of between 16.5% and 20.5%. Finally, the high CP group included breeding events that occurred when pasture CP levels were 20.5% or more. Means for each of these groups were 14.95%, 18.90% and 24.38% CP respectively (table 16).

To determine if a relationship existed between the amount of protein in the diet and the likelihood of conception, a Chi-square test was performed on these three groups. To be able to observe if a similar conception rate existed in those animals that were not sampled, all non-sampled breeding events that occurred during the same period as sample events, were also grouped under the different CP groups and Chi-square tests performed.

Table 16. Percentage of conceptions for the different CP groups and lactation groups of sampled, non-sampled and total breeding events.

Lactation number	Low crude protein[1]			Medium crude protein[2]			High crude protein[3]		
	Sampled	N.S.[a]	Total[b]	Sampled	N.S.	Total	Sampled	N.S.	Total
First lactation	50	52	51	29	42	38.4	36	37.1	37
Second and third	65	54.5	59.5	51	39	42.3	42	38.5	40
Fourth or greater	55.5	66.6	60.6	31.6	47	42.4	37	41	39.5
All lactations	56.3	57.3	60	36.7	42.6	41.1	38.4	39.4	39

[1] Low = <16.5% dietary crude protein
[2] Medium = 16.5% to 20.5% dietary crude protein
[3] High = 20.5% dietary crude protein or more
[a] Not sampled
[b] All breeding events, sampled and non-sampled

Table 16 shows the percentage of all conceptions grouped under each of the CP groups for sampled, non-sampled and total breeding events that occurred during the survey period regardless of lactation number as well as conception per lactation group. It can be observed in this table that as pasture CP levels go up, the conception rate decreases. The medium CP group showed the lowest percentage of conceptions for sampled breeding events regardless of lactation number followed by low percentage of conceptions for the animals in the high CP group.

When a Chi-square test was performed, a highly significant difference between CP groups was observed for sampled, non-sampled and total breeding events ($P<0.005$), as can be seen in table 17. This indicates that there is a strong probability that the amount of DCP has an association with conception efficiency.

To determine if levels of dietary ME could be related to conception, three ME groups were formed in the same way the CP groups had been formed. Breeding events were classified under the low (less than 9.6 Mj/kg DM), medium (9.6 to less than 10.2 Mj/kg DM) and high (10.2 Mj/kg DM or more) pasture energy groups. However, results obtained showed that pasture ME levels were not significantly associated with the probability of conception ($P>0.05$) for either sampled or non-sampled animals.

7.0 DETERMINATION OF THE CRUDE PROTEIN AND METABOLIZABLE ENERGY RELATIONSHIP WITH FERTILITY IN DIFFERENT LACTATION GROUPS

The lactation number, grouped as first lactation, second and third lactations and fourth or greater lactation, was also used as a variable in the model since age and lactation number are known to influence conception (Kaim *et al*, 1983;

Randel, 1989) and are further known to influence the impact of dietary protein on conception rate (Ferguson *et al*, 1989).

In table 16, the conception rate grouped under each of the CP groups and lactation number groups for sampled, not sampled and the total number of breeding events can be observed. The medium CP group had the lowest conception rate for first lactation sampled breeding events as well as for fourth and greater lactation sampled events.

In figure 27 the conception rate, classified by lactation number and grouped by DCP intake, can be seen for sampled, non-sampled and the total number of breeding events that occurred during the sampling period. All 3 groups show the highest conception rate when levels of CP are low. Animals in their first lactation and those with 4 or more lactations show a reduced conception rate when their diet contains levels of CP that are greater than 20.5% although these results were only statistically significant in animals in their fourth or greater lactation. The conception rate increases slightly, when cows are fed a diet with a lower percentage of CP. Animals in their second or third lactations showed a decrease in conception rate as DCP percentage increased.

Observing the chi-square results in table 16, it can be seen that no statistical difference in conception rate was observed between the different CP groups for first lactation animals while, on the other hand, a significant difference was observed for those in their fourth or greater lactation ($P<0.05$).

Similarly, non-sampled breeding events showed comparable results to those of the sampled breeding events. Significance was found in the fourth or greater lactation animals ($P<0.05$), while no significant difference in conception rate of cows in their first lactation was found between the high,

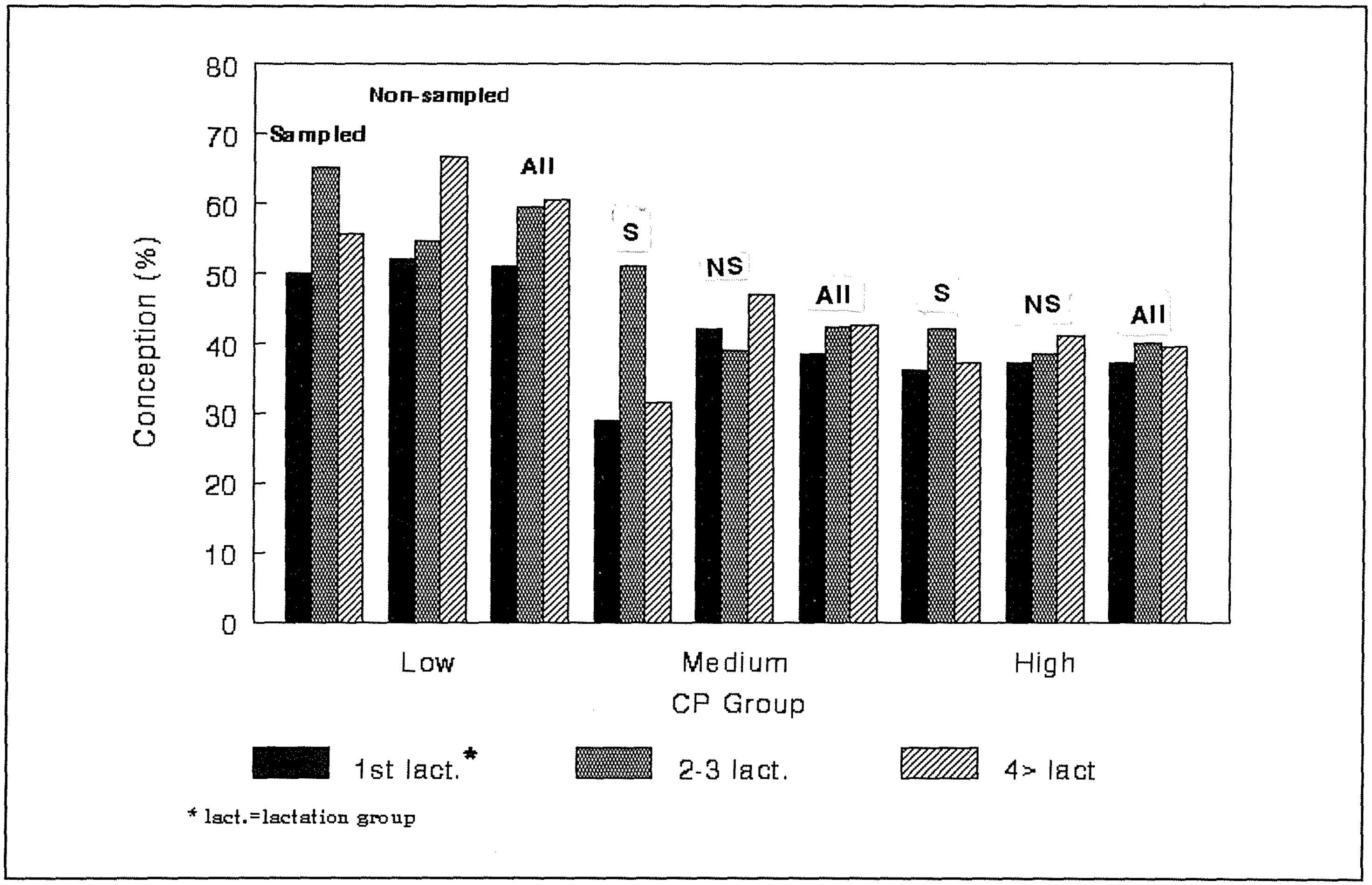

Figure 27. Conception rate per lactation group for each of the crude protein groups for sampled, non-sampled and total breeding events.

Table 17. Chi square test stratifying for age in sampled, non-sampled and all animals to observe the difference in conception within breeding events with the same lactation number but fed diets with low, medium and high protein content.

	First lactation			Second and third			Fourth and larger			**All lactations**		
	S[a]	NS[b]	All[c]	S	NS	All	S	NS	All	**S**	**NS**	**All**
No. Cases	148	236	384	139	245	384	203	334	537	490	815	1305
Concept (%)	38.5	42.8	41.1	51.8	41.6	45.3	40.4	47	44.5	43.1	44.1	43.7
Overall Chi-sq	4.52	2.54	5.24	4.68	3.69	8.93	7.46	9.09	13.12	14.5	12.96	25.71
P Value	0.10	0.28	0.07	0.09	0.16	0.01	0.02	0.01	0.001	0.001	0.001	0.000

Significant difference between crude protein groups at $P<0.05$ level.
Highly significant difference between crude protein groups at $P<0.005$.

[a] Sampled breeding events.

[b] Non-sampled breeding events.

[c] Sampled and non-sampled animals for each of the lactation groups.

[d] Animals from a,b and c without looking at lactation groups.

medium or low CP groups. All breeding events in their second or third lactation as well, did not show a significant difference in conception rate between CP groups.

8.0 PROGESTERONE LEVELS IN COWS INCLUDED IN THE STUDY

Due to the small amount of serum extracted from some blood samples, after performing all of the blood metabolite analyses, inadequate sample volume was left to process for progesterone levels.

From 649 samples, 591 (91% of samples) were processed, 365 corresponding to non-conceptions while the rest were for conceptions. Only 2% of the samples had values higher than 1.00 ng/l. As the percentage was low, it was considered that the level of erroneous heat detection was very low, and thus, it was concluded that no new statistical analysis excluding these services was necessary as the correct detection rate for the 365 services not resulting in conception was apparently 98%.

V

DISCUSSION

The purpose of this study was to investigate the effect of DCP and energy levels in the feed on fertility in New Zealand dairy cattle. There were 8 main objectives:

1) Investigation of the relationship between dietary crude protein levels and conception rate in New Zealand dairy cattle.
2) Investigation of the influence of metabolizable dietary energy on any effect of protein on conception rate.
3) To study the influence of the age of cows as an interacting factor with dietary metabolizable energy and protein on conception rate.
4) To determine the relationship between urea-N levels in blood and/or vaginal mucus and conception rate.
5) To determine if blood and vaginal mucus nitrogen levels are related to dietary protein intake.
6) To establish any relationship between energy levels in blood and dietary metabolizable energy and protein.
7) To investigate the effect of levels of dietary crude protein and/or metabolizable energy on the levels of blood components associated with energy metabolism.
8) To study the relationship of the energy related blood components with conception efficiency.

Among the original objectives to be studied was the influence of body condition score, linked to a combined measurement of CP and ME on conception efficiency. It was not possible to obtain body condition scores for all the cows sampled. During a one and one half week period of the sampling process, three of the farms were visited by a technician instead of a veterinarian. This technician had not been trained to condition score, so during this sampling period, no body condition scores were recorded for the farms under her charge.

It was also noticed that criteria for body condition scoring were not always equally applied among the participating veterinarians. It would have been advisable if only one person could have been in charge of scoring of all animals, however this was not possible.

The ten farms selected to participate in this study were representative of the average seasonally calving farm population of the Manawatu area. Thus, results obtained can be considered to be representative of the 1990 spring breeding season in this area.

The size of the farms in this study was variable, ranging from the smallest one, with 115 cows, to the largest one with nearly 400 cows and first-calf heifers. Management among the farms was also variable.

As in a number of seasonal farms in the area, more than half of the farms were under the owners charge. The remaining farms were either under the charge of a manager or a share milker. All but one showed concern about farm events. It was noticed that in this one farm, the person in charge showed little interest in the management and handling of the animals and the hygiene around the farm was less than satisfactory. This was not only observed by the veterinarian in charge of the sample collection but also an opinion shared by one of the inseminators. When analyzing the results, this point had to be ignored as it could have been a bias when this was done.

All farms were serviced by the Massey University Veterinary Clinic and therefore, due to this acquaintance with Massey University personnel, the majority of farmers were very cooperative during the whole length of the survey, making the collection of samples a relatively easy task.

The conception rate achieved in past breeding seasons by these farms had been within the average recorded in New Zealand dairy cattle and during the 1990 spring breeding season. No extraordinary event that would have later affected conception rate was recorded, such as drastic climatic changes altering pasture nutrient content.

1.0 DIETARY CRUDE PROTEIN AND METABOLIZABLE ENERGY CONTENT IN PASTURES

The mean levels of pasture CP and ME obtained in this study were 15% to 17% below those reported by Holmes and Wilson (1987) as common for spring pastures composed of ryegrass and clover mixtures, the same pasture mixtures found in the farms included in this study. This can be observed in table 1 when comparing the DCP and ME levels for spring pasture shown there with the mean values (table 4) obtained during the survey (24% CP and 11.8 MJ/kg DM of ME vs. 20.4% CP and 9.83 MJ/kg DM of ME).

However, the closest values to those presented by Holmes and Wilson are the ones found in the high CP group formed when results from pasture analysis for all farms were classified under low, medium and high dietary CP groups. Here mean values were 24.12% for dietary CP and 10.24 MJ/kg for ME. Metabolizable energy levels were lower than those commonly found, not only for the mean value but for the individual values. Metabolizable energy levels increased as dietary CP increased. Nevertheless, the difference in content of ME did not seem to vary as much as the dietary CP levels. Pastures with low CP levels had ME levels not far below those found in pastures with a high content of CP.

In general, the mean high CP group values are in accordance with those reported in the literature as common in New

Zealand (Holmes and Wilson, 1987) while the other two groups had levels below those reported. However, it was yet to be seen if these levels provided the correct amount of dietary CP and energy for an efficient reproductive performance in the dairy cows.

2.0 THE RELATIONSHIP BETWEEN CRUDE PROTEIN AND METABOLIZABLE ENERGY IN PASTURE AND METABOLITES IN BLOOD AND VAGINAL MUCUS

Blood and vaginal mucus metabolites were measured to assess their usefulness in estimating the protein and energy status of dairy cows by establishing if concentrations of these metabolites reflected the concentrations of DCP and/or energy in the feed. This investigation was to precede the most important objectives of this study which were to investigate the relationship between levels of blood and vaginal mucus metabolites with conception efficiency and their relationship with the concentrations of DCP and/ or energy levels in pasture.

To be able to relate these parameters to pasture dietary CP percentage and ME proportions in pasture, the relationship between blood and vaginal mucus metabolites and CP and ME levels had to be established. Three metabolites that reflect the protein status of the cow, BUN, MUN and ALB, were measured; while energy status was assessed by levels of BOHB, NEFA and GLUC.

From the results obtained in this study, it can be observed that there is a direct relationship between pasture DCP levels and the concentration of urea-N in blood plasma. As the levels of CP increased in the diet, BUN significantly increased ($P<0.001$). Crude protein accounted for 9% of the variation of BUN. This finding agrees with previous reports by various authors (Manston *et al*, 1975; Jordan *et al*, 1983; Ferguson and Chalupa, 1989; Macleod *et al*, 1986).

In these reports, urea-N is not always measured as blood urea-N (BUN) as it was in the present study. Some authors measure plasma urea-N (PUN) or serum urea-N (SUN). However, levels of urea-N in whole blood, serum and plasma have been seen to be comparable (Coles, 1980). Therefore, it is valid to compare the SUN (serum urea-N), PUN (plasma urea-N) and BUN (blood urea-N) concentrations found in the different studies reported.

With an increase of DCP intake, an increase in the levels of ammonia in the rumen occurs and this may exceed that which can be used by ruminal micro-organisms. This excess ammonia is absorbed by passage across the rumen wall and is transported to the liver where it is converted to urea. This results in higher levels of urea in blood and tissue fluids such as uterine and vaginal mucus and milk.

Numerically, BUN levels found in this study followed the same trend as those reported by studies done overseas. Higher levels of BUN occurred as the pasture dietary CP percentage increased. However, when looking at mean BUN levels for each of the CP groups formed (low, medium and high CP groups), the mean BUN value observed in the animals in the high protein group, feeding on pastures with a DCP content of 20.5 % or more (with a mean value of 24.12% CP), was higher than that reported by authors such as Manston *et al* (1975). These authors observed serum urea-N concentrations of approximately 20-22 mg/100 ml in animals being fed a diet containing 24.2 % digestible CP while in the present study the mean blood plasma urea-N level, when converted from mmol/l to mg/100 ml, was 36.02 mg/100 ml.

The mean BUN level observed in the medium CP group was also higher than that observed by Carroll *el al* (1988) for animals on a diet with DCP levels similar to levels observed in the medium protein group where levels ranged from 16.5% CP to 20.5% CP. While they reported a mean blood plasma urea-N concentration of 20.9 mg/100 ml for animals fed a 20% CP ration, in the present study, the medium CP group had a mean BUN of 34.1 mg/100 ml (5.66 mmol/l). On the other hand, the maximum BUN values observed in this study (table 9), are similar to those reported by Olter and Wiktorsson (1983) in Sweden and by Moller (1990) in New Zealand.

One reason why differences in urea-N concentration in blood plasma were observed in the present study compared to those found in the literature, could be the degradability of protein in the diet. BUN is known to be a sensitive indicator of dietary CP intake, especially in the form of degradable dietary protein (Manston *et al*, 1975). Pasture is known to contain large amounts of RDP which, according to Holmes and Wilson (1987), is assumed to be of a degradability (dg) of 0.8. Once ingested, RDP is rapidly converted to ammonia by the ruminal microorganisms. This large amount of RDP is converted into an excess of ammonia that subsequently is converted to urea. This urea is measured as urea-N concentrations in blood plasma or serum.

Rations formulated in studies by Carroll *et al* (1988) and Manston *et al* (1975), contained ingredients such as barley and corn that have been proven to have low degradability, combined with ingredients such as soybean and oats that are more degradable. Although degradability in pastures used was not known, it is known that in general, pastures contain large amounts of degradable protein. In summary, therefore, it is possible that the higher BUN levels recorded in the cows in this study compared with cows in the three reported studies on a similar DCP level, may result from a greater proportion of readily degradable protein. To confirm this, it would be necessary to analyze the amount of RDP in the pastures fed to the animals used in this study.

Time of sampling does not seem to be the cause of the difference between studies. In all the studies, including this one, blood samples were collected about 2 or 3 hours after feeding, a time when BUN might be at its highest levels (Oltner and Wiktorsson, 1983; Manston *et al*, 1975). Weather conditions could be a factor influencing levels of BUN. A combination of high temperatures and no access to water during the time the animals were waiting to be inseminated could influence the levels of blood urea-N. However, in this study, during the sampling period, temperatures were mild, or cold and rainy as often occurs in the Manawatu area in spring, so this not likely to have been a factor that played part in the variation of levels of BUN in the animals seen in this study.

Pasture ME levels had a highly significant positive effect on the levels of BUN but only explained 4% of the variation. This disagrees with reports by Macleod *et al* (1984). They observed that as they increased energy density ratios of forage:concentrate from 75:25 to 55:45, and 35:65; plasma urea nitrogen decreased linearly. However, their rations were formulated based on energy concentration of the diet and calculated as energy densities from forage:concentrate ratios, while in the present study, pasture ME was used.

In this study, ME which was measured as a proportion of pasture DM, while Macleod *et al* (1984) estimated energy balance and the amount of digestible energy consumed. This makes the observations in both studies different and therefore, not directly comparable.

In this study, pasture ME levels increased as pasture CP increased. Nevertheless, total intake of carbohydrates and other sources of energy by the animal could have been low and therefore not sufficient to be utilized both for milk production and detoxification of ammonia and urea. Excess ammonia would increase pH in the reproductive tract, thus reducing fertility due to the toxic effects of ammonia and urea-N on ova, sperm and early embryos.

BUN levels could have remained high and energy levels in the animal low without pasture ME concentrations reflecting this situation. The significantly positive relationship between pasture ME and BUN might have been explained if the true energy intake in these animals was known.

However, the existence of an interaction between pasture crude protein concentrations and energy levels on BUN could well explain the reason why a significant positive relationship was found. In this study, a negative interaction between pasture CP and ME significantly affected the relationship of these variables with BUN. The interaction between pasture CP and ME for the total model including the interaction accounted for 12 % of the variation of BUN. This was the highest R-square obtained among all the variables.

ALB levels showed a significant negative linear relationship with CP%, and a negative quadratic relationship with ME. The results contrast with those reported by Jordan and Swanson (1979) and Jordan *et al* (1983) where significant positive relationships were found.

The findings also disagree with reports by Manston *et al* (1975) where they observed that high intakes of DCP were reflected as hyperalbuminemia. In the present study it was observed that when CP increased, ALB decreased making this an inverse relationship. This finding is similar to that reported by Parker and Blowey (1976). They observed no consistent correlation between digestible CP intake and plasma ALB. In their study, when the overall herd means for all samplings were compared, the herd with the highest percentage of DCP underfeeding had the highest mean ALB whereas the herd with little underfeeding of DCP showed the lowest mean (3.62 vs 3.19 g per cent).

One possible reason for low levels of ALB occurring in association with high levels of dietary CP mentioned by these authors and by Manston *et al* (1975), could be that ALB levels do not reflect the protein intake at the time of sampling, as animals were constantly rotated into pastures with different CP percentages. It is known that ALB responds slowly to an increase in dietary protein (Wilson *et al*, 1985) and even then, it does not increase above normal with high CP intakes (Blauwiekel and Kincaid, 1986).

Only one percent of the variation in ALB concentration could be accounted for by the variation in levels of ME. This result was obtained when using the quadratic ME term and proved a significant negative relationship. However, when pasture DCP and ME were both included in the model, the amount of the variation that could be explained rose to 7.8%. Again, a significant negative relationship was observed between ALB and dietary CP but this time combined with a significant positive relationship with ME.

These results could possibly be explained by a slow response of serum ALB to an increase in DCP. Also, the existence of an interaction between pasture CP and ME might have affected the relationship with ALB.

No significant relationship was observed between MUN and pasture CP showing that in this study, MUN was not a good indicator of pasture DCP intake. This does not agree with the results reported by Carroll *et al* (1988) where a significant positive relationship was observed in MUN concentrations and DCP.

Glucose was observed to have a significant negative relationship to both pasture CP and ME. However, only 1.9% of the decrease in serum GLUC that occurred when pasture CP increased could be explained by this means.

The results observed for serum GLUC levels agree with findings by Hibbit *et al* (1969). They observed that animals on a low protein diet (10% digestible CP) had a higher GLUC level than those fed on diets containing 15 and 25% digestible CP. The mean serum GLUC levels for each of the groups were 78.8, 71.0 and 70.5 mg/100ml respectively. In the present study, when all breeding events were separated into high, medium and low CP groups, the highest mean GLUC level (3.036 mmol/l) at breeding was observed in the low protein group fed pastures containing less than 16.5% dietary CP (table 8), while the lowest mean GLUC level was observed in breeding events found in the high CP group (2.845 mmol/l).

Animals fed pastures with low levels of CP appeared to have a sufficient energy intake which was reflected in high levels of serum GLUC. Another factor that may have intervened in the increase in GLUC concentrations in serum could have been oestrus (King, 1971), but this would have applied equally to all groups.

When high intakes of pasture DCP occur, especially of rumen degradable protein, dietary energy supplies may not be sufficient to support disposal of the excess ammonia and urea produced. If, like in this study, animals are at peak lactation, an energy deficit may occur in those animals feeding on pastures high in CP and this would be reflected in a decrease in serum GLUC concentration.

According to Hibbit *et al* (1969), feeding large quantities of protein leads to an increase in the intake of potential ketone body precursors in the form of the ketogenic amino acids. In an animal which is lacking available carbohydrates at peak lactation and mobilizing fat reserves, these additional ketone body precursors would be incompletely oxidised in the tricarboxylic acid cycle leading to ketone body formation.

No study has been reviewed where a negative relationship with dietary energy and serum GLUC was observed. Pasture ME may inadequately reflect the dietary energy intake of animals. However, this negative relationship must be regarded cautiously as it was observed that there was an interaction between pasture CP and ME. Although only 3% of the variation in NEFA levels could be accounted for by the interaction between CP and ME, it was significant ($P<0.01$).

Pasture ME does not appear to be the best indicator of digestible energy available for intake by the dairy cow. Therefore, the effectiveness of serum GLUC concentrations as indicators of the animal's energy status can not be confirmed. Some authors found serum GLUC concentrations to be good indicators of energy status. Nachtomi *et al* (1991), mentioned that GLUC concentration in whole blood or plasma from cows has been used extensively as an indicator of energy status. However it is used mainly as an individual indicator. King (1971) mentions that blood GLUC levels are good indicators of the carbohydrate status of an animal and herd mean blood GLUC concentrations are valuable in that not all hypoglycaemic herds suffer from dramatic clinical signs.

Blowey *et al* (1973), report that plasma GLUC levels indicate starch intake and increased starch intake facilitates microbial protein synthesis in the rumen which reduces ammonia concentration and blood urea levels. Parker and Blowey (1976), observed that although positive correlations were found in three herds between starch equivalent (SE) intake and plasma GLUC, the highest occurred in a herd with the most serious underfeeding of SE. No significant correlation occurred in three other herds where SE underfeeding also was common. Even when observations were confined to inadequate levels of SE intake, there was no constant association.

Some studies however show opposite findings. Oltner and Wiktorsson (1983), observed that the concentration of GLUC in plasma varied little and did not change with the diet. Changing the energy supply for cows in their study, from 86% to 124% of the requirement did not affect plasma GLUC levels. Therefore, they concluded that when no dramatic under-or over-feeding in relation to production is evident, plasma GLUC concentrations seem to be of limited value as an indicator of energy supply.

It would be interesting to further study the role of serum GLUC as an indicator of the dairy cow's energy status taking in to consideration pasture water soluble carbohydrates and fibre, as well as the animal's milk production.

Results obtained in this study show a linear increase in serum NEFA levels as pasture CP increased. This is clearly observed in table 8 where the means for each of the CP groups is observed. NEFA was observed to have a significant positive relationship to pasture CP. Only 1% of the increase in NEFA that occurred when pasture CP increased could be explained by this relationship.

On the other hand, the relationship between serum NEFA and pasture ME is significantly negative. This relationship is of a quadratic nature that can not readily be observed in the table. In figure 24, a curvilinear tendency is observed.

In the literature, serum NEFA has been shown to be negatively correlated with energy balance (Blauwiekel and Kincaid, 1986). NEFA are released to blood plasma when adipose tissue is mobilized to meet the energy demands of the animal (Holmes and Lambourne, 1970; Bowden, 1971). Thus, they are considered to be sensitive indicators of the mobilization of body fat (Kronfield, 1982). NEFA levels have also been observed to be affected by the carbohydrate content of the ration fed to the animals and thus, have been proposed as useful indicators of carbohydrate utilization (Bowden, 1971).

The results in the present study agree with those obtained by Nachtomi *et al* (1991). They studied the effect that protein-energy intake had on blood metabolites of 46 multiparous Israeli Holstein cows. These animals were fed 3 dietary concentrate levels (50, 65 and 80%) and 2 CP levels (17 and 21%) in DM. They observed that increasing the energy intake by raising the concentrate level from 50 to 65 and 80% in the diet, lowered ($P<0.05$) the plasma NEFA.

In the present study, pastures with the highest ME levels were reflected in the animals as lower NEFA levels. The above observations raise the question as to why NEFA concentrations show a relationship with pasture ME more in accordance with what is found in the literature than do serum GLUC levels. It could be because they are more sensitive indicators of undernourishment than GLUC or ketones (Bowden, 1971).

From the literature reviewed it is known that the levels of NEFA will rise when there is a deficit of energy supply and this can happen with diets high in protein, especially in the form of DIP, since a great amount of energy is needed for the detoxification of ammonia. Pasture is known to contain high quantities of DIP. In the current study, it is proposed that the inverse relationship between NEFA and ME indicates the energy deficit that results from pasture diets which are high in CP.

In summary, if the results obtained in the present study are compared with those observed in the literature, only serum NEFA levels were found to reflect dietary energy intake accurately. NEFA was the only metabolite to show a significant relationship with ME levels in accord with reports mentioned above.

However, although NEFA were shown to be significantly related to ME, the doubt still exists as to whether pasture ME was an accurate indicator of the amount of dietary energy ingested by the dairy cow.

There is the possibility that pasture ME levels were sufficient for maintaining a proper protein - energy balance in the cow. This seems probable as it can be observed in table 5 that pasture ME levels showed a tendency to increase as CP levels increased. However, voluntary food intake or feed allowance may have been low and thus, restrict the amount of dietary energy voluntarily ingested by the animal. The majority of the breeding events included in this study were the second of the lactations and the voluntary food intake of cows would thus be reaching maximum levels. However, as Holmes and Wilson (1987) explain, the delay in reaching maximum voluntary food intake after parturition is longer than the delay in reaching maximum milk production and hence maximum requirement for dietary energy, and intervals of up to 10 weeks occur between peak milk yield and maximum food intake.

Further studies should be done using other pasture energy measurements mentioned in the literature such as fibre and water soluble carbohydrates to clarify this point. Both have been observed to better reflect dietary energy intake. Furthermore, levels of GLUC and NEFA in the blood are usually closely related in ruminants, with NEFA increasing while GLUC decreases. Blood GLUC levels have been observed to decline more slowly than NEFA increases. Thus, when looking at the these metabolites as reflections of the animal's energy status, the use of pasture water soluble carbohydrate levels as reflectors of dietary energy availability would certainly be of interest as they may better reflect the dietary energy intake.

No significant relationship was found between BOHB concentrations in serum and levels of pasture CP or ME. These results agree with those of Cody *et al* (1990), who fed 4 concentrate supplements differing in CP and UDP content to 16 lactating Friesian cows, together with grass silage ad libitum. The supplement treatments were: 1) Barley 122 g CP per kg DM, dg 0.77; 2) barley/soya-bean meal, 210 g CP per kg DM, dg 0.69; 3) barley/soya-bean meal/fish meal 190 g CP per kg DM, dg 0.61; 4) barley/soya-bean meal/fish meal 219 g CP per kg DM, dg 0.59. They observed BOHB levels of 0.40, 0.38, 0.38 and 0.36 mmol/l respectively for each of the 4 treatments. In the present study, mean levels of BOHB in cows fed low, medium and high pasture DCP were of 1.616, 1.570 and 1.604 mmol/l respectively. Although these results were non-significant like those found by Cody *et al* (1990), they are still higher. This is consistent with the view that pastures low in dietary energy are associated with high serum BOHB levels.

MUN, in this study was not a good indicator of pasture CP as no significant relationship was observed between the levels of urea-N present in vaginal mucus and the pasture CP levels. MUN levels were very inconsistent as in some cases where animals were fed pasture with high CP percentage, MUN levels could be lower than those observed in animals feeding on low CP diets. It was surprising to find that although dietary crude protein intake did not seem to influence MUN in a significant way, the levels of MUN were related significantly to conception efficiency. This point is discussed later on in this chapter.

Age, or in the case of the present study lactation number, was not a factor that significantly influenced the relationship between pasture CP and ME and blood metabolite levels. Table 7 shows that the levels of all metabolites were similar between the different age groups.

Other factors that could have influenced the outcome of this study were climate, milk production and handling stress. Since no outstanding changes in the weather were observed, this is an unlikely factor. Milk production could have played a part in the amount of energy available for efficient protein metabolism. If energy was insufficient in the diet, part of that which was available would be diverted to milk production, not leaving enough for efficient detoxification of ammonia in the diets that contained high amounts of CP.

3.0 CONCEPTION EFFICIENCY AND ITS RELATIONSHIP TO BLOOD, VAGINAL MUCUS METABOLITES, PASTURE CRUDE PROTEIN AND METABOLIZABLE ENERGY

Conception rates at all sampled and non-sampled breeding events that occurred during the sampling period were calculated to observe if any difference existed between them. The results presented in this study show that in the 10 farms of the Manawatu area that were surveyed, no significant difference was found ($P>0.05$) between sampled and non-sampled breeding events overall nor within farms. It can thus be concluded that the sampling methods (especially the vaginal mucus collection procedure) did not interfere with conception.

A significant difference was observed between farms, for each of the groups. This difference was found to be due to time of insemination. Since not all farms started their AB at the same time, some had started one or two weeks before this study began, some of the cows of these farms had improved chances of conceiving as a number of cows were being inseminated for the second time.

Nevertheless, although these findings are of interest, they do not have an effect on the main object of this study, that was to investigate the relationship between conception efficiency and pasture dietary CP and energy levels.

The low conception rate obtained in some of the farms during the collection period is a surprising observation. It is lower than commonly reported for the average New Zealand herd. The lowest conception rates were observed mostly in farms that started their breeding season at the same time as this study commenced. However this was not universally true as farm 5, that also fell in to this group, had one of the highest conception rates (66%).

Lactation number, on its own, did not have any significant effect on conception as observed in both sampled and non-sampled animals. De Kruif and Brand (1978) mention that low conception rates have been reported for primiparous cows. They state that primiparous cows have a longer interval to the first oestrus that is usually the consequence of ovarian inactivity rather than a weak expression of oestrus. However, there are reports that conception efficiency decreases with age of cow; for example, these authors note that low conception rates have been reported in cows over 7 years of age. Ball (1978) and Azzam *et al* (1989) report in both dairy cattle and beef cattle, that a lower conception efficiency is observed in 4-year old cows onwards. In the present study, in percentage terms, conception was higher in animals in their fourth or greater lactation.

The study of the relationship between blood metabolites and conception rate provided results that agreed with those previously reported by Ferguson *et al* (1989), Ducker (1984), and Blanchard *et al* (1990). At the same time, a relationship between concentrations of pasture CP and conception efficiency was also found to agree with reports by Ferguson *et al* (1989), Kaim *et al* (1983) and Jordan and Swanson (1979a).

Moller (1991) observed in his study, higher blood urea levels in herds feeding on pasture containing higher levels of CP (24.09 and 23.62% DM) compared to those feeding on pasture with lower levels of protein (20.91 and 21.87% DM). He also observed that elevated blood urea levels were associated with reduced conception rate. This would also seem to agree with the results obtained in the present study, however his findings must be looked at cautiously as no statistical analyses were performed on his work to determine if these findings were significant.

In the present study, when observing the results obtained from the logistic regression analysis, only BUN and MUN showed a significant relationship with the probability of conception. All of the other metabolites were excluded from the model as non-significant. Even BOHB, that has been reported in some studies (Armstrong *et al* 1990; Ducker *et al*, 1985b) as being significantly correlated with the number of services required per conception, was not a significant factor influencing the probability of conception in this study.

Serum GLUC levels were not significantly related to conception. However, it was observed that some of the GLUC levels were below 2.9 mmol/l (the lowest being 1.0 mmol/l), a level which has been reported by Miettinen (1991) as a limit below which fertility problems can present themselves.

The relationship between BUN and conception was a negative one which agrees with prior reports (Moller, 1991; Canfield *et al*, 1990) while in the case of MUN, the relationship was positive. MUN results are contrary to observations in all other works that have been reviewed. However, it has been considered previously that MUN cannot be trusted as a real indicator of protein intake and thus does not truly relate to conception (Carroll *et al*, 1988). These doubts increase when results from the linear regression of MUN on CP are analyzed and the relationship is shown to be non-significant.

Few studies have been done using MUN, and the relationship between MUN and the protein-nitrogen intake is still unknown. More studies have been done using BUN as an indicator of nutritional status, specifically of DCP intake, and its validity is much better established. In the present study, the relationship between MUN and conception, although significant, was weak. Breeding events with high MUN levels were 1.02 times more likely to conceive than breeding events with low levels of MUN.

BUN and plasma urea-N (PUN) have been related in many works to the probability of conception. In most of these studies, similar findings to the ones in the present study have been observed. Low conception rates have been reported in association with high BUN levels in animals fed excess CP in the ration.

Ferguson *et al* (1988) found that cows with PUN greater than 20 mg/dl were 3 times less likely to conceive than those with lower concentrations. Canfield *et al* (1990) found that animals that conceived at first service had a lower plateau for Plasma urea-N than those that did not conceive (15.7% vs. 18.6 mg%, $P<0.02$). In the present study, breeding events leading to conception were associated with lower BUN levels than those that did not conceive (mean BUN levels of 5.43 mmol/l vs 5.66 mmol/l). The odds of a breeding event leading to conception as BUN levels decreased (from 11.73 mmol/l to 2.45 mmol/l) were 1.2 times greater. As can be observed, levels of BUN found in the present study followed a similar pattern to those observed by these authors.

In summary, systemic urea elevation caused by excess dietary protein intake as reflected by elevated urea-N in blood, can be associated with a low conception rate.

Age was another factor that was significantly related to conception rate when included in the logistic regression model. As lactation number increased, conception decreased in a significant way when blood urea levels were high.

The high levels of BUN reflect an excess of ammonia and urea in the animal due to an imbalance in the protein:energy supply for example, as is possibly the case in the present study, when high pasture CP - low dietary energy occurs. This, according to Ferguson and Chalupa (1989), may cause toxic by-products of nitrogen metabolism from the rumen to impair sperm and ova, or cause early embryonic death.

A significant negative relationship was observed between high intakes of dietary protein and reduced conception rates in dairy cattle. These results agree with previous reports on a dietary protein - fertility relationship that strongly suggest that feeding diets with high CP concentrations may depress fertility (Ferguson and Chalupa, 1989; Sonderegger and Schurch, 1977; Ferguson *et al*, 1986; Folman *et al*, 1981).

Chi-square calculations showed that a significant difference ($P<0.005$) in conception efficiency existed between the different CP groups. This was not only true in the sampled breeding events but also in the non-sampled breeding events. Looking at both results, it can further be concluded that sampling methods had no effect over the conception outcome in the sampled cases.

Levels of feeding, including those of CP, around the time of insemination can markedly influence the reproductive performance of dairy cattle. As dietary CP increased, conception decreased as is reflected by the fact that samples grouped under the low CP label (<16.5% CP), had the highest percentage of conceptions compared to the medium (<20.5) and the high CP groups (>20.5), 56.3% vs. 36.7 and 38.4%, respectively in the sampled group and 57.3% vs. 42.6 and

39.4% CP for the non-sampled group.

Canfield *et al* (1990) and Ferguson *et al* (1988) also observed that animals fed high levels of dietary CP (18-19% CP) had lower conception rates. However, these authors studied first service conception rate while in the present study, conception rate to breeding on the day of sampling was observed. It was not possible to study first service conception rate because breeding events sampled during the study period were not all first services. Some were second or even third services.

Aside from this, Ferguson *et al* (1988) studied not only the effect of dietary CP on fertility but also the effect of different levels of degradable protein on fertility. In the present study, degradability of the pasture based diet was not known. Still, if it is assumed, as reports by Holmes and Wilson (1987) state, that it could be around 0.80, then it can also be expected that these high levels would affect fertility. This assumption can only be investigated if the levels of degradable protein in the pastures grazed are known.

Another difference between the present study and that performed by Canfield *et al* (1990),is that they found that energy balance was affected by protein intake in excess of that required, especially if the protein was highly degradable. In this study, pasture ME was shown to be of little use to assess the pasture dietary energy content or its effect on fertility as no significant relationship was found between energy levels and conception efficiency. Besides, protein - energy balance was not studied. Nevertheless, it would have been interesting to know the energy balance of the cows at the time of breeding events sampled in the present study and see if it was affected by excess protein intake, then further investigate its relationship with conception. However, knowing the normal nutritional requirements of dairy cattle for maintenance and milk production and that pastures can have a low energy content, it can be assumed that a similar situation to that observed by Canfield *et al* (1990) occurred in this study.

No significant relationship was found between conception and ME levels in this study. No combined effect between dietary CP and ME concentrations was observed either. However, even when no significant combined effect was observed, this is not a guarantee that this effect does not exist, but pasture ME just might not show it. Moller (1991) has suggested that pasture soluble carbohydrates better reflect the combined effect of CP and energy and their relationship to fertility. He observed that dietary imbalances of protein and soluble carbohydrates seemed to contribute to fertility problems (conception rate and anoestrum). However, his conclusions are based on visual observation of the numeric data he obtained. No statistical analysis was performed to give weight to his assumptions. McClure (1961b), suggested that high total nitrogen levels in young rapidly growing pasture would be associated with a nutritional and lactational stress infertility. In a later paper, the same author (McClure, 1970a), adds carbohydrate and energy content as probably affecting reproduction.

There is evidence that spring pasture is high in protein and sometimes carbohydrate is low (Varta and Bailey, 1974). Thus, it would have been interesting to know the soluble carbohydrate content in the pastures analyzed in this experiment to investigate the effect it had on conception.

Though soluble carbohydrates had been considered initially as a variable for measuring energy in this work, ME was chosen because analysis of soluble carbohydrates was not performed in the Batchelar Animal Health Laboratory and because other works had been conducted where the effect of dietary energy intake on fertility was studied using ME as a measure of energy levels in the diet (Ducker *et al*, 1985a, b).

Varta and Bailey (1974), noted that in the North Island of New Zealand, much lower values of soluble carbohydrates have been reported for perennial rye grass when compared to the South Island. These values would most likely be for leafy herbage and would not include much of the basal tissues of the grass where soluble carbohydrate is stored. Thus, it can be assumed that these values would be useful as a reflection of the soluble carbohydrate to which the cow has access, since that in the leafy herbage is at grazing level for cows.

The mechanisms by which fertility was affected are unknown but based on studies, like those performed by Carroll *et al* (1988), Ferguson and Chalupa (1989), Ferguson *et al* (1988), Jordan and Swanson (1979) and Swanson (1989) among others, some assumptions can be made.

In this study, the effect of high dietary CP on fertility could be as proposed by Ferguson and Chalupa (1989), Blanchard *et al* (1990) and Jordan and Swanson (1979). They reported that high amounts of dietary CP in the form of excess DIP, cause ruminal alkalosis and increases of rumen ammonia, which result in an energy deficit and disorders of liver cells. Feeding excessive dietary protein increases liver glutamine dehydrogenase and ornithine carbamyl transferase in plasma of dairy cows, thus reflecting cellular damage throughout the body and possibly creating a sub-optimal uterine or ovarian environment. This occurs with the increase of urea-N and ammonia levels in the blood that may elevate the pH in the reproductive tract and this may affect fertility of the ova or cause early embryonic death that occurs before the time of maternal recognition of pregnancy. It could also affect the fertility of the sperm directly by decreasing the ability of spermatozoa to penetrate bovine cervical mucus, as has been observed in experiments in vitro

(Blanchard *et al*, 1990). All of these factors would thereby reduce conception efficiency.

These theories could explain why non-conception occurred more in animals fed pasture high in CP than in those eating medium or low levels of pasture CP.

This study was not designed to look at number of services per conception. In this survey, samples and information were collected to specifically estimate if, as had been observed in North America and Israel, pasture CP levels show any relationship with conception efficiency in New Zealand dairy cows. Observations were taken as individual conceptions or non-conceptions to an insemination. This did not permit us to calculate conception rates per service but rather conception rate at the date of sampling. Due to this, the effect of CP on number of services per conception and days open could not be observed and compared to results where an increase in days open and services per conception occurred when protein increased (Blauwiekel *et al*, 1986) as information on dietary CP content was not available for all AI dates.

The effect of pasture CP intake on conception efficiency within the different lactation groups was studied as reports of the existence of a relationship between age, DCP intake and conception efficiency have been made by authors like Kaim *et al* (1983), Howard *et al* (1987) and Ferguson and Chalupa (1989).

No significant difference was observed between the three age groups when they were not classified in the different CP groups. However, once pasture CP is included in the statistical model as an effect on conception rate, a significant difference was observed.

Sampled and non-sampled breeding events of cows in their first lactation, appeared to have a lower conception efficiency as pasture CP increased but this effect was not significant when a Chi-square analysis was performed. Likewise, there was no significant influence of the percentage of pasture CP on conception efficiency of breeding events for cows in their second or third lactation.

A significant difference (P<0.05) between CP groups was found only when both sampled and non-sampled breedings were included in the chi-square analysis. Both of these findings agree with the study performed by Ferguson and Chalupa(1989) where they analyzed the results from other studies on the effect of amount and degradability of dietary CP on fertility. However, our study did not look at the degradability of the pasture protein. They observed that fertility of cows in their second and third lactations was not affected greatly by either amount or degradability of dietary protein.

Breeding events of cows in their fourth or greater lactation, fed low pasture CP diets, had conception rates of 55.5% and 66.6%. However, as pasture CP levels went up, there was a drop in conception efficiency to 31.6 and 47% in the medium CP group and 37 and 41% in the high CP group for sampled and non-sampled groups respectively.

Fourth or greater lactation cows showed a significant effect of levels of pasture CP on conception efficiency. When looking at conception rates, it becomes apparent that there might be an interaction between CP intake and lactation number and the occurrence of a reproductive health problem. Hence, as CP increased, conception rate decreased in this older group of cows.

Results observed in cows in their fourth or greater lactation agree with observations by Kaim *et al* (1983), Ferguson *et al*

(1986) and Ferguson *et al* (1988). Ferguson *et al* (1988) report that maturity in cows (fourth or greater lactation) and protein degradability may influence the effect of dietary CP on fertility. Degradability of protein plays an important role in fertility and the assumption is that in this study, it also plays an important role in the effect of protein on conception rate in older cows.

In this study the physiological mechanisms by which CP intake and age interact to influence conception rates have not been specifically studied. Although there is a significant interacting relationship between both of these factors and conception, why it occurs can only be postulated. Randel (1989) suggests that excessive protein intake may depress postpartum rebreeding performance, especially in older cows. One of the probable causes of lower fertility in older cows fed high CP, as Kaim *et al* (1983) report, could be a reduced feed intake by the older animal or an aging process affecting the endocrine or reproductive systems. Also, this reduced feed intake of diets high in CP combined with a high milk production, may create an energy:protein imbalance. If the nutritive requirements for production (milk yield, gestation and general body health) are met, the dairy cow's nutritive requirements for reproduction should be adequate (de Kruif and Brand, 1978). However, in this case where an energy:protein imbalance occurs, not enough energy would be available for detoxification of excess ammonia and the excretion of urea. Excess urea-N and ammonia would increase pH in the reproductive tract and reduce fertility.

Pasture crude protein levels, according to the results obtained in this study, have an effect on conception that becomes statistically significant as the lactation number increases. This effect of pasture CP is reflected in the levels of BUN, as could be seen in our results. Dairy cows feeding on pastures with high levels of CP could have an elevation in their BUN levels and these high BUN levels, combined with low levels of MUN increase the probability of non-conception, especially as the lactation number of the animals increased.

Other factors could have also influenced the outcome of the results. Weight loss has been considered as a factor that can play a part in conception efficiency. In older cows, increased weight loss combined with high milk yields may interact with the increased energy for detoxification of ammonia and urea-N when cows are fed diets high in CP content (Kaim *et al*, 1983). Also, as in the case of New Zealand dairy cattle, cows fed on lush young grass or forage oats may lose excessive amounts of body weight (between 5 and 10% of their immediate post-calving weight) by the time of mating and fail to hold to service (McClure, 1970a). Further studies should be carried out considering weight, to see if a relationship exists between weight, pasture CP, pasture ME levels and conception efficiency. For this, what should probably be done is record body weight immediately after calving and then prior to insemination. The body weight loss would be then known and this could be related to pasture CP and dietary energy intake, milk production and conception efficiency.

Weather conditions and their effect over pasture quality should be studied. Wet, rainy weather could have some effect over the nutritional composition of pastures and therefore in a future study, weather conditions should also be considered when assessing variations in DCP, dry matter content and energy levels.

VI

CONCLUSIONS

From the findings in this study, it can be concluded that the high crude protein levels found in the pasture based diet fed to dairy cows in the Manawatu area in New Zealand, have a negative influence on conception efficiency of cows in their fourth or later lactation.

Blood urea-N was a sensitive indicator of dietary crude protein intake and the cow's energy status, while vaginal mucus urea nitrogen did not reflect dietary crude protein intake in a significant way.

The only blood metabolite that appeared to reflect the protein - energy levels in the animal and the energy status of the cow, in accordance with the majority of the literature reviewed on the subject, was NEFA levels.

Pasture CP levels influenced BUN levels which in turn influenced fertility. Conception efficiency decreased as BUN levels increased in the cow. This was especially true in older cows. The results in this study confirm reports by Ferguson *et al* (1989), Canfield *et al* (1990) and Blanchard *et al* (1990) where similar observations have been made in entirely different environmental and managemental conditions.

The mechanisms by which dietary protein intake affected conception efficiency might have been due to an increase in concentrations of ammonia, urea and other nitrogenous compounds in the blood and in uterine fluids due to an excess of protein degraded in rumen. These compounds would have created an unfavourable environment for the ova, spermatozoa or embryos.

There was no apparent influence of pasture metabolizable energy levels on conception efficiency. It has been suggested that pasture soluble carbohydrates better reflect the relationship of fertility to energy intake and the combined effect of CP and energy. Thus, the use of pasture soluble carbohydrates as an additional measure of pasture energy availability in future studies of this relationship would be of great interest.

Weight loss combined with high milk yields are factors that have been reported to play a part in conception efficiency, especially in older cows by Kaim *et al* (1983). Further studies should be carried out to determine the relationship of pasture crude protein and dietary energy intake to conception rate.

Ferguson *et al* (1988) and Blanchard *et al* (1990) have suggested that a way to decrease the effect of protein intake on conception would be to reduce not only the concentration of dietary crude protein on the diet to levels below 19% CP but also to reduce the amount of DIP in the diet and increase the amount of UIP.

This in New Zealand would imply the supplementation of cows with concentrates high in UIP such as fish meal or the use of other feed with higher dietary energy content to balance the diets high in CP. However, this would also require an increase in maintenance costs which the dairy farmer might not be prepared to undertake.

Recent research reported by Chalupa (1980) and Shelling (1984), proposes the use of ionophores as a way of reducing the ruminal degradation of dietary protein. Monensin has been suggested to have a protective effect with respect to dietary protein. It has been found to decrease the rate of free amino acid degradation in the rumen fluid. As ruminal ammonia-N production is decreased, more dietary protein escapes degradation and can be available for digestion in the small intestine. If this is true, ruminal degradation could be reduced without having to alter the actual pasture based diet. Further studies on this point would be of great interest.

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CLINICAL SCIENCES

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4 October 1990

Dear Client

We are writing to you to ask if you are willing to take part in a study we plan to conduct in some spring breeding herds in the Manawatu.

Many studies here and overseas have shown a close relationship between nutrition and reproduction in high producing dairy cows.

One nutritional factor studied recently in Israel and the United States which has been found to depress fertility is feeding of diets with high content of readily digested protein. Levels of 19-23% dietary crude protein have been found to reduce conception rates and therefore should be considered to be excessive for optimum reproduction.

Milk production in this country is based on grazing pastures. Many spring pastures contain protein levels far in excess of this (up to 35% crude protein). These pastures also tend to have a lower energy level than that recommended which can in itself restrict reproduction efficiency. It is therefore likely that fertility is related to pasture composition.

The objectives of the proposed study are to:

1. Determine if any relationship exists between dietary crude protein and conception rate.
2. See if any relationship exists between nitrogen levels in blood and vaginal mucus and conception rate.
3. See if a combined measure of dietary energy and protein can be related to conception rate.
4. Study if the above relationship linked to age and/or body condition relates to conception rate.
5. See if blood and vaginal mucus nitrogen relate to dietary protein.

The study will be conducted only in the Manawatu area. We intend to have a minimum of ten farms participating and from these a total of approximately 600 cows will be selected.

Tail blood and vaginal mucus will be collected on the day of insemination and submitted to a variety of analyses. Pasture samples will also be taken approximately weekly from each co-operating farm and analysed for crude protein and energy content.

Appendix 1. Letter

Additional data collected will be the age of the cow, body condition, overall health, reproductive history and previous milk yield. Confirmation of pregnancy by rectal palpation will be carried out.

Samples taken should not represent a risk to the animal and will be taken as early as possible during the morning. If You agree to become part of this study you will receive the results from the calculations and any conclusions that may be derived from them. Participating farms will not be identified in any publication or dissemination of results from the study.

We will be following this letter up with a telephone call should you not return the tear-off slip below and can answer any question you might have at this time. We hope we can count on your co-operation in this study. If so would you please tear of the slip below, sign and return it in the enclosed envelope. Thank you for your co-operation.

Yours sincerely

Edith Fernández-Baca.
Dr Edith Fernandez-Baca

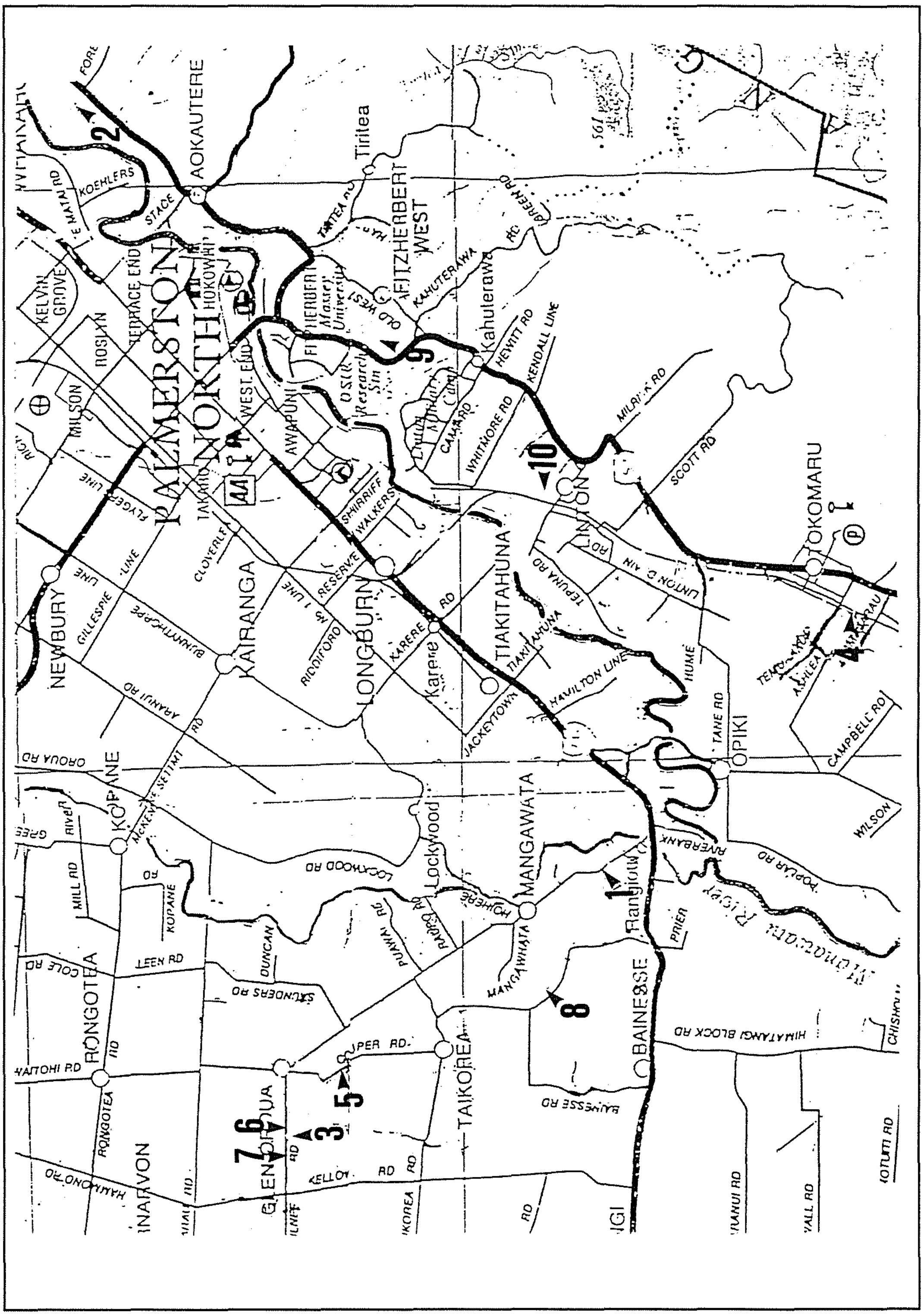
Max Merrall
Dr Max ~~Merrall~~

I agree to participate in the study

____

(name and signature)

Appendix 1. Letter.. (Continuation)



Appendix 2. Location of farms sampled during the survey. Numbers indicate farms

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