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RELATIONSHIP OF CORTICOID, THYROID AND METABOLIC PROFILES WITH FERTILITY OF BEEF FEMALES ON SOURVELD AND MIXED SWEETVELD

by

JOHANN ANDRE ERASMUS

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BLOEMFONTEIN

(Promoter: Prof. P.I. Wilke)

(Co-promoter: Prof. J.P.C. Greyling)

(September 2000)

DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work, and has not previously in its entirety or in part been submitted at any university for a degree.

[signature] ....

Signature

16 September 2000

Date

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My Creator, for providing insight and guidance, and granting me the health to conclude another chapter in my life.

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INTRODUCTION

The natural pasture of the high rainfall areas around the Dohne Agricultural Development Institute in the Eastern Cape of South Africa is described as a dense, sour grassveld, known as the Dohne Sourveld (Acocks, 1988). This pasture is characterized by vigorous grass growth during Spring and is reported to be of high quality. However, it meets the nutritional requirements of animals for only a portion of the year (October to February), whereafter the nutritive value declines markedly and has a protein content of between 3.5 and 4% at the end of March (Pienaar *et al.*, 1951). This leads to low annual production and reproduction rates in beef cattle, imposing severe economic limitations for beef producers in this region.

It is this substandard performance of beef cattle production on the Dohne Sourveld which has captured the imagination of animal scientists in this region for many decades. It is especially during the winter season that body weights of beef females on this natural pasture declined significantly; up to a third of body weight being lost during this period. It is against this background that previous research has centered around ways and means to curb the excessive weight loss, and a lot of effort was put into developing a lick for animals during the winter season. Urea-energy supplementation was found to have a beneficial influence on animal production during winter (Bishop, 1959). Since then, much time was devoted to study the effect this non-protein nitrogen and energy lick had on animal production (Bishop, 1963; Kreft, 1963; Kemm & Coetzee, 1964; Pieterse & Lesch, 1964; Pieterse & Preller, 1965; Schoeman & Lishman, 1965; Von La Chevallerie, 1965; Bishop *et al.*, 1966, 1968; Pieterse, 1967; Lesch *et al.*, 1969; Erasmus & Barnard, unpublished data, 1978). In spite of increased animal production resulting from the supplementation of this lick, animal performance still suffered limitations; later reports revealed the average weaning weight (7 months of age) of Bonsmara calves to be 194 kg (Erasmus *et al.*, 1986), whilst the calving percentage fluctuated between 35 and 87% (Erasmus, 1988).

Henceforth, it was decided to pursue the avenue of unsatisfactory reproduction rates because of its severe eroding influence on beef cattle enterprises in the high rainfall sourveld areas of the Eastern Cape. In a series of experiments, Erasmus (1988) compared different variables relating to reproductive efficiency between beef females on the Dohne sourveld and mixed sweetveld. The latter is a drier and sweeter version of the Dohne Sourveld, and corresponds with the dry thornveld in the regions of Alice and Fort Beaufort (Pienaar *et al.*, 1951). The reproductive rates of females on this native pasture were found to be in excess of 90 %. Erasmus (1988) concluded that the reproductive efficiency is hampered on the Dohne Sourveld because of the latter's low nutritional status. Findings reported thus far are amongst others:

- Heifers under sub-maintenance free-ranging conditions had a higher incidence of irregular oestrus or indicated no oestrus at all, indicating that the occurrence of sub-oestrus may be prevalent in herds kept on the Dohne Sourveld
- Peak levels of oestradiol and FSH surges of heifers on the sourveld did not always coincide with the preovulatory LH surge
- FSH surges were significantly lower in heifers on the sourveld when compared to mixed sweetveld heifers (3.68 vs. 2.29 mIU/ml)
- In most cases, basal LH concentrations of heifers on the sourveld were higher, but the preovulatory LH surges 26.6% lower than those of heifers on the mixed sweetveld.

It was clear that the natural pasture of the Dohne Research Station lacked in sufficient nutritive quality to properly maintain reproductive function. The question arose as how to quantify this nutritive insufficiency in terms of fertility of the beef female. It seemed a logical step to assess the metabolic function of these females in relation to the reproductive inefficiency reported in this Bonsmara herd.

Although the metabolic status of individuals and groups of animals were monitored previously, it was a later trend - during the late 1960's and early 1970's - to rationalize the use of blood chemistry, and to combine a number of these parameters into a single package (Payne, 1978). It appears that the metabolic profile test was designed to study blood chemistry in dairy cows, and it soon became apparent that high milk yield was often associated with low fertility and/or metabolic disorders. Thus, it was found in German Black Pied cattle that fertility disorders were more common in cows with a milk production of higher than 5 000 kg (Aehnelt *et al.*, 1968). Kali and Amir (1970) found that production parameters such as milk yield, butterfat percentage and butterfat production were higher in repeat breeders than in cows conceiving at first insemination.

Metabolic profile studies assumed a wider application when it was extended to sheep and beef cattle. Energy (Russel, 1978) and protein status (Sykes, 1978) were determined in ewes, whilst hormone and energy profiles were studied in lactating beef cows (Coggins & Field, 1978). However, it would seem that most of the work concerning blood metabolite concentrations were performed on dairy cows, with the emphasis on controlled environmental conditions, including feeding regimes. Although much work has been done to relate various feeding regimes to fertility (Morris, 1980), little is known about the nutritional status of beef animals as measured by circulating concentrations of various blood parameters of animals under natural grazing conditions.

The present study was thus undertaken to provide norms of different blood and plasma metabolites and hormones for beef females grazing on 2 different natural pasture types. Bonsmara females on the mixed sweetveld, having high annual reproductive rates, served as a control against which females on the sourveld were evaluated. It is hoped that these norms will provide useful background information regarding the problems pertaining to the low nutritional status of the sourveld pasture, and relating this to overwintering and low fertility of large stock on the sourveld of the Eastern Cape region. Recommendations and concrete solutions concerning this problem of low reproductive efficiency are of the utmost importance, and it was the purpose of this study to make a meaningful contribution in this regard.

EXPERIMENTAL PROCEDURES

1. EXPERIMENTAL TERRAIN

The present study was conducted simultaneously at two experimental sites, each representing the sourveld and mixed sweetveld respectively. The first location is the Dohne Research Station, which is situated 6 km. north-east of Stutterheim, at grid reference 270 28' East, and 320 31' South. It lies east of the Amatola mountains in the Eastern Cape, and is 80 km. from the coast, East London being the nearest harbour. The topography can be described as undulating with a middle slope. The height above sea level is 925 m.

The second experimental site is the farm Campagna, situated about 3 km north-east and adjacent to the Dohne Research Station at grid reference 270 29' East, and 320 29' South. It has a transitional native pasture type from sourveld to sweetveld, known as mixed sweetveld. The altitude is 777 m (Dohne Farm Plan, 1985).

2. SOIL

The most dominant soil series in the area are Williamson, Rietvlei, Mispah and Soetmelk (Hartman, 1985). These soils are characterized by, among others, shallow effective depths and low estimated available moisture capacities. According to Pienaar *et al.* (1951), the soils are acid and the pH levels vary between less than 5.0, and 6.5. Fertility of the soil is low with phosphorus and nitrogen being the main deficiencies.

3. NATURAL PASTURE

The natural pasture type at Dohne is classified as the Dohne Sourveld (veld type 44) and is described by Acocks (1988) as a dense, sour grassveld. The grassveld is mainly dominated by *Themeda triandra, Heteropogon contortus, Tristachya hispida, Eragrostis capensis, Sporobulus africanus, Elionurus argenteus, Microchloa caffra, Senecio retrorsus, Harpochloa flax, Eragrostis plana, Brachiaria serrata* and *Rhynchosia totta* (Acocks, 1988).

Grass growth is vigorous during spring when the pasture is of high quality. The sour grassveld meets the requirements of animals for only a portion of the year, i.e. from October to the end of February. Thereafter, the nutritive value declines markedly and has a protein content of between 3.5 and 4% at the end of March (Pienaar *et al.*, 1951).

The natural pasture at Campagna can be described as a drier and sweeter version of the Dohne Sourveld (Danckwerts, personal communication, 1986) and is known as the mixed sweetveld. According to Pienaar *et al.* (1951), mixed veld corresponds with the dry thornveld in the regions of Alice and Fort Beaufort. The latter veld type is described by Acocks (1988) as the Valley Bushveld (Veld Type 23), which is surrounded by the False Thornveld of the Eastern Province (Veld Type 21).

The grass species occurring in the mixed sweetveld are: *Digitaria eriantha*, *Eustachys mutica*, *Heteropogon contortus*, *Sporobolus fimbriatus*, *Eragrostis chloromelas*, *Eragrostis curvula*, *Eragrostis rasemosa*, *Pennisetum sphacelatum*, *Sporobulus africanus*, *Brachiaria serrata*, *Eragrostis capensis*, *Panicum stapfianum*, *Themeda triandra*, *Alloteropsis semialata*, *Andropogon appendiculatus*, *Harpochloa flax*, *Cymbopogon plurinodus*, *Elionurus argenteus*, *Hyparrhenia hirta*, *Aristida congesta*, *Cynodon dactylon*, *Eragrostis plana*, *Microchloa caffra* and *Sporobolus nitens*, with *Chloris gayana* being the invader species.

The condition of the pasture was moderate during the study. Because of overgrazing in the past, an increase in species such as *Eragrostis plana* and *Eragrostis chloromelas* occurred.

4. CLIMATE

According to the temperatures taken at Dohne, the climate can be classified as moderate. The mean summer temperatures vary between 18 and 20°C, and the mean winter temperatures between 13 and 14°C (Figure 1). The mean monthly temperatures during the experimental period (1979 to 1983) are compared with the average temperatures over a period of 34 years (1951 to 1984).

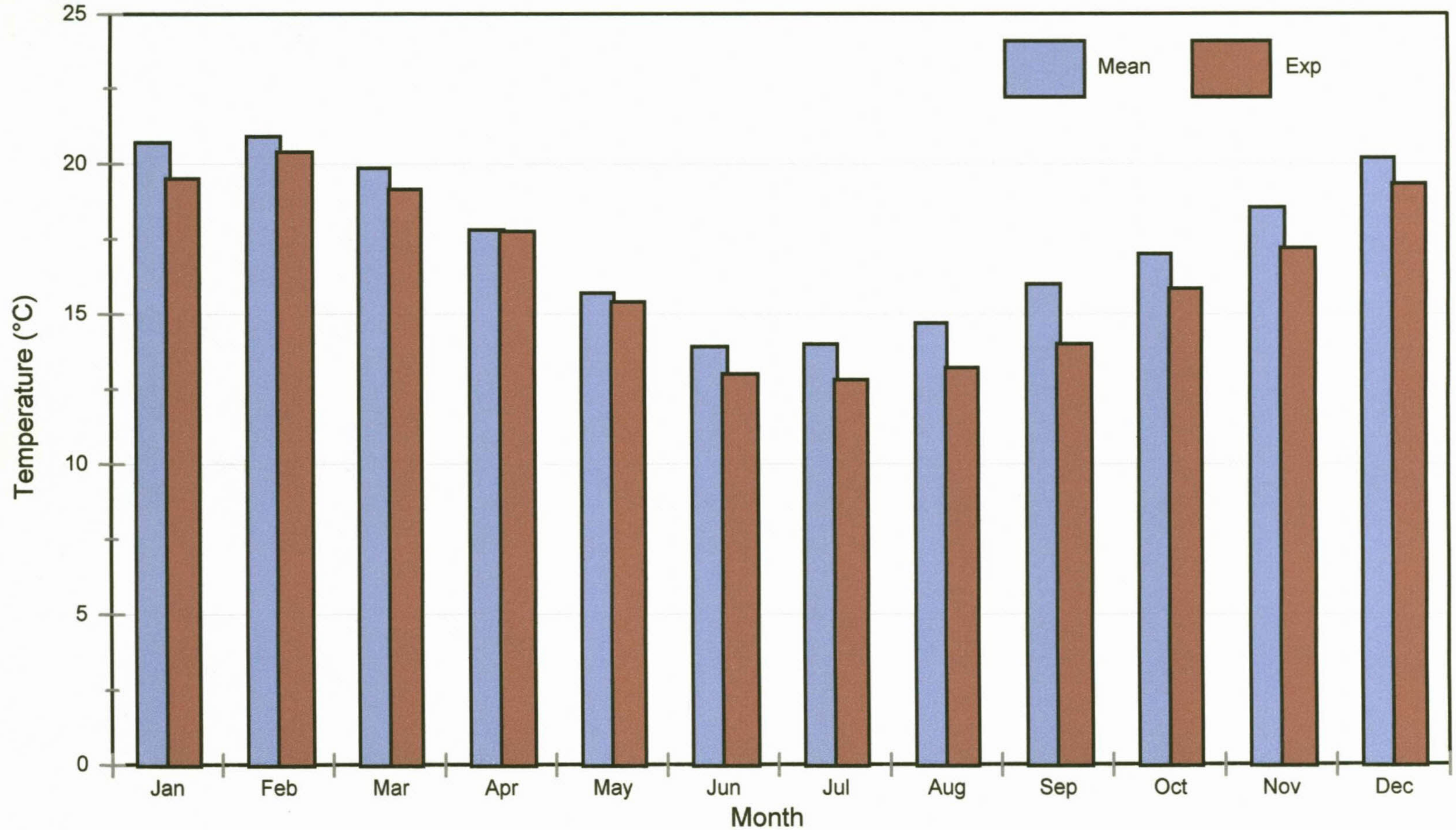


Figure 1 Mean monthly temperature from 1951 to 1983, and during the experimental period 1979 to 1983

Climatic data at Campagna was not recorded. However, being a drier phase of the Dohne Sourveld, higher temperatures are expected at this location.

A 30% chance of experiencing frost (2°C in a Stevenson screen) exists from 27 May until 26 September, and a 50% chance exists from 20 June to 15 September (Dohne Farm Plan, 1985). The Dohne Development Institute is subject to frequent misty conditions of the Amatola mountains.

Dohne experiences summer rainfall: the mean rainfall of 700 mm is mostly recorded between September and May. However, showers may also occur during the winter months, ranging from May to August. The mean monthly rainfall recorded at the Dohne Development Institute during the experimental period, and from 1951 to 1984, is presented in Figure 2. The approximate annual rainfall at Campagna is 650 mm.

The monthly maximum humidity at Dohne is illustrated in Figure 3.

5. EXPERIMENTAL ANIMALS

Bonsmara heifers and cows were used for this study. The number of animals used for blood collection, as well as the total number of heifers and cows involved in the experiment during the different years, are shown in Table 1

Table 1 Total number of experimental heifers and cows, and number used for blood sampling

Year	Total number of animals		Total number sampled	
	Heifers	Cows	Heifers	Cows
1979/80	19	23	12	8
1980/81	14	8	8	8
1981/82	45	-	18	-
1982/83	-	45	-	45

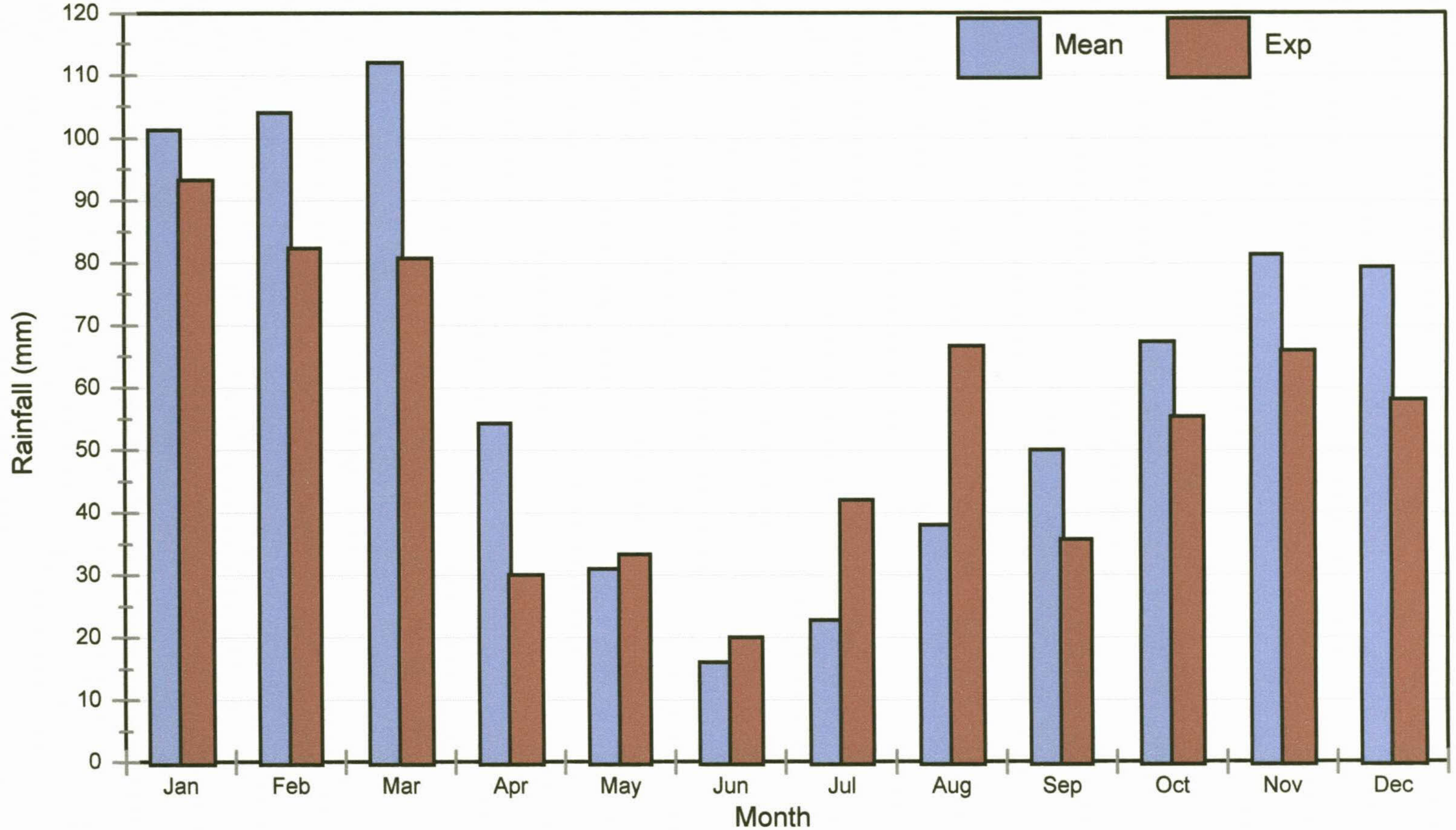


Figure 2 **Mean monthly rainfall from 1951 to 1983, and during the experimental period 1979 to 1983**

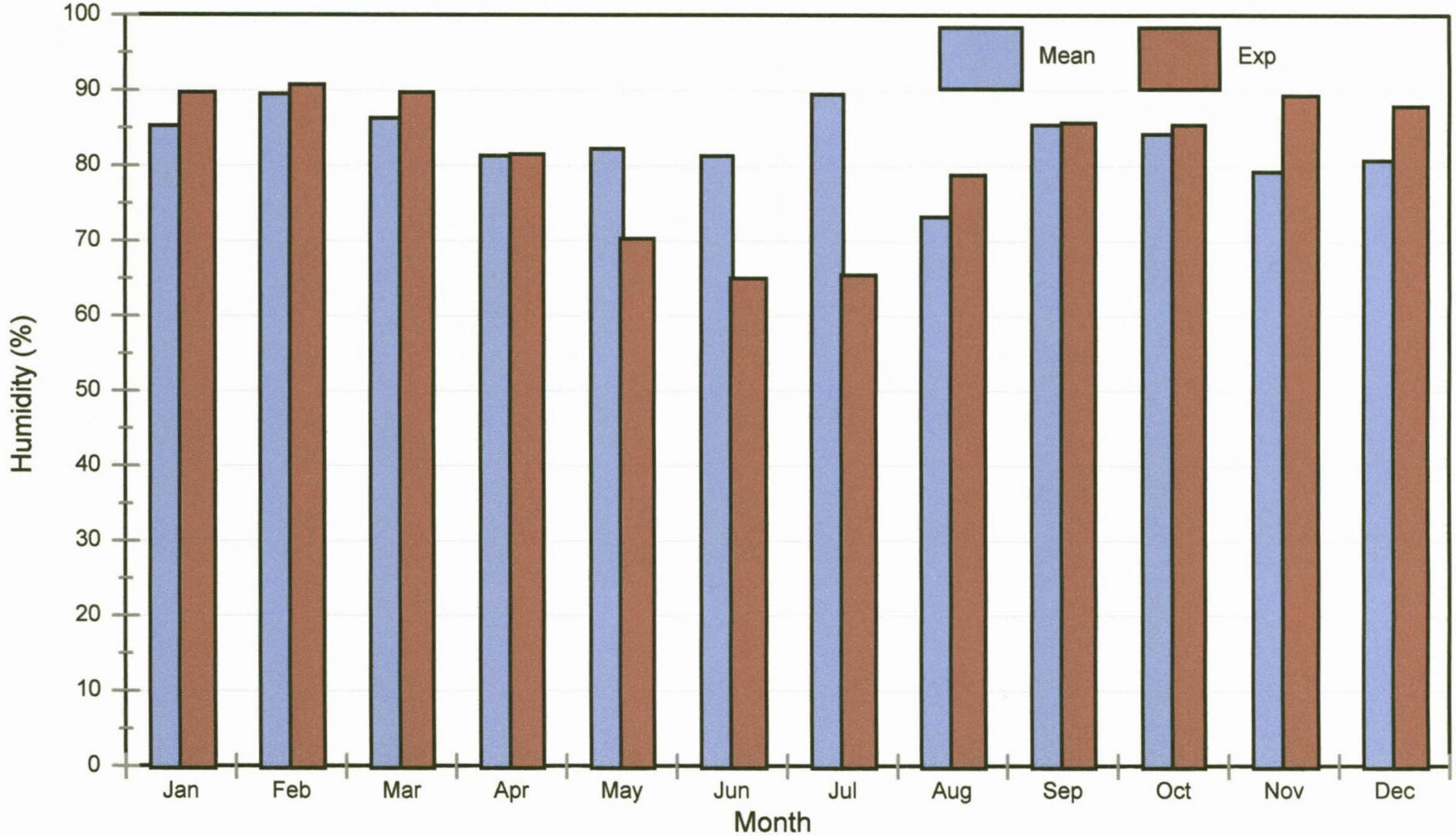


Figure 3 **Mean monthly humidity from 1951 to 1983, and during the experimental period 1979 to 1983**

It should be noted that at the beginning of 1981, Bonsmara heifers were used that had been mated from beginning of November 1980 up to the end of January 1981. These heifers were thus pregnant during most of the year (1981), and calved down from August to October 1981. These animals were referred to as first-calf cows, or first calvers in the text. During the subsequent season, the females were referred to as cows.

6. EXPERIMENTAL TREATMENTS

The trials began during September 1978. During 1978/79, heifers and cows were run on the Dohne Sourveld only, of which 7 heifers and 4 cows were randomly selected and bled for blood and blood plasma analysis. During 1979/80, heifers and cows were run on the Dohne Sourveld and mixed sweetveld at Campagna, of which 5 heifers and 5 cows were selected for blood analyses. During September 1980, 45 Bonsmara heifers were divided into 3 groups (thus 15 animals per group):

Group 1: Bonsmara females were run on the Dohne Sourveld under normal management conditions. The designation sourveld + N was applied to this group of experimental animals.

Group 2: Females were run on the Dohne Sourveld as in Group 1, but received the summer lick (bone meal 50% and salt 50%) during the winter months, i.e. May to August. This group was thus deprived of the high protein energy lick (non-protein nitrogen) usually fed to beef females during the winter season. The designation sourveld - N was applied to this group of females.

Group 3: Females were run on mixed sweetveld under normal management conditions. The designation sweetveld + N was applied to this group of experimental animals.

The same animals were subsequently used until the termination of the trial in May 1983.

7. MANAGEMENT OF THE BEEF HERDS

During the summer season (September to April), the experimental animals on both the sourveld and mixed sweetveld had access to the summer lick consisting of bone meal and salt (1:1). During the winter period, animals had access to a high protein and energy lick consisting of urea (15%), maize meal (30%), bone meal (25%) and salt (30%).

Animals were removed from a camp when approximately a third of the foliage of the grass had been grazed. Animals were stocked at a rate of 2.1 large stock units per hectare.

Under normal management conditions, the females were dosed with a broad spectrum anthelmintic after being weaned at 7 months of age. However, all experimental animals were given an anthelmintic prior to being grouped into treatments to obviate any influence helminths may have had on the experimental animals.

Regarding mating seasons, heifers and cows were either inseminated or mated during the standard mating seasons of three months, viz. November to January. During the 1979 season, oestrus was induced when seven heifers and four cows on the sourveld were injected with two injections of prostaglandin F2α at 11 days interval to synchronize oestrus. These animals were artificially inseminated at the occurrence of the second and subsequent oestrous periods.

During the 1980 season, heifers and cows on the sourveld and mixed sweetveld were treated with two injections of prostaglandin F2α 11 days apart. Animals were artificially inseminated on the 11th day. Fertile bulls of were used to cover animals during the subsequent oestrous periods. From 1981 onwards, natural mating took place. Thirty cows were exposed to a bull.

8. METHODS AND DATA COLLECTION

8.1 Glucose concentrations

Glucose concentrations were determined in the blood and plasma by means of the Merckotest Blood glucose (O-Toluidine method) kit (Merck Cat. No. 3335). The blood was drawn in test tubes packed on ice, and analysed for blood glucose concentrations immediately.

8.2 Total protein concentrations

The Merckotest Total protein kit (Biuret method) was used to determine total protein concentrations in the blood plasma (Merck Cat. No. 3327).

8.3 Albumin concentrations

The albumin concentrations in the blood plasma were determined using an Albumin kit (Procedure No. 630, Sigma Diagnostics).

8.4 Urea concentrations

The Merckotest Urea (Berthelot Reaction) kit was used to determine plasma urea concentrations (Merck Cat. No. 14315).

The red blood cell glucose concentrations were calculated by subtracting the blood glucose concentrations from the plasma glucose concentrations. Similarly, for the calculation of the globulin concentrations in this study, the plasma albumin concentrations were subtracted from the total plasma protein concentrations.

Above-mentioned analyses were performed on a Philips spectrophotometer, model PYE Unicam PU 8600 - UV/VIS.

8.5 Cortisol concentrations

Plasma cortisol concentrations were determined in duplicate by the competitive protein binding technique, as described by Wentzel *et al.* (1975).

8.6 Total thyroxine (T4) concentrations

Plasma samples were assayed using the Amerlex T4 RIA kit according to the manufacturer's instructions (The Radiochemical Centre, Amersham).

8.7 Body weights

The body weights of the cattle were recorded every fortnight, on the day of blood sampling.

In this metabolic profile study, the experimental animals were bled at fortnightly intervals. During the 1978/79, and 1979/80 seasons, blood plasma samples were analysed for cortisol and thyroxine (T4) concentrations. From 1981 to 1983, samples were analysed for cortisol, T4, blood and plasma glucose, total plasma protein, plasma albumin and plasma urea concentrations.

9. STATISTICAL ANALYSIS

The data were analysed by least-squares analysis of variance computer programme LSML76, as described by Harvey (1977). This programme provides for unbalanced data with unequal number of subclasses.

CHAPTER 1

BLOOD AND BLOOD PLASMA METABOLIC PROFILES OF BONSMARA FEMALES ON DOHNE SOURVELD AND MIXED SWEETVELD

1.1 INTRODUCTION

In most pastoral regions, a period of low rainfall occurs each year, during which pasture growth ceases and the nutritive value of the herbage declines (McDonald, 1968). Extensive research has been conducted in order to combat body weight loss and decreased production of farm animals grazing on natural pasture, known as sour grassveld, during this period (Kotze, 1950; Bishop, 1959, 1963; Bishop *et al.*, 1969; Lesch *et al.*, 1969; Nel & Van Niekerk, 1970; Erasmus & Barnard, unpublished 1979). Many of the studies involved the feeding of urea or biuret to cattle and sheep grazing under extensive conditions on natural pasture. Beef producers in these areas have been reluctant to improve upon this regime; the feeding of NPN (non-protein nitrogen) supplements largely still serves as the only source of supplementary feeding. According to Economides *et al.* (1973), agreement exists that sub-maintenance nutrition in commercial beef herds is prevalent, since the maternal plane of nutrition is not supplemented in order to minimize production costs.

The problem in many beef herds is: How can the adequacy of the diet be determined? Hand-fed animals are given diets of known composition and volume; the adequacy of the diet can thus be determined. According to McDonald (1968), research with animals grazing under genuine pastoral conditions has been discouraged because of the technical difficulties in studying the grazing animal, as well as the high cost involved in such work. Consequently, a large part of past research has been directed at metabolic diseases, rather than production problems. Yet, it is of great importance from an economic point of view to assess the nutritional status of the animal; substandard performance may result from the increased incidence of metabolic disorders and reduced reproductive performance (Coggins & Field, 1978). In this respect, Hlasny (1996) is of the opinion that increased blood urea levels may be symptomatic of various reproductive disorders in dairy cows.

Although changes in body weight and body condition are useful indicators to measure nutritional status, the time taken for such changes to become evident is too long (Russel, 1978). In extensive management systems, indirect measurements of nutritional status provide an alternative to measure nutrient adequacy. According to Russel (1978), the determination of the circulating concentration of one or more metabolites offers a relatively simple means of measuring nutritional status. The latter is the extent to which the animal's requirements are met by its nutrient intake. Indirect measures of intermediary metabolism would be easily justifiable financially if suitable techniques were available (Elsley, 1978). There is no doubt that reliable blood or plasma parameters which indicate energy and protein status of free-grazing livestock, will be of immense value to determine requirements and deficiencies of these 2 nutrients.

In view of the above-mentioned, the present study was undertaken to ascertain to what degree shortfalls in nutritional status occur in beef females run on natural sourveld grazing. In order to achieve this objective, animals on the mixed sweetveld (higher nutritional status) served as a control against which certain blood metabolites were compared.

1.2 EXPERIMENTAL PROCEDURES

Five Bonsmara first-calf cows were used in each of the following 3 experimental groups: Group 1, females were run on the Dohne Sourveld, supplemented with a bone meal and salt lick in the summer season, and an NPN lick during the winter season (sourveld + N); Group 2, females were run on the Dohne Sourveld, supplemented with bone meal and salt lick throughout the year (sourveld - N); Group 3, females were run on the mixed sweetveld, supplemented with a bone meal and salt lick in the summer, and an NPN lick in the winter (mixed sweetveld + N).

Blood samples were obtained via the jugular vein at fortnightly intervals (09:00) in heparin tubes, packed on ice, and immediately analysed for blood and plasma glucose, total plasma protein, plasma albumin and plasma urea concentrations. Aliquots of plasma samples from each experimental animal were stored at -20°C for the later determinations of cortisol and thyroxine concentrations. The methods used for the analyses of the mentioned blood and plasma metabolites and hormone concentrations, were previously described (Experimental Procedures, paragraph 8 Methods and Data Collection, pp 11 to 12).

1.3 RESULTS

1.3.1 Production performance of beef females

Production performance (body weight) of experimental animals on the Dohne Sourveld is low throughout most of the year (Figure 1.1). Meaningful body weight increases of heifers and cows are limited to the spring period, following directly after the main calving period (August).

The vast difference in body weights between females on the sourveld and mixed sweetveld is evident from Figures 1.2 to 1.4. These illustrations also depict the vast differences in trends in body weights between the various years. During 1980, females on the mixed sweetveld were able to maintain body weight during the winter, whilst their counterparts on the sourveld were in a negative energy balance until the beginning of November (Figure 1.2).

During the following year, much lower rainfall was recorded, and the animals were subjected to drought conditions (Figure 1.3). It is clear that the spring nutrition of the natural pasture was insufficient to allow the animals to recover from the stresses of winter feeding and parturition. Cows on the mixed sweetveld + N also stagnated during this period, although at a higher body weight level. The effect of withholding an NPN lick from the cows during the winter limited production in the subsequent spring and summer seasons. The differences in body weight between the 2 sourveld groups of cows were maintained until the following spring, when compensatory growth took place and the differences were annulled (Figure 1.4).

1.3.2 Metabolic profiles

Metabolic protein and energy concentrations of first-calf cows on the sourveld and mixed sweetveld during 1981/82 are presented in Table 1.1. These are mean values recorded throughout the year.

1.3.2.1. Influence of pasture type on energy profiles

During the first year (1981/82), blood, plasma and red blood cell (RBC) glucose concentrations showed a positive relationship with the nutritional status, i.e. glucose concentrations were always highest in first-calf cows on the mixed sweetveld, and lowest in cows on the sourveld deprived of an NPN lick during the winter period (Table 1.1). In all these cases, differences were statis-

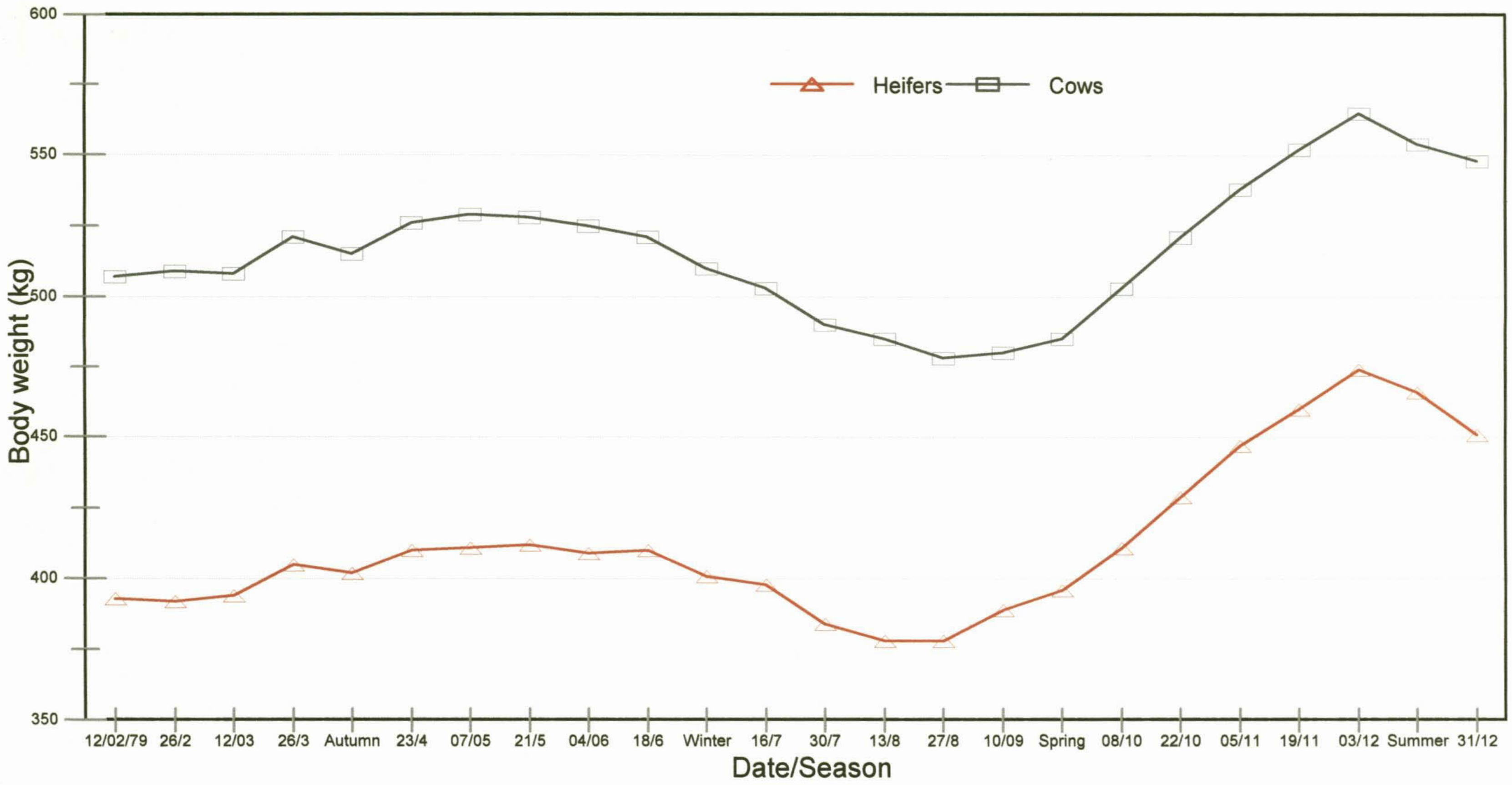


Figure 1.1 Mean body weight (kg) of Bonsmara heifers and cows on Dohne Sourveld during 1979

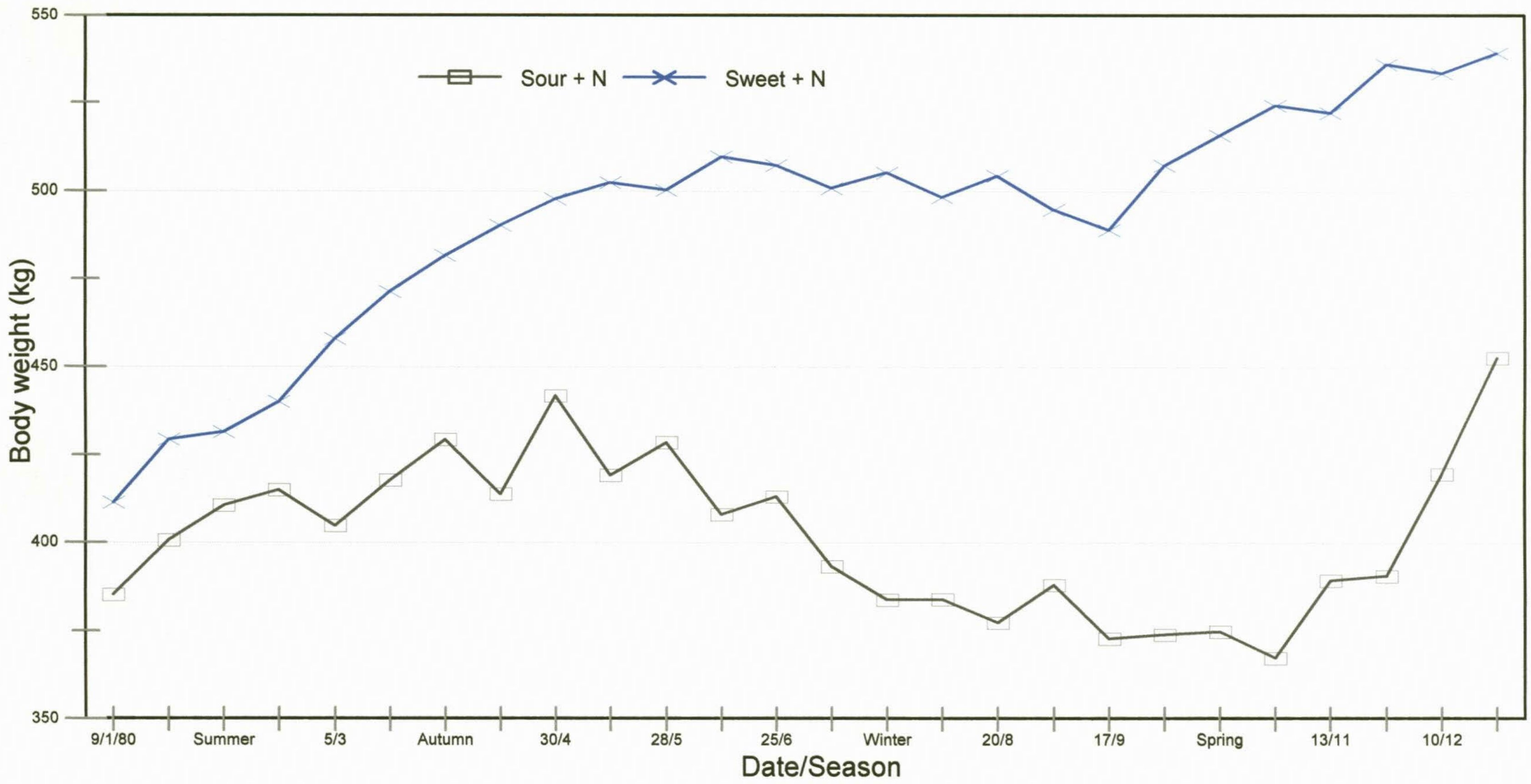


Figure 1.2 **Mean body weight (kg) of Bonsmara females on Dohne Sourveld and mixed sweetveld during 1980**

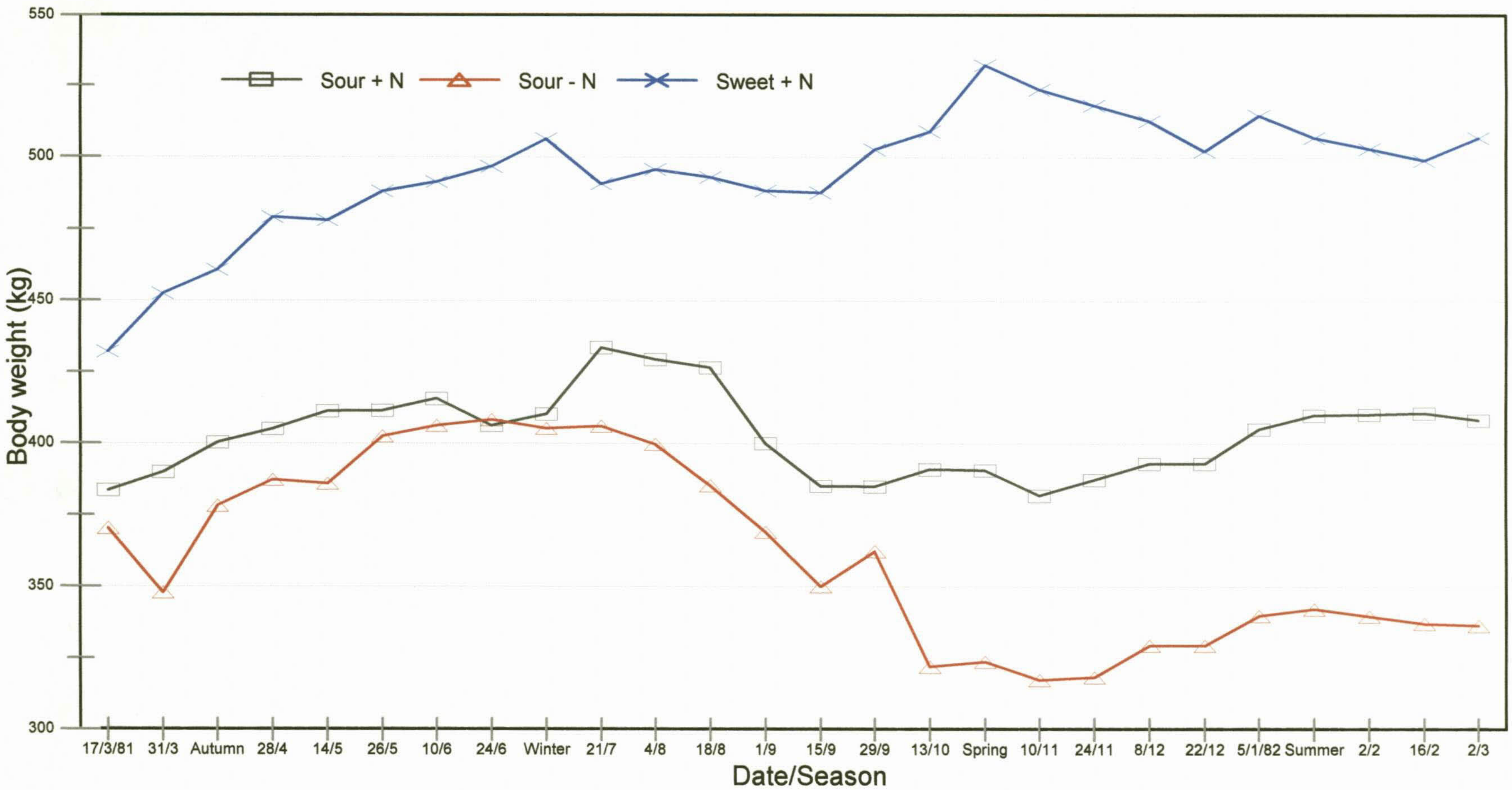


Figure 1.3 Mean body weight (kg) of first-calf Bonsmara cows in 3 treatment groups during 1981/82

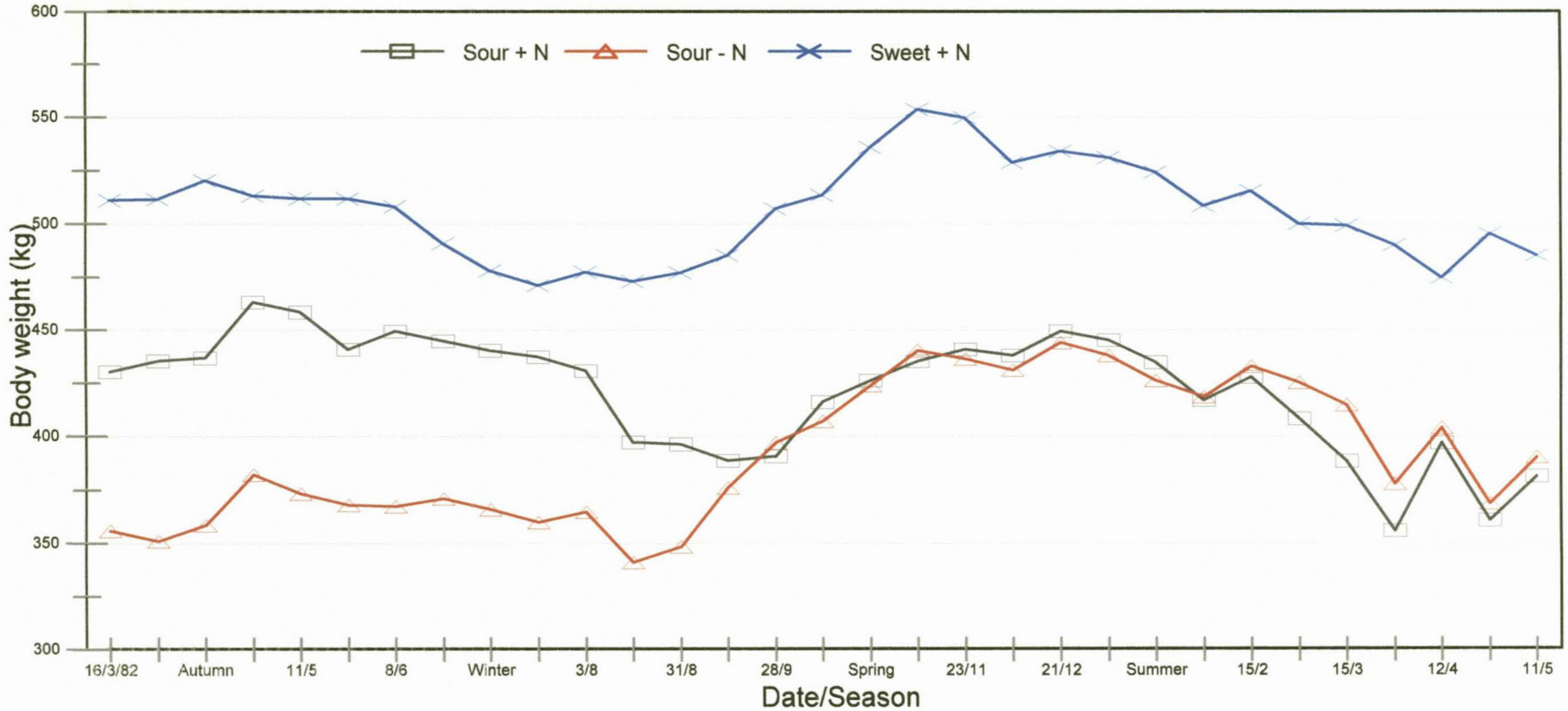


Figure 1.4 Mean body weight (kg) of second-calf Bonsmara cows in 3 treatment groups during 1982/83

Table 1.1 Mean metabolic concentrations of Bonsmara first-calf cows on the sourveld supplemented with an NPN lick, sourveld without an NPN lick, and the mixed sweetveld with an NPN lick during their first productive year (1981/82)

Constituent	Treatments		
	Sourveld + N	Sourveld - N	Mixed sweetveld + N
Blood glucose (mmol/l)	2.13 ± 0.02[a]	2.12 ± 0.02[b]	2.30 ± 0.02[c]
Plasma glucose (mmol/l)	3.20 ± 0.03[d]	3.10 ± 0.03[e]	3.38 ± 0.03[f]
Red blood cell glucose (mmol/l)	1.06 ± 0.03[g]	0.98 ± 0.03[h]	1.09 ± 0.03[i]
Total plasma protein (g/l)	66.0 ± 0.52[j]	68.0 ± 0.52[k]	72.0 ± 0.53[l]
Plasma albumin (g/l)	32 ± 0.30[m]	32.0 ± 0.30[n]	34.0 ± 0.30[o]
Plasma globulin (g/l)	35.0 ± 0.50[q]	36.0 ± 0.50	38.0 ± 0.50[r]
Plasma urea (mmol/l)	1.75 ± 0.11[s]	1.35 ± 0.11[t]	3.75 ± 0.11[u]

c > a, b ($P < 0.01$)
f > d, e ($P < 0.01$)
g > h ($P < 0.05$)
i > h ($P < 0.05$)
l > j, k ($P < 0.01$)
o > m, n ($P < 0.01$)
r > q ($P < 0.01$)
u > s, t ($P < 0.01$)

tically significant. It appears that of the 3 parameters, the blood and the plasma glucose concentrations give the best reflection of the nutritional differences between the 2 pasture types.

The influence of the sourveld + N, sourveld - N and mixed sweetveld + N on blood glucose concentrations of first-calf cows during 1981/82 is depicted in Figure 1.5. It is clear that, with a few exceptions, blood glucose concentrations in cows on the mixed sweetveld were higher throughout the period. As the nutritive value of both the sourveld and mixed sweetveld decreased as the season progressed, blood glucose concentrations decreased and reached minimum levels towards the end of July (winter). Maximum blood glucose levels were obtained during February/March (late summer) (Figure 1.5).

Plasma glucose levels were also significantly ($P < 0.01$) influenced by feeding regimes (Figure 1.6). The increased nutritive value of the mixed sweetveld + N promoted an increase in levels throughout the year. The sourveld group of cows without any NPN supplementation generally had the lowest plasma glucose concentrations. The influence of seasonal nutritive status is not as obvious in this parameter as was the case with blood glucose concentrations.

Although less obvious, RBC glucose concentrations followed a similar pattern, with much more variation between treatments being noted (Figure 1.7). It is clear that this parameter is not a sensitive barometer of energy status in the animal body. Although concentrations were higher in females on the mixed sweetveld, values were not consistently higher, and the low concentrations experienced during January and February (summer) 1982, are not in accordance with observed blood glucose concentrations.

During the second year (1982/83), energy metabolites appeared not to reflect the nutritional status of the natural grazing in the same manner as during the 1981/82 season (Table 1.2). It is interesting to note that blood and plasma glucose concentrations were highest in cows not receiving an NPN supplementation during the winter season. The influence of veld type on blood glucose levels in second-calf cows contrasting somewhat with the results obtained during the first year, is evident

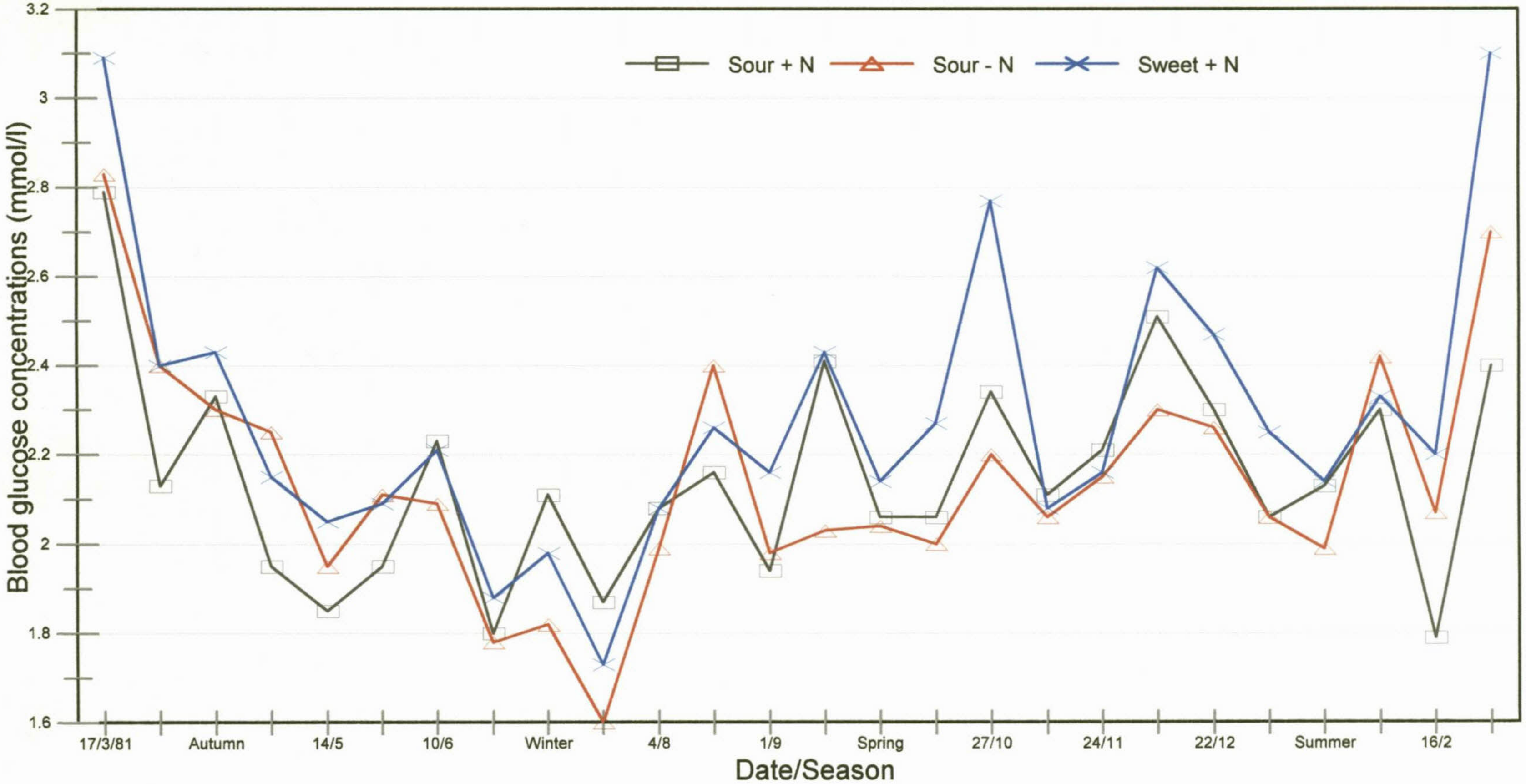


Figure 1.5 **Mean blood glucose concentrations (mmol/l) of first-calf cows in 3 treatment groups during 1981/82**

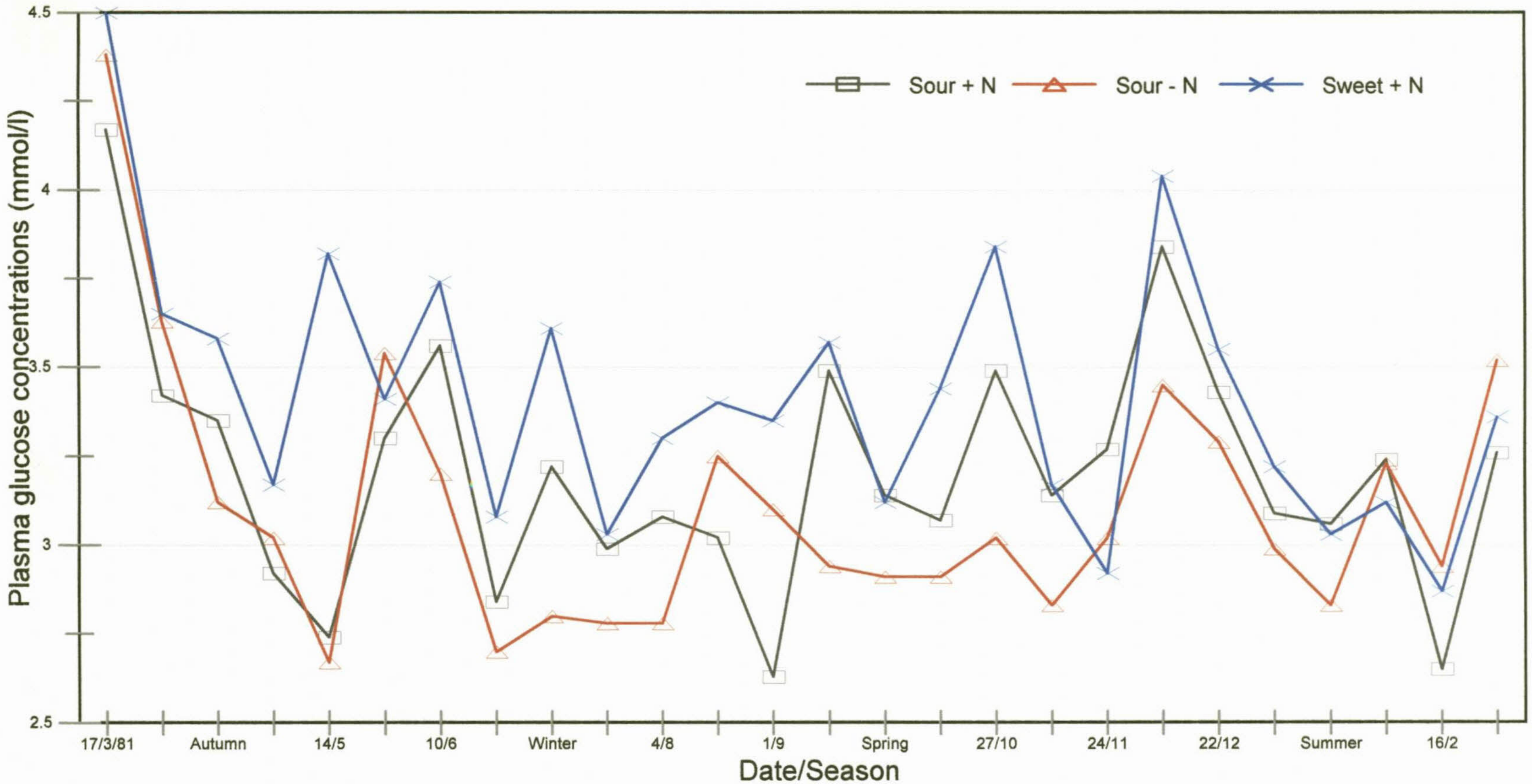


Figure 1.6 **Mean plasma glucose concentrations (mmol/l) of first-calf cows in 3 treatment groups during 1981/82**

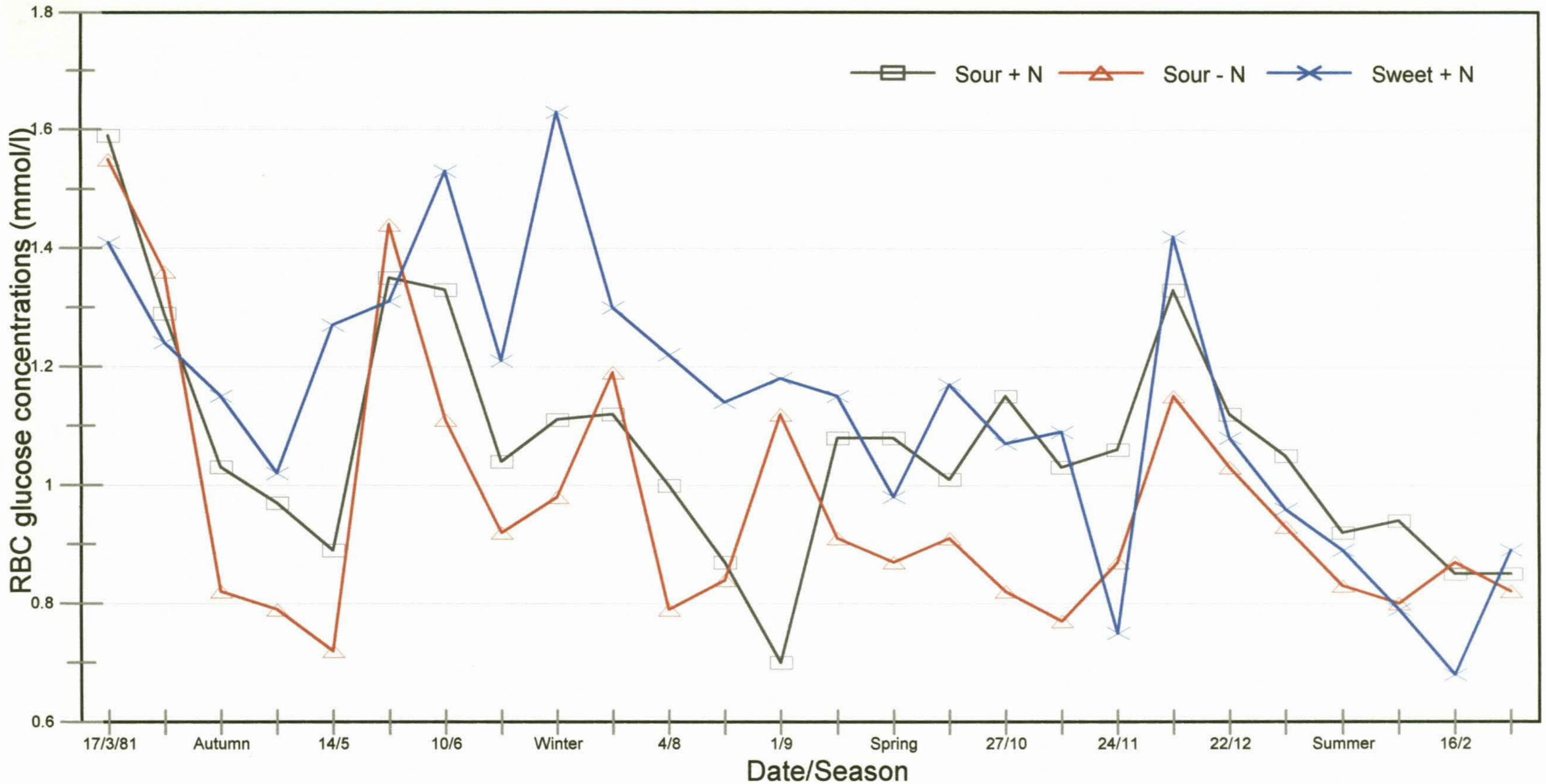


Figure 1.7 Mean red blood cell glucose concentrations (mmol/l) of first-calf cows in 3 treatment groups during 1981/82

Table 1.2 Mean metabolic concentrations of Bonsmara second-calf cows on the sourveld supplemented with an NPN lick, sourveld without an NPN lick and the mixed sweetveld with an NPN lick during their second productive year (1982/83)

Constituent	Treatments		
	Sourveld + N	Sourveld - N	Mixed sweetveld + N
Blood glucose (mmol/l)	2.03 ± 0.03[a]	2.21 ± 0.03[b]	2.13 ± 0.03
Plasma glucose (mmol/l)	3.21 ± 0.03	3.34 ± 0.03	3.31 ± 0.03
Red blood cell glucose (mmol/l)	1.17 ± 0.03	1.14 ± 0.03	1.20 ± 0.03
Total plasma protein (g/l)	75.0 ± 0.68	77.0 ± 0.68	77.0 ± 0.69
Plasma albumin (g/l)	30.0 ± 0.29[c]	31.0 ± 0.29	32.0 ± 0.30[d]
Plasma globulin (g/l)	45.0 ± 0.70	46.0 ± 0.71	46.0 ± 0.72
Plasma urea (mmol/l)	1.81 ± 0.08[e]	1.68 ± 0.08[f]	3.13 ± 0.08[k]

a < b (P < 0.01)

c < d (P < 0.05)

e < k (P < 0.01)

f < k (P < 0.01)

from Figure 1.8. No clear pattern of the effect of treatment on these concentrations was found. Initially, blood glucose concentrations decreased over time, and reached minimum levels towards end of July (winter) 1982. As was the case during the previous year, concentrations immediately increased to reach maximum levels during September (spring), and steadily decreased as the summer season progressed.

Plasma glucose concentrations did not reflect nutritional status of the second-calf cows either (Figure 1.9). While it is true that the levels of all 3 groups reached low values during the beginning of August, and were thus in line with trend of body weight (Figure 1.4), subsequent glucose concentrations did not reflect the increased nutritive value of the natural grazing during the active growth period. In contrast, the upward trend in glucose concentrations from January to April (summer to autumn) 1983 coincided with a downward trend in body weight.

As is evident from Figure 1.10, red blood cell glucose concentrations were not influenced by the natural pasture, or by the variation in nutritive value of the grazing between seasons.

1.3.2.2 Influence of pasture type on protein profiles

As was the case with glucose concentrations, plasma protein metabolites were all significantly elevated in first-calf cows on mixed sweetveld during the first year (Table 1.1). A surprising phenomenon is that, except for plasma urea concentrations, cows receiving an NPN supplementation during the winter had lower mean protein levels than animals not receiving the supplement.

Total plasma protein profiles of the 3 groups of first-calf cows are illustrated in Figure 1.11. The higher protein content of the mixed sweetveld is reflected virtually throughout the year in the total protein content of the blood plasma of cows on this treatment.

Initially, sourveld + N cows had lower plasma protein concentrations than the sourveld - N group. NPN supplementation was made available from May to September (throughout the winter), but did not seem to be effective in immediately raising the plasma protein levels. It is interesting to note that,

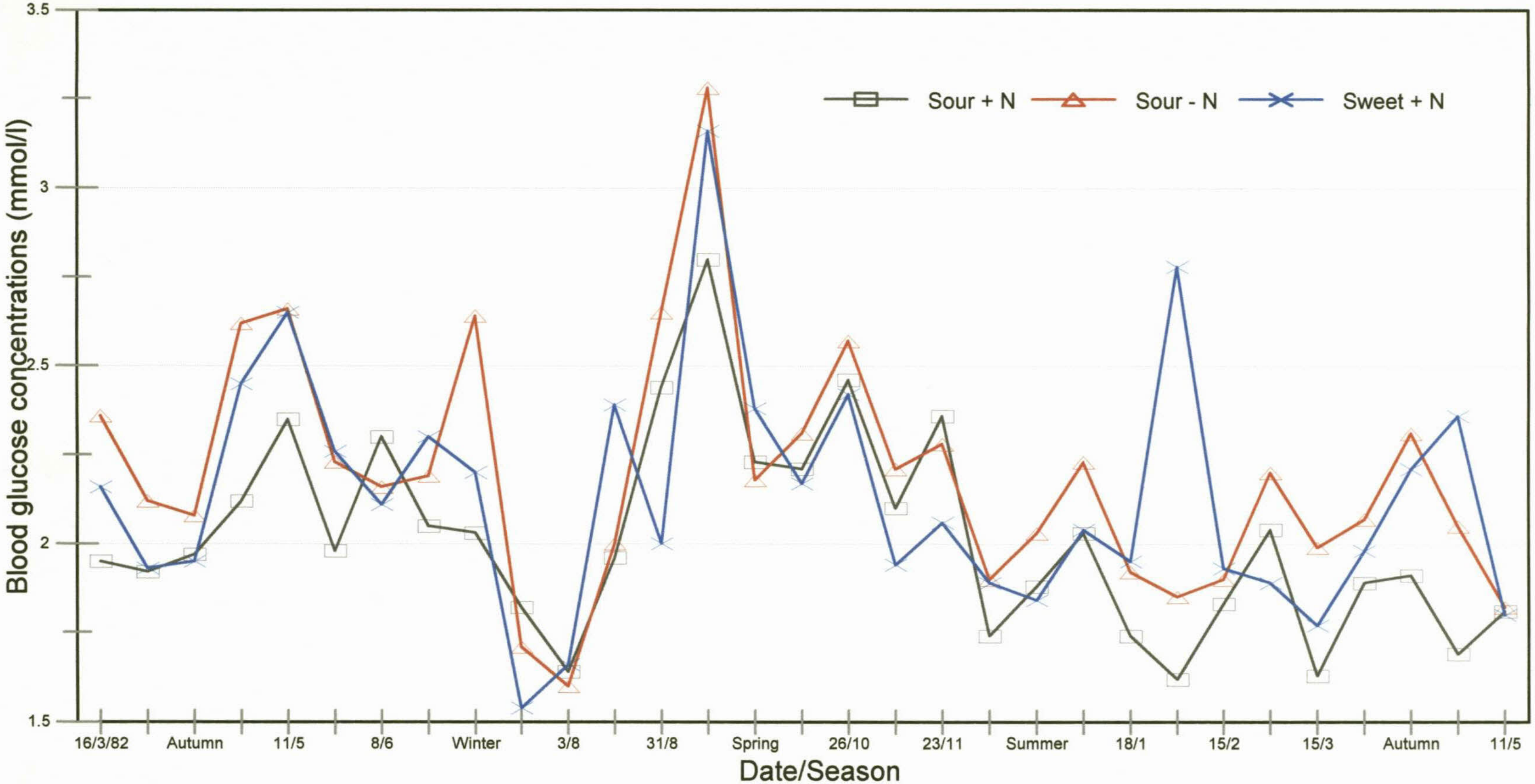


Figure 1.8 Mean blood glucose concentrations (mmol/l) of second-calf cows in 3 treatment groups during 1982/83

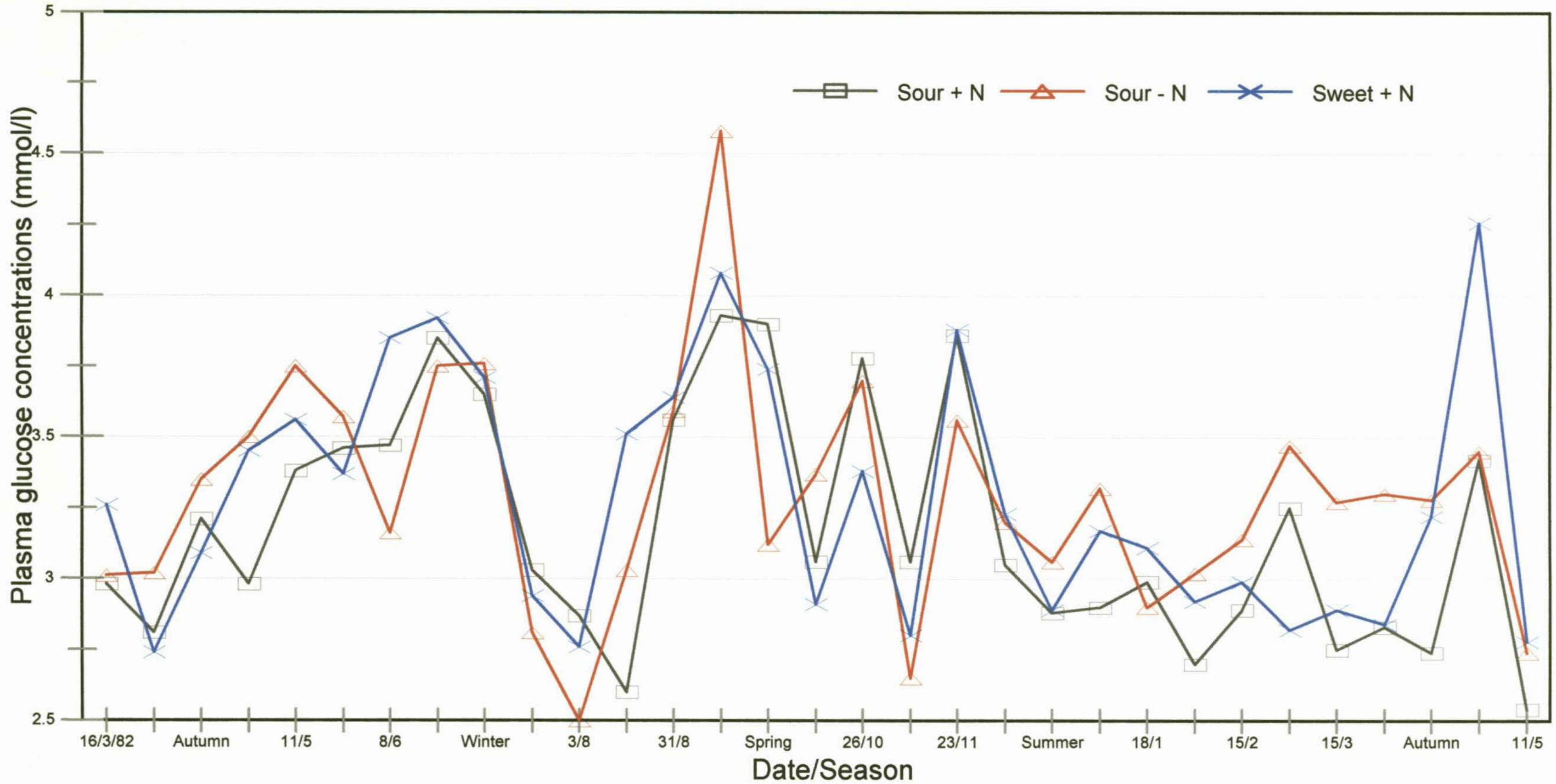


Figure 1.9 Mean plasma glucose concentrations (mmol/l) of second-calf cows in 3 treatment groups during 1982/83

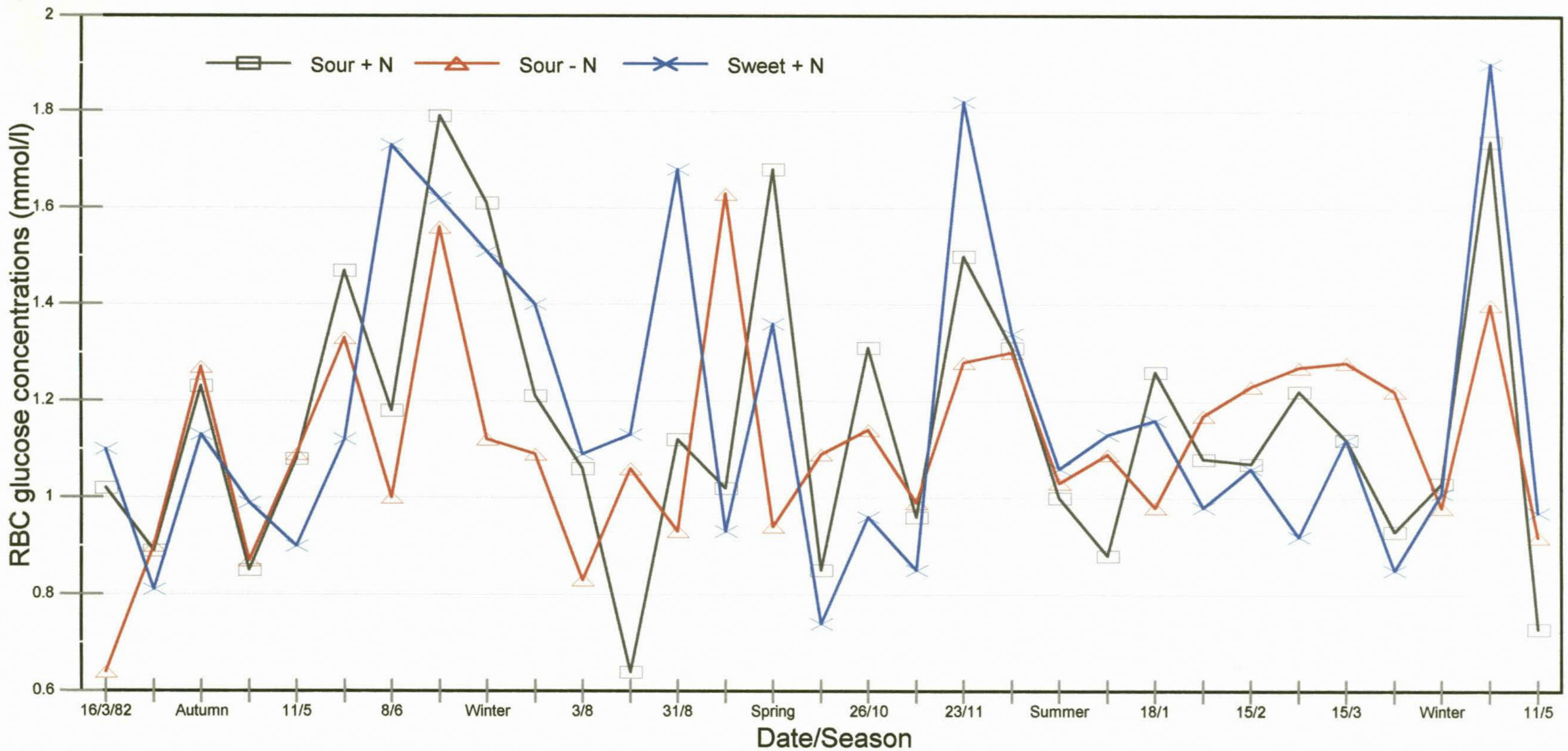


Figure 1.10 Mean red blood cell glucose concentrations (mmol/l) of second-calf cows in 3 treatment groups during 1982/83

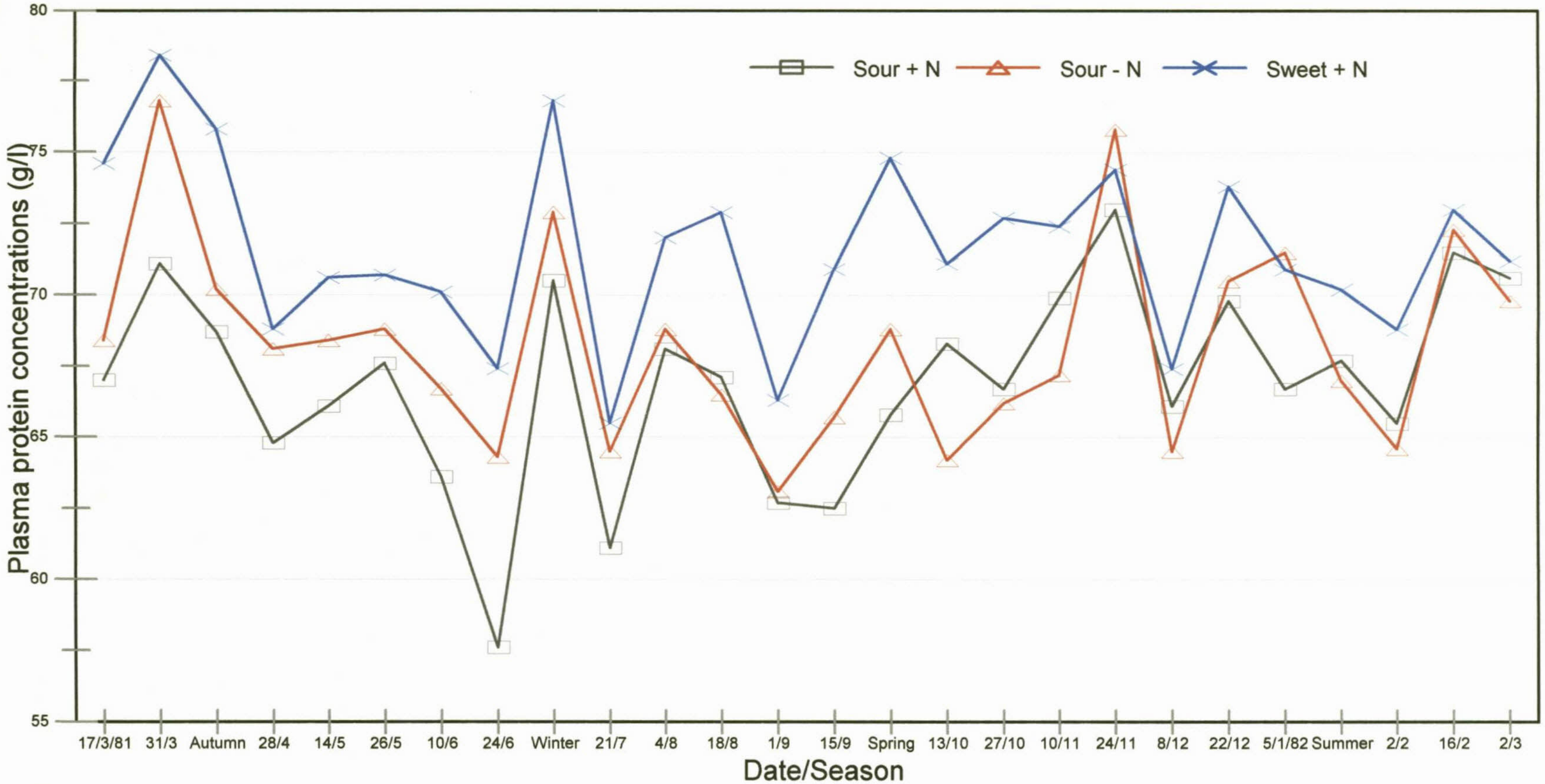


Figure 1.11 Mean plasma total protein concentrations (g/l) of first-calf cows in 3 treatment groups during 1981/82

whilst plasma protein concentrations were elevated in the mixed sweetveld group of the cows, this parameter did not respond to increases and decreases in the protein content of the natural grazing as influenced by season.

Initially, little difference was noted between treatments in albumin levels from March to June (autumn to winter) (Figure 1.12). However, as the season progressed, differences became more marked, with mixed sweetveld first-calvers having higher values compared to those of the other 2 groups. It appears that plasma albumin is more sensitive to the nutritional status of the animal than the plasma protein concentrations. When the albumin concentrations are superimposed on body weight changes, remarkable correspondence is noted, especially in the 2 sourveld groups of cows (Figure 1.3). Albumin levels and body weights increased from March to July, followed by a sharp decline at the beginning of September (spring), and levelled off as the season progressed. Although there was a vast difference in body weights between cows on the mixed sweetveld and those on the 2 sourveld groups, this was not distinctly reflected in the albumin concentrations.

Globulin profiles are presented in Figure 1.13. In contrast to the albumin levels recorded, globulin concentrations in cows on the 3 treatments differed much more at the onset of the period. Regarding both these metabolites, it is noted that females on sourveld with NPN supplementation had lower levels than the sourveld group without NPN supplementation during the first part of the season. Until May (autumn), both these groups received a bone meal and salt lick, and were thus managed under similar conditions. These differences can possibly be ascribed to camp effect.

It appears that the trend in globulin concentrations is in direct contrast with that of albumin profiles, e.g. the increase in albumin concentrations from March to August (autumn to winter) coincided with a decrease in globulin levels. Likewise, a decreasing trend in albumin concentrations from September to January (spring to summer) 1983 coincided with increased globulin levels. Negative correlation coefficients ($P < 0.01$) were found between these 2 constituents (Table 1.4 & 1.5). Globulin concentrations decreased during autumn, and reached minimum levels during June/July (winter) (Figure 1.13), whereafter levels increased as spring and summer months progressed.

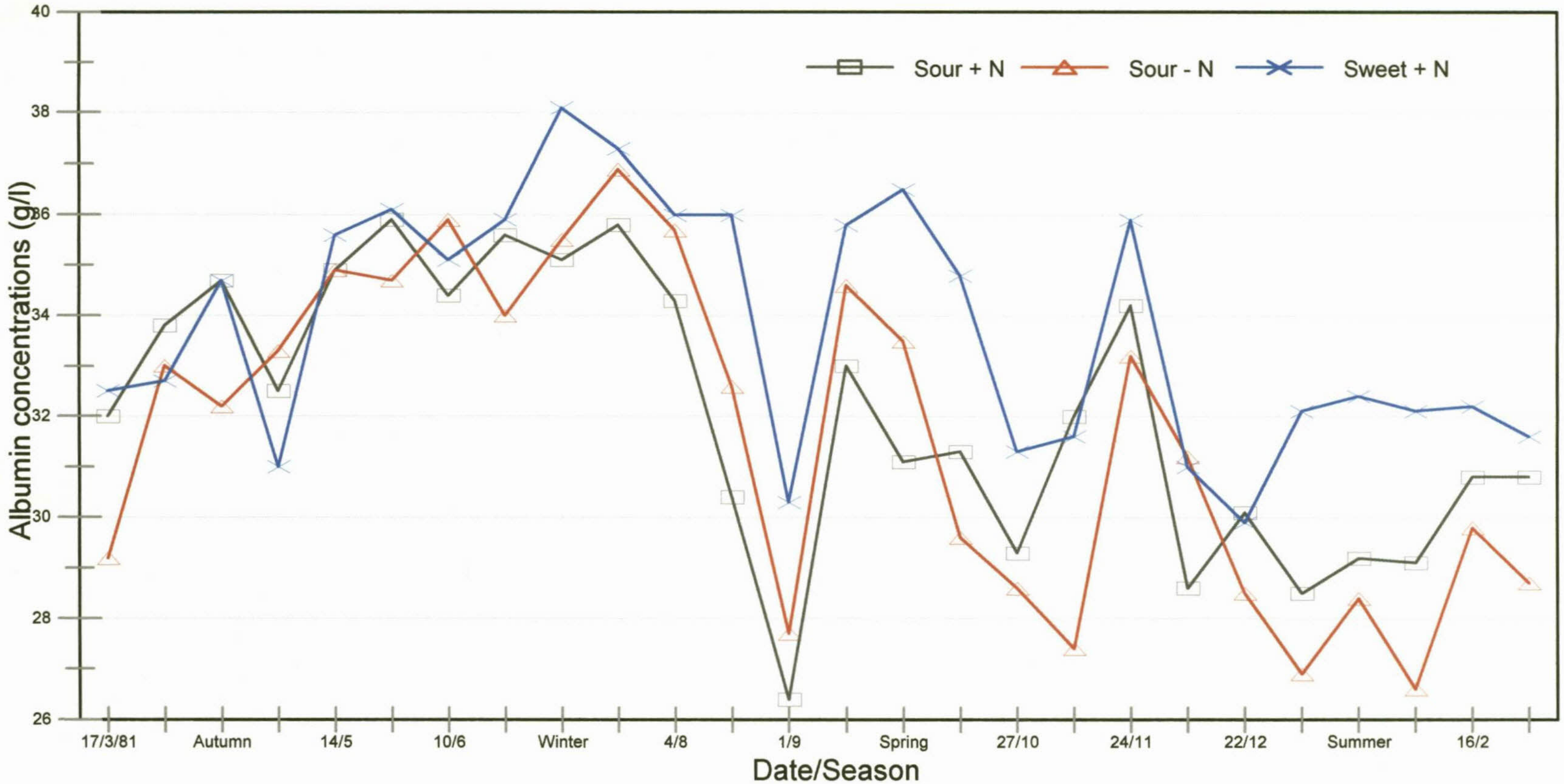


Figure 1.12 Mean plasma albumin concentrations (g/l) of first-calf cows in 3 treatment groups during 1981/82

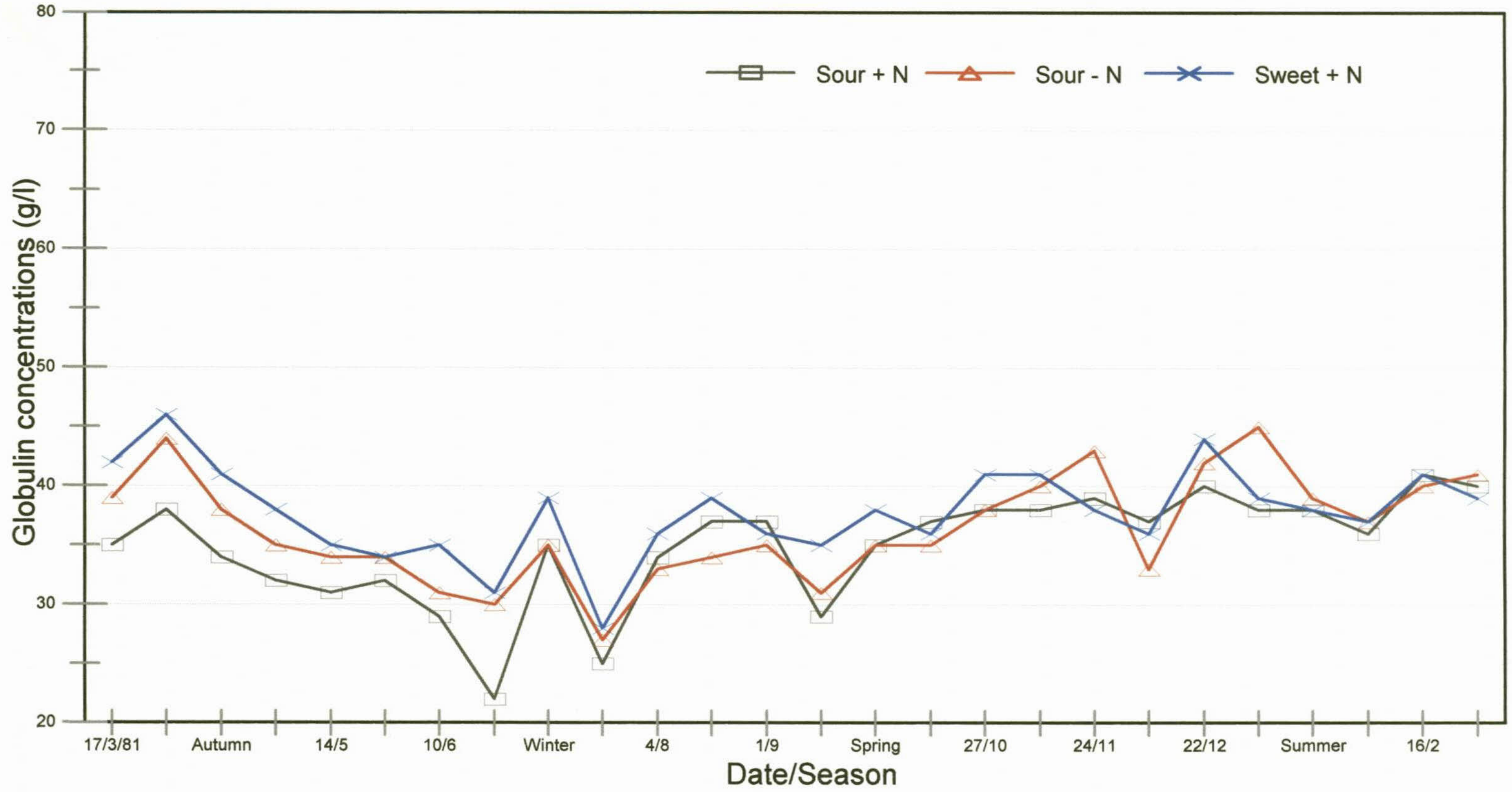


Figure 1.13 Mean plasma globulin concentrations (g/l) of first-calf cows in 3 treatment groups during 1981/82

Urea concentrations appear to be a good indicator of protein status in plasma of beef females in the sense that levels in cows on the mixed sweetveld were elevated above those of cows on the sourveld (Figure 1.14). In this parameter, the superior protein status of cows on the mixed sweetveld is well illustrated. Although fluctuating, levels decreased during the period of low nutritive value of the natural grazing during winter, and sharply increased during spring. Plasma urea concentrations of the 2 sourveld groups did not differ initially. Though the urea lick was made available to one group during the beginning of May (autumn), blood urea levels did not rise significantly until July (winter), and remained above those of the sourveld - N group until the beginning of September (spring), when the lick was withdrawn. It thus appears that the intake of urea is reflected in the blood plasma of the first-calf cows. After withdrawal of the winter lick, plasma urea levels of both sourveld groups followed similar trends, and did not respond to increased nutritive contents of the grazing during the summer season.

As second-calf cows, differences in plasma protein, albumin and globulin concentrations between the different groups of cows were much smaller than during the first year (Figure 1.15 to 1.17). Of these 3 blood constituents, only albumin concentrations were indicative of the higher nutritive value of the mixed sweetveld. An interesting phenomenon during this year was the increase in total plasma protein and globulin concentrations during winter (July to August), and the sharp decrease of these levels during early spring. The striking similarity between these two parameters is evident from Figure 1.15 and 1.17. It is evident that both these metabolites did not reflect the nutritive value of either veld type or nutritional status within veld type as influenced by season.

Similar to the first year, plasma urea concentrations were consistently elevated in cows on the higher feeding regime (Figure 1.18). As in the previous year, the supplementation of an urea lick during the winter period on the sourveld led to an increase in plasma urea concentrations from June to end of August 1983.

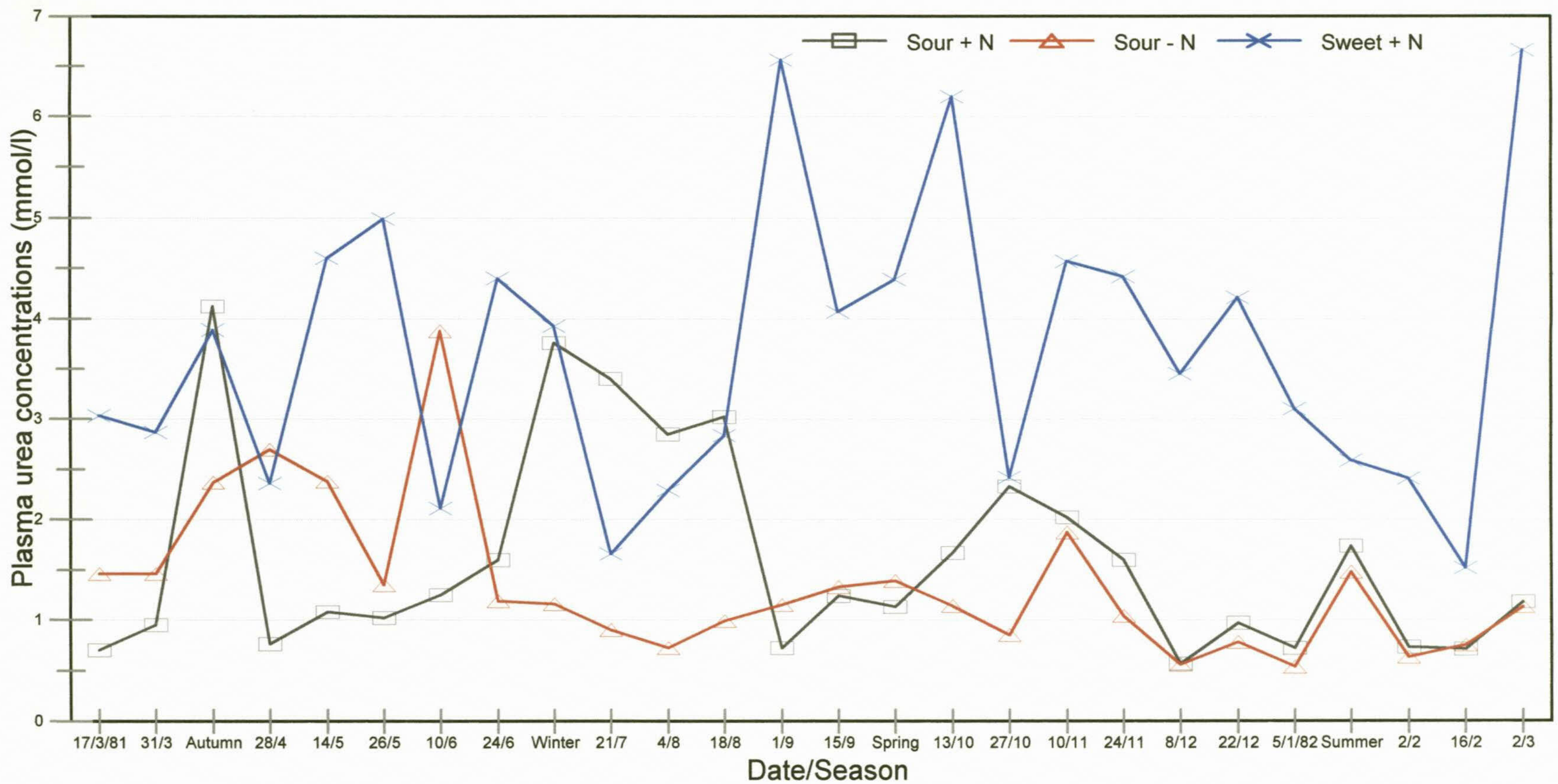


Figure 1.14 Mean plasma urea concentrations (mmol/l) of first-calf cows in 3 treatment groups during 1981/82

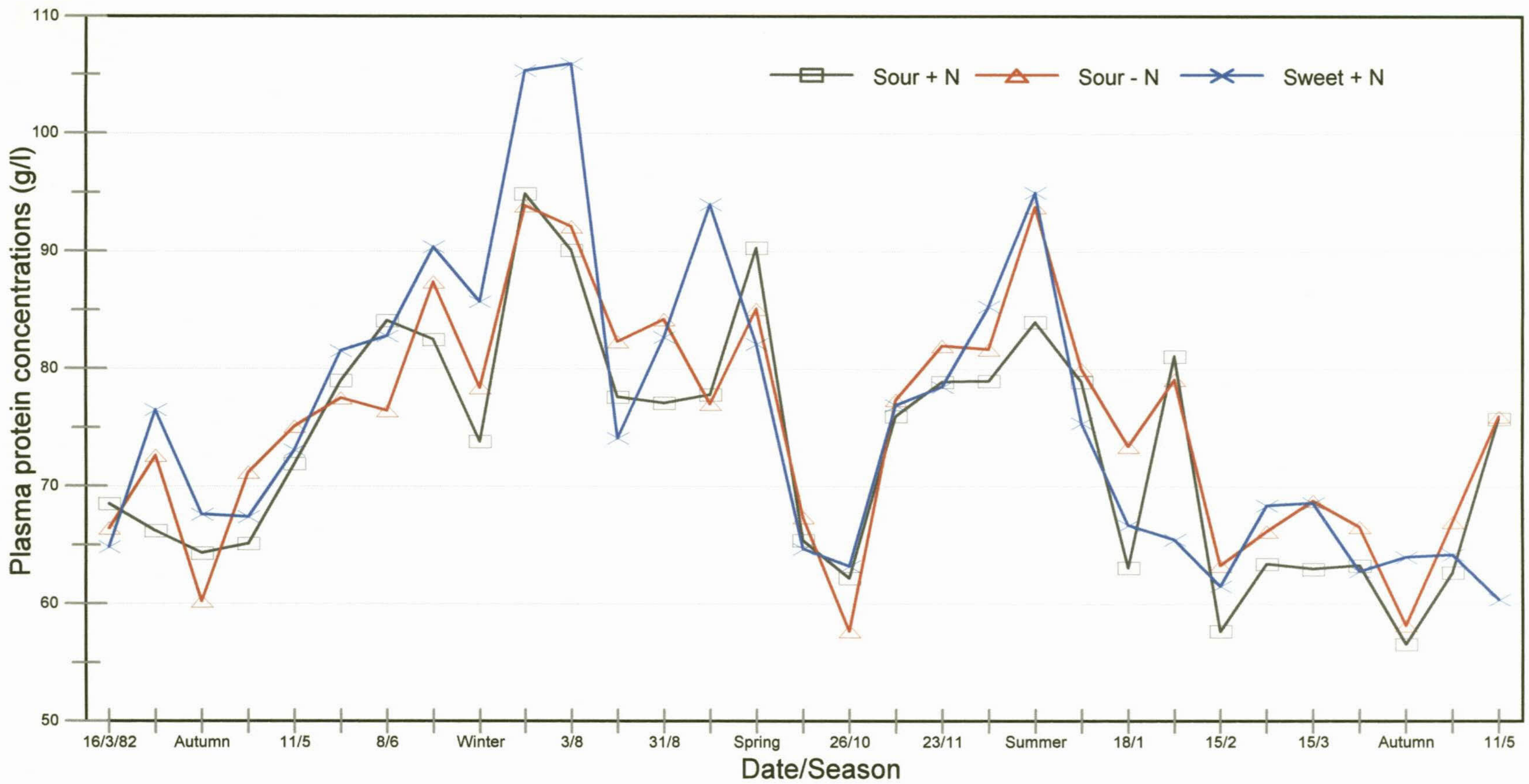


Figure 1.15 Mean plasma total protein concentrations (g/l) of second-calf cows in 3 treatment groups during 1982/83

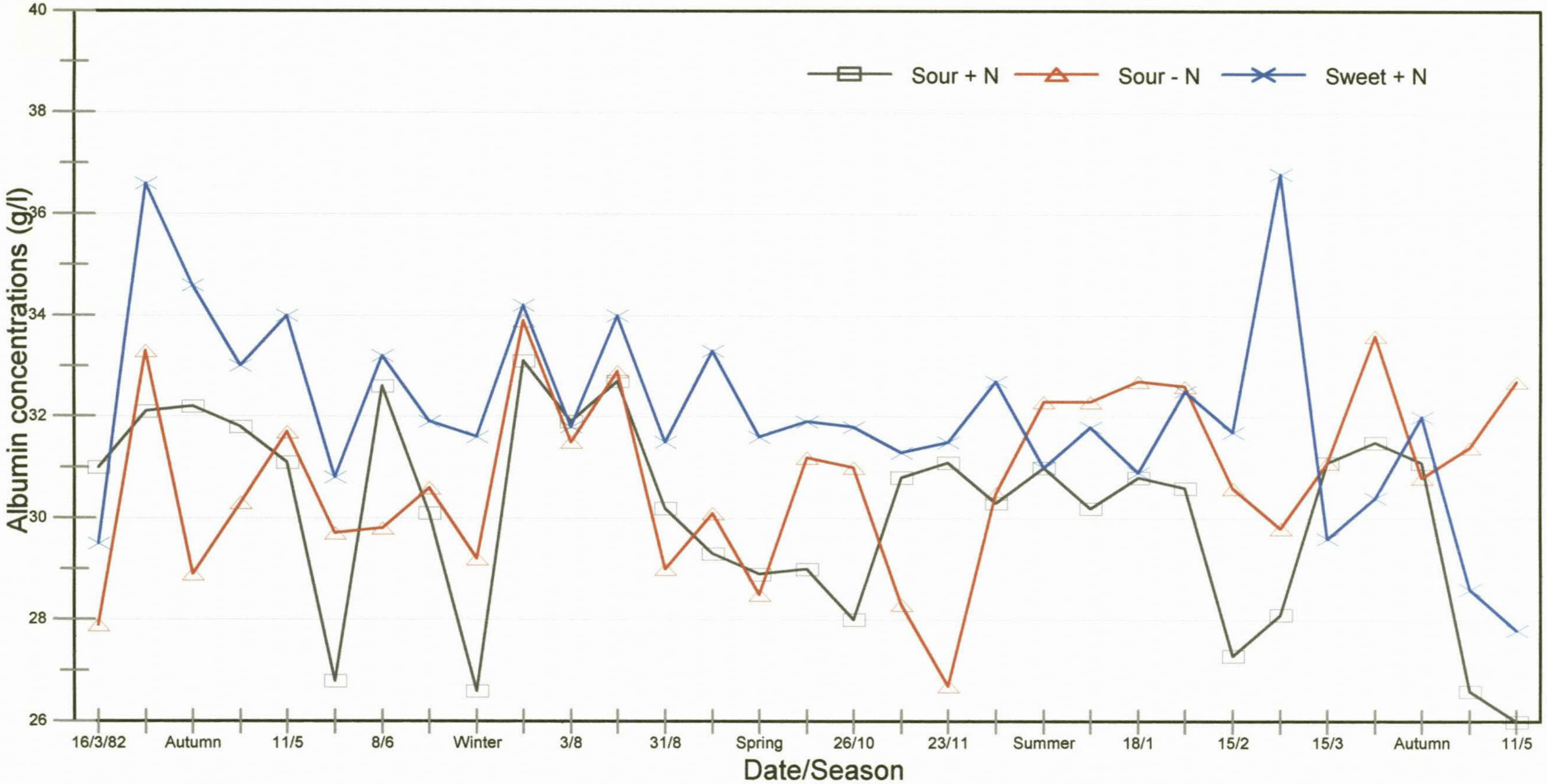


Figure 1.16 Mean plasma albumin concentrations (g/l) of second-calf cows in 3 treatment groups during 1982/83

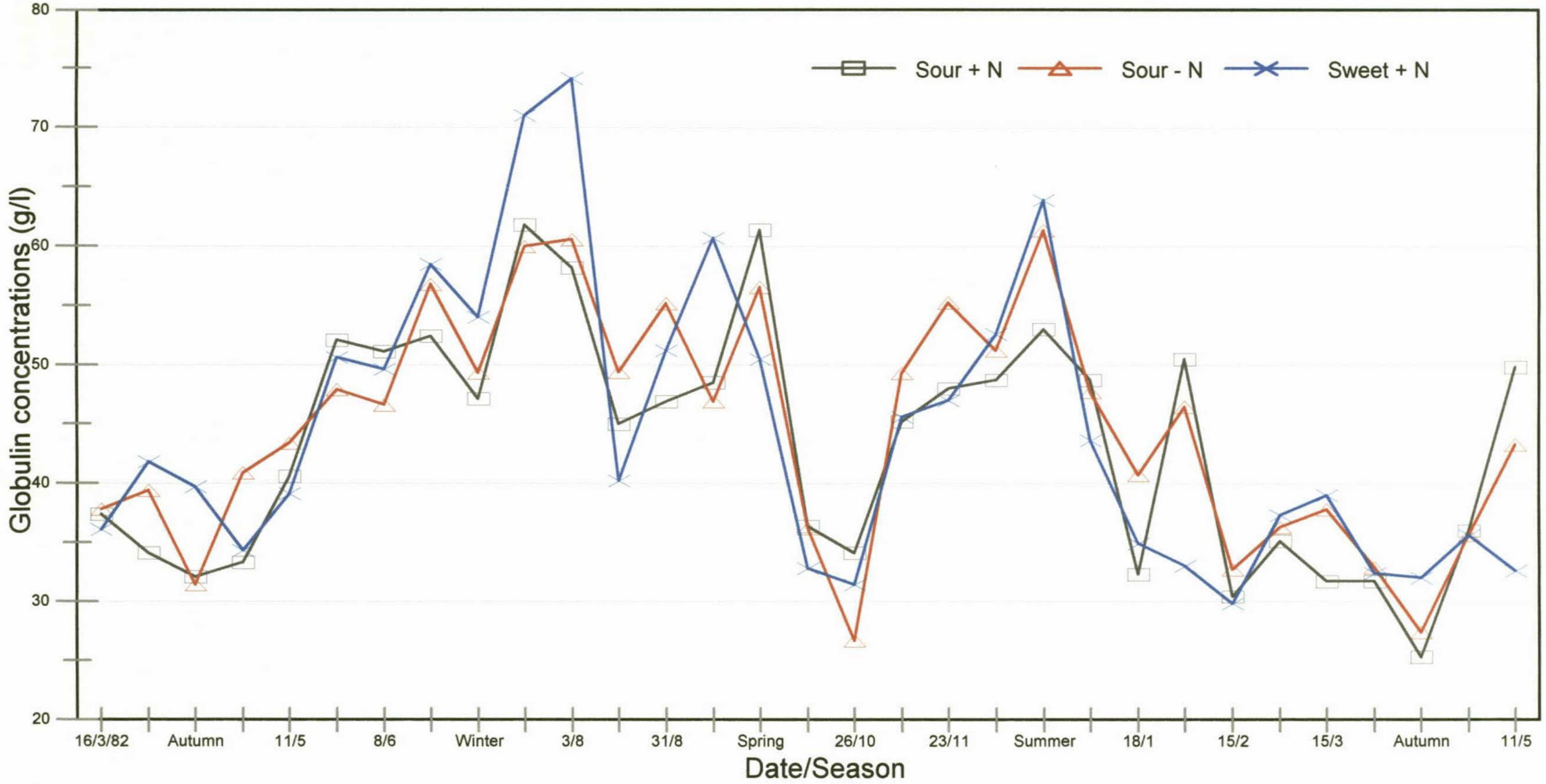


Figure 1.17 Mean plasma globulin concentrations (g/l) of second-calf cows in 3 treatment groups during 1982/83

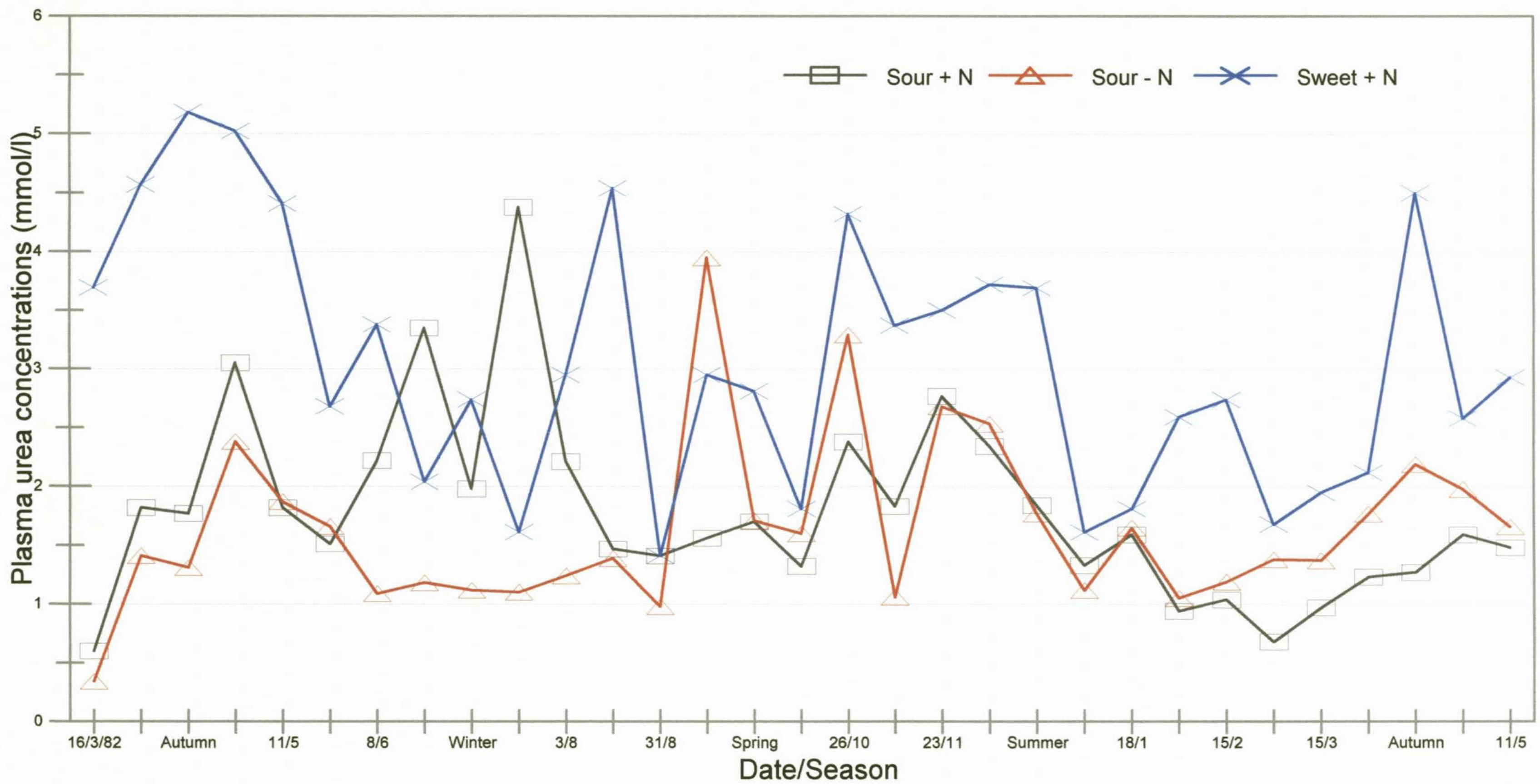


Figure 1.18 Mean plasma urea concentrations (mmol/l) of second-calf cows in 3 treatment groups during 1982/83

1.3.3 Influence of NPN supplementation on blood and plasma metabolites

The influence of non-protein nitrogen supplementation on blood and plasma metabolites of first-calf cows on the sourveld was subjected to a separate analysis of variance. The results are presented in Table 1.3.

Regarding the energy profiles, NPN supplementation led to increased plasma and red blood cell glucose concentrations during 1981/82. During the following year (1982/83), females on the sourveld not receiving an urea lick in winter had significantly ($P < 0.01$ and $P < 0.05$ respectively) higher blood and plasma glucose concentrations when compared to their counterparts.

With regard to protein metabolites, only plasma urea concentrations were influenced by urea supplementation during both seasons. As was the case with the influence of veld type on blood profiles, metabolites were elevated in cows not receiving the non-protein nitrogen supplement during 1981/82 and 1982/83.

1.3.4 Relationships between the various blood and plasma constituents

The relationship between the individual blood and plasma constituents for the 1981/82 and 1982/83 seasons are presented in Table 1.4 and 1.5. Most of the relationships were positive. During both 1981/82 and 1982/83, plasma glucose x blood glucose ($P < 0.01$), red blood cell glucose x plasma glucose ($P < 0.01$) and especially globulin x total plasma protein ($P < 0.01$) concentrations were highly correlated. Interestingly, body weight was well correlated with most constituents during the first year; however, it is apparent that only body weight x album ($P < 0.01$) and body weight x urea ($P < 0.01$) showed consistent and significant relationships during both years.

1.4 DISCUSSION

Because the Compton Metabolic Profile Test was designed with dairy herds in mind, limited investigations have been carried out with other farm animals. Nonetheless, literature reveals a wide variation in results regarding the influence of feeding regimes on energy metabolites.

Table 1.3 Influence of NPN supplementation on mean blood and plasma metabolic concentrations in Bonsmara females

Constituent	1981/82		1982/83	
	Sourveld + N	Sourveld - N	Sourveld + N	Sourveld - N
Blood glucose (mmol/l)	2.14 ± 0.02	2.13 ± 0.02	2.02 ± 0.03^{k}	2.18 ± 0.03^{l}
Plasma glucose (mmol/l)	3.20 ± 0.03^{a}	3.09 ± 0.03^{b}	3.21 ± 0.04^{m}	3.44 ± 0.04^{n}
Red blood cell glucose (mmol/l)	1.06 ± 0.02^{c}	0.97 ± 0.02^{d}	1.20 ± 0.04	1.25 ± 0.04
Total plasma protein (g/l)	67.0 ± 0.56^{e}	68.0 ± 0.56^{f}	73.0 ± 0.90	75.0 ± 0.90
Plasma albumin (g/l)	32.0 ± 0.28	32.0 ± 0.28	30.0 ± 0.30	31.0 ± 0.30
Plasma globulin (g/l)	35.0 ± 0.52^{g}	36.0 ± 0.52^{h}	43.0 ± 0.89	44.0 ± 0.90
Plasma urea (mmol/l)	1.74 ± 0.10^{i}	1.38 ± 0.10^{j}	1.78 ± 0.07	1.63 ± 0.07

b < a (P < 0.05) j < i (P < 0.05)

d < c (P < 0.01) k < l (P < 0.01)

e < f (P < 0.05) m < n (P < 0.05)

g < h (P < 0.05)

Table 1.4 Correlation coefficients between the various protein and energy constituents in the1981/82 season

	Blood glucose	Plasma glucose	RBC glucose	Plasma protein	Plasma albumin	Plasma globulin	Plasma urea
Body weight	0.0391	** 0.3640	** 0.4138	** 0.3436	** 0.2534	* 0.1669	** 0.3933
Plasma urea	** 0.2234	** 0.3040	* 0.1730	** 0.2929	0.0510	** 0.2449	
Plasma globulin	* 0.1880	0.1471	0.0099	** 0.7678	** -0.4318		
Plasma albumin	-0.0746	0.0712	0.1548	** 0.2243			
Plasma protein	0.1408	* 0.1800	0.0922				
RBC glucose	-0.1368	** 0.7108					
Plasma glucose	** 0.5974						

* P < 0.05

** P < 0.01

Table 1.5 Correlation coefficients between the various protein and energy constituents in the 1982/83 season

	Blood glucose	Plasma glucose	RBC glucose	Plasma protein	Plasma albumin	Plasma globulin	Plasma urea
Body weight	-0.0086	0.0608	0.0777	* 0.1016	** 0.2280	0.0357	** 0.4367
Plasma urea	** 0.1734	** 0.2147	** 0.1243	0.0560	** 0.2214	-0.0050	
Plasma globulin	0.0509	0.0739	** 0.1576	** 0.9497	** 0-1229		
Plasma albumin	-0.0140	-0.0114	-0.0073	** 0.1563			
Plasma protein	-0.0502	0.0737	** 0.1548				
RBC glucose	-0.0583	** 0.6679					
Plasma glucose	** 0.6880						

* P < 0.05

** P < 0.01

During the first season, an increase in feeding regime led to an increase in blood and plasma glucose concentrations. Similar results were obtained in mature, pregnant Hereford cows (Rasby *et al.*, 1982), dairy cows (Fisher *et al.*, 1975; Herdt *et al.*, 1982; Blum *et al.*, 1983; Blum *et al.*, 1985; Pelletier *et al.*, 1985; Halse & Tveit, 1994; Grimard *et al.*, 1995) and sheep (Parr & Williams, 1982; Everts, 1990; Beam *et al.*, 1992; Peterson *et al.*, 1992). In Merino rams fed lupin grain, glucose concentrations in the cerebrospinal fluid were higher than in control rams (Boukhliq *et al.*, 1996). Branum *et al.* (1996) reported that refeeding wethers led to an increase in serum glucose concentrations after being maintained at a body weight of 28 kg.

On the other hand, reducing the dietary intake led to decreased blood glucose concentrations in ewes (Grizard *et al.*, 1979; Oddy & Holst, 1991), cows (Bertoni & Calamari, 1979; Bertoni *et al.*, 1983; Grimard *et al.*, 1994; Mostaghni & Askari, 1996), growing heifers (Smith *et al.*, 1992; Yambayamba *et al.*, 1996) and growing steers (Beeby & Swan, 1979). Interesting results were obtained by Sawadogo *et al.* (1991), who observed decreased plasma glucose concentrations in Gobra Zebu under poor natural pasture grazing conditions. These conditions may be similar to those experienced with the 2 sourveld treatments of the present study.

McCann and Hansel (1986) also reported that concentrations of glucose decreased significantly in fasted heifers, and returned to control levels 4 to 7 days after fasting was terminated. Similarly, feed deprivations led to decreased plasma glucose concentrations in dairy heifers (Mills & Jenny, 1979) and crossbred beef steers (Schaefer *et al.*, 1990). However, another group of workers found that the level of nutrition had little or no influence on blood glucose concentrations in dairy cows (Treacher *et al.*, 1979; Oltner, 1983; Uremovic *et al.*, 1995; Belibasakis & Tsirgogianni, 1996), heifers (Ambatkar *et al.*, 1996), sheep (Doize *et al.*, 1979), growing lambs at pasture (Rhind *et al.*, 1984) and beef cows (Coggins & Field, 1976; Shrick *et al.*, 1990). McNiven (1984) reported that in lean sheep, plasma glucose levels decreased as feed intake decreased. In contrast to the above results, another group of researchers found that glucose concentrations decreased as energy levels in the diet increased (Champredon *et al.*, 1974), whilst others found that nutrition had no significant influence on blood or plasma glucose concentrations in ewes (McCrabb *et al.*, 1990) lambs (Holcombe *et al.*,

1992) or wethers (Nosbush *et al.*, 1996).

The discrepancy observed regarding the influence of different feeding regimes on blood glucose concentrations could be attributed to the influence of climate, variation in nutritive qualitative and quantitative levels of the natural grazing between years, sensitivity of parameter used and influence of pregnancy and/or lactation.

The influence of climate and nutritive value of the pastures in the present study may have been responsible for the variation in glucose concentrations between the 2 years. In a previous study, it was reported that the total non-structural carbohydrate (TNC) content of mixed sweetveld fistula samples was constantly higher than that of sourveld (mean values of 4.10 vs. 3.34% in summer [$P < 0.01$], and 2.91 vs. 2.82% in winter over both years) (Erasmus, 1988). During 1981/82, the differences in blood and plasma glucose concentrations in cows between the sourveld and mixed sweetveld treatments were more pronounced than during the following year (1982/83); in fact, relatively small differences were noted in energy levels of the experimental animals on the 2 veld types during the latter year. Thus, the reaction of the energy profiles to treatment during the second year may be attributed to a reduced difference in nutritive values of the 2 veld types, or a factor influencing metabolism that was absent during the first year.

During 1981/82, the animals on the mixed sweetveld were probably in a positive energy balance, as reflected by body weight (Figure 1.3). The intake of nutrients exceeded growth and reproductive requirements. Glucose concentrations of 2.29 and 3.38 mmol/l for blood and plasma respectively could thus be considered as a norm against which these 2 processes will adequately function. During 1982/83, the nutritional value of this veld type was unable to meet the increased nutritional requirements of the second-calf cows for processes such as pregnancy, calving, lactation and rebreeding. Cows grazing on the mixed sweetveld during this year, as well as the 2 sourveld groups during both seasons, were in a negative energy balance as indicated by body weight trends. It is thought that, because of reduced feed intake or higher demands made by the animal body, precursors to gluconeogenesis were absorbed at a reduced rate. Consequently, peripheral uptake of glucose was reduced, apparently as a result of decreased insulin.

Pregnancy and lactation may have played a significant role regarding glucose metabolism. During 1982/83, 2 cows from the sourveld + N and 4 cows from the sourveld - N groups did not conceive. It, therefore, follows that, although the mixed sweetveld + N group was on a higher plane of nutrition, the glucose concentrations of these cows were lower than that of the group that was on the lowest plane (sourveld - N; Table 1.2). This latter group had only 1 cow suckling a calf. This aspect of lactation will receive further attention when discussed in Chapter 3.

The choice of glucose concentrations as an indicator of energy status deserves attention. It is known that the ruminant has a general requirement for energy, and a specific requirement for glucose. One can, therefore, assume that differentiation in energy intake will not necessarily be reflected in the glucose concentrations. Also, plasma glucose concentrations are subject to homeostatic control. According to Russel (1978), large changes in the rate of glucose utilization are reflected in only relatively small changes in peripheral circulating concentrations. Another factor which might influence the sensitivity of glucose concentrations as an indicator of energy status, is that it is to some degree stress sensitive. Hyperactivity of the adrenal cortex can cause relatively large increases in plasma glucose concentrations; the latter may thus not necessarily reflect the energy status of the animal (Russel, 1978).

In view of the above, plasma-free fatty acids (FFA) are preferred as a more sensitive index of feed intake (Lindsay, 1978; Russel, 1978). It is thought that plasma FFA concentrations might be less affected by homeostasis than plasma glucose concentrations (Kronfeld, 1972). However, it is pointed out by Lindsay (1978) that the major disadvantage of FFA's is the indication that FFA's, too, are stress-sensitive. According to Holmes and Lambourne (1970), plasma FFA's were more sensitive to short-term fluctuations related to time of feeding and excitement.

Present total plasma protein results could not substantiate the general view of previous researchers. Protein concentrations in blood serum of cows fed different amounts of protein remained constant

(Marinov, 1981) and in 2 groups of rams fed 14% protein concentrate and forage respectively, no change was noted in total protein concentrations (Thomas & Chiboka, 1984). Similarly, diet had no significant influence on total protein levels of high-producing Holstein-Friesian cows (Esievo & Moore, 1979; Belibasakis & Tsirgogianni, 1996), Angus and Hereford cows (Shrick *et al.*, 1990) or heifers (Oldham *et al.*, 1979). Blum *et al.*, (1983) found that total protein did not differ significantly between 3 groups of lactating cows fed different energy diets. In fact, plasma total protein and albumin concentrations decreased after lactating cows were introduced to spring pasture (Sato *et al.*, 1981). On the other hand, it was noted that total protein levels rose with increasing dietary intake in steers (Evans, 1972), Hereford cows (Rasby *et al.*, 1982) and dairy cows (Russel, 1974). Sawadogo *et al.* (1991) also reported a decrease in plasma protein and urea concentrations of Zebu cattle under poor natural pasture conditions. These conflicting results indicate that total plasma protein levels are probably not a good indicator of protein status in the ruminant.

Literature reveals albumin concentrations to be a much more sensitive indicator of varying levels of dietary protein. In late pregnant sheep, albumin concentrations ranged from 35 g/l in adequately fed, to 12 g/l in most severely undernourished sheep (Sykes, 1978). Similar results with sheep (Lynch & Jackson, 1983), rams (Thomas & Chiboka, 1984) and Friesian heifers (Oldham *et al.*, 1979) indicate a decrease in albumin levels with reduced protein intake, and *vice versa*. Interestingly, Bertoni and Calamari (1979) found that albumin levels decreased with a diet low in energy, when fed to dairy cows. Similarly, restricted diets (hay) led to reduced serum albumin concentrations of crossbred Friesian cows on days 13 to 28 after calving (Mostaghni & Askari, 1996). Another group of workers found that albumin levels were not influenced by diet (Belibasakis & Tsirgogianni, 1996) or the protein content of the diet (Esievo & Moore, 1979; Pelletier *et al.*, 1985). Sato *et al.* (1981) found that albumin concentrations decreased after lactating Holstein heifers were gradually introduced to spring pasture. Although it may appear that the level of nutrition does not consistently influence protein profiles, the process of lactation seems to influence these parameters, and will be discussed in more detail in Chapter 3.

Not much information is available on the influence of nutrition on globulin concentrations. In Friesian cows fed different diets, no significant differences were found in globulin levels (Belibasakis & Tsirgogianni, 1996). Similarly, Esievo and Moore (1979) found that dietary protein also had no significant influence on globulin concentrations. According to Payne *et al.* (1970), albumin levels are by far the most important member of the total protein-albumin-globulin group, and it is doubtful whether globulin profiles are worth including in a metabolic profile test.

Perhaps the most striking is the influence dietary protein has on plasma urea nitrogen concentrations. Present results, and those from related studies, indicated an increase (Evans *et al.*, 1980; Sato *et al.*, 1981; Jordan *et al.*, 1983; Thomas & Chiboka, 1984; Janicki *et al.*, 1985; Pelletier *et al.*, 1985; Holcombe *et al.*, 1992; Ozpinar *et al.*, 1995; Barton *et al.*, 1996; Fekete *et al.*, 1996; Okada *et al.*, 1997) or decrease (Oldham *et al.*, 1979; Lynch & Jackson, 1983; Bortolussi *et al.*, 1996) of urea nitrogen levels as the dietary intake of protein increased or decreased. Milk urea N concentrations were found to be linearly and positively associated with dietary crude protein content and intake (Cannas *et al.*, 1998). Similar to results obtained in the present study, poor pasture conditions caused a decrease in urea concentrations in Zebu cattle (Sawadogo *et al.*, 1991). Similarly, other researchers found positive relationships between urea concentrations and dietary concentrate levels in lambs (Beam *et al.*, 1992) and sheep (Peterson *et al.*, 1992). Marinov (1981) noted that the urea concentrations followed the pattern of the amount of protein in the feed. It was found that serum urea levels increased with fertilizer application of N, P and K to pasture (Bakanov *et al.*, 1976). Cows fed green lucerne forage alone without any supplementation, showed a marked drop in live weight, but had a high blood urea level (Pres *et al.*, 1975). It was reported that plasma urea nitrogen was lower in starved ram lambs (Horton *et al.*, 1996) and higher in cows receiving hay, compared to wheat straw (Ozpinar *et al.*, 1995).

It is interesting to note that energy levels in the diet appear to have an opposite effect on urea concentrations to that of protein. Increasing the supply of energy caused a reduction in serum urea to dairy cows (Lebeda & Prikrylova, 1978; Lavandera & Gagliostro, 1995), ewes (Catalano *et al.*, 1995) and lambs (Holcombe *et al.*, 1996), whilst Shrick *et al.* (1990) reported that cows fed low-energy diets had higher concentrations of blood urea nitrogen. These results are of interest to the present study, for it is accepted that both protein and energy contents were higher on the mixed sweetveld than sourveld, and during the summer compared to the winter on both pasture types. If increased energy supply leads to a decrease in urea levels, this process is of secondary importance as plasma urea levels increased with increased nutritive content of the natural pasture. This increase must, therefore, be ascribed to increased protein content of the grazing.

In contrast to the above-mentioned, diet did not have any significant effect on urea concentrations of Friesian cows (Belibasakas & Tsirgogianni, 1996). Graml *et al.* (1995) reported that fasting bulls resulted in higher plasma levels of, among others, urea. In a study with lambs, Bunting *et al.* (1992) found lambs fed high-fat diets, to have significantly ($P < 0.05$) lower plasma urea nitrogen concentrations in comparison with the controls. It thus appears that conflicting reports on the influence of feeding regime on urea concentrations are by far in the minority, and the present study is in agreement with Eggum (1974) that blood urea levels can give good indirect measurements of protein adequacy in the diet.

Varying results were obtained concerning the influence of urea supplementation on glucose concentrations. In pregnant beef heifers and cows, urea significantly increased plasma glucose concentrations (Fishwick *et al.*, 1974). In a study, Treacher *et al.* (1979) found that blood glucose concentrations were similar in 2 groups of cows fed urea or fish meal and groundnut 6 weeks before calving, through to the end of lactation. Hugi *et al.* (1997) reported that blood glucose concentrations were not associated with protein and fat intake, but correlated with lactose and total sugar intake.

Plasma urea levels were the only protein indicators to reflect NPN supplementation. Generally, the literature reveals that urea supplementation promotes increased levels of urea in the blood of lactating dairy cows (Champredon *et al.*, 1974; Mostafavi *et al.*, 1974; Materikin & Tishenkov, 1983). Soya bean meal and urea gave similar values for plasma urea, and these were higher than those given by a protein-deficient diet (Muller, 1973). When urea-nitrogen was replaced by fish meal-nitrogen, blood urea concentrations decreased with increasing amounts of fish meal in the diet (Oldham *et al.*, 1985).

However, it was also found that urea supplementation did not increase urea concentrations in the milk or blood (Atkeshev, 1983).

Urea supplementation is known to increase intake of roughage (Campling *et al.*, 1962; Pieterse *et al.*, 1963; Fishwick *et al.*, 1973; Fishwick *et al.*, 1974; Hennesy, 1984), gross energy, digestible energy and TDN (Lesch *et al.*, 1963). Since it is possible that energy supplementation may result in increased urea-nitrogen concentrations (Boling *et al.*, 1979), the higher plasma urea concentrations in the experimental animals on the sourveld + N treatment may thus be ascribed to both increased protein and energy intakes.

Of the 4 protein parameters studied thus far, only albumin and urea concentrations reflected the higher protein contents of the natural pastures in first- and second-calf cows on the mixed sweetveld. This indicates the overriding influence of dietary protein over processes such as pregnancy, calving and lactation which may also influence protein status. These results thus suggest that in first-calf cows, total protein, albumin, urea and, to a lesser extent, globulin concentrations are indicative of protein status. However, this would only apply to groups of animals where rather large differences in feeding regimes are prevalent. No clear differences existed between the 2 sourveld groups. In lactating second-calf cows, it appears that albumin, and especially urea concentrations, may be used to monitor protein status.

According to Mackie and Fell (1971), the half-life of albumin is 13 to 18 hours. However, this may be reduced during lactation. It, therefore, follows that albumin concentrations could be used as an indicator only during periods of long-term protein sufficiency or deficiency.

In contrast, urea has a half-life of only a few hours (Cocimano & Leng, 1967), suggesting that it could be the best indicator to use to detect short-term changes in nutritional status (Sykes, 1978). From the present results, it appears that urea concentrations in the blood plasma may effectively be used as long-term or short-term indicators of protein status in the grazing animal.

1 151 251 8X

Correlation coefficients should be interpreted with care, as blood metabolites are influenced by species differences, breed, age, nutrition, lactation and physiological status of animals. Similarly, in the present study, it is expected that not only the differences in natural pastures, but also the effect of pregnancy and lactation may have influenced the level of the blood and plasma metabolites in the experimental animals.

The most significant relationship was established between total plasma protein and globulin concentrations ($r = 0.77$ and $r = 0.95$ for the 2 years respectively). This is in agreement with $r = 0.91$ reported by Coggins and Field (1976) and $r = 0.96$ and $r = 0.90$ reported by Hewett (1974). In the present study, the negative correlation between albumin and globulin during both years is in contradiction with the results obtained by Coggins and Field (1976) and Heyns (1965). Heyns (1965) also found a significant positive correlation of $r = 0.71$ between the 2 constituents. However, present results are in agreement with those of Hewett (1974) and Payne *et al.* (1973). The latter authors considered that a compensatory mechanism was possibly involved in maintaining the total protein concentrations.

Plasma urea consistently showed a positive and significant relationship with all 3 energy metabolites. These results are in agreement with those of Coggins and Field (1976), but in disagreement with those of other workers (Heyns, 1965; Hewett, 1974). The differences obtained in present results between the 2 years underline the important influence that certain factors such as season and physiological state of the animal might have on metabolite homeostasis.

The relationship between body weight and different constituents were not always consistent. During the first year (1981/82), body weight was positively and sometimes strongly correlated with energy metabolites. During the following year, the relationships were much less prominent, and body weight showed a tendency to be negatively correlated to blood glucose. However, it is evident that body weight shows a strong tendency to be positively correlated with albumin and plasma urea. The results of albumin do not correspond, but those of urea nitrogen do correspond with the findings of Heyns (1965). It thus appears that plasma urea concentrations increase in conjunction with body weight in growing, pregnant and lactating beef first-calvers.

1.5 CONCLUSIONS

Blood glucose concentrations in growing Bonsmara first-calvers may be a sensitive enough barometer of energy status in the animal body to distinguish between nutritive values of sourveld and mixed sweetveld, and to indicate differences in seasonal nutritive value of the natural pastures. The fact that glucose concentrations tended to be higher in first-calf cows on the mixed sweetveld, (and thus a higher nutritive feeding regime), reflected an increase in the rate of gluconeogenesis. It could thus be concluded that in animals in this group, the basic metabolic demands had been satisfied. Judging from the body weight increase of these animals, additional energy intake was thus canalised into growth function.

The drastic decline of 43% (from 2.8 to 1.6 mmol/l) in the blood glucose concentrations from mid-March to end of July, is a clear indication that the experimental animals experienced nutritional stress during this period. It is evident that the provision of an NPN lick was insufficient to maintain the females, and that processes such as growth and reproduction may be seriously hampered if access to additional nutrition is denied.

The sudden upswing in glucose concentrations during mid-August (late winter) cannot be due to increased nutritive status of the grazing, as the latter usually occurs at a much later time of the year. It is postulated that the concentration of blood glucose is not only affected by level of nutrition, but that the metabolic response is also influenced by photoperiodic stimuli, when glucose concentrations increase as day-length increases.

If this is true, the interpretation of glucose concentration relating to the energy status of an animal should be approached with caution in the grazing animal. From Figure 1.15 it is clear that glucose concentrations in all 3 groups of experimental animals decreased during autumn to reach minimum levels on 21/7/82 (winter). During this period, the same females were gaining in live weight. However, it should be pointed out that during July and August (winter), the animals were in late pregnancy; because the weight of the pregnant uterus and its contents may be as much as 60 kg towards the end of pregnancy, these females in fact lost body weight.

Although the weight of the experimental animals did not decrease immediately, the downward trend in blood glucose concentrations, from March to July, may have signalled the advent of lowered nutritive value of the pastures and shorter day-length. Similarly, the early upswing in glucose levels in August and September (late winter to early spring) may have signalled the increase in day-length and rise in nutritive status of the natural grazing, with an increase in live weight following later.

Plasma glucose and red blood cell glucose concentrations did not reflect the nutritive status of the natural pastures, and are probably of less value as indicators of energy status. For instance, the high RBC glucose concentrations of first-calvers on the mixed sweetveld during midwinter is not a true reflection of energy status as the animals were maintaining body condition during this period. When animals were in a positive energy balance and gaining weight (September and October) (spring), RBC glucose levels were in decreasing phase.

During pregnancy and lactation, none of these metabolites were positive indicators of energy status in the animal (1982/83). It is concluded that the process of lactation may influence the metabolism to such a degree as to render the use of glucose concentrations for energy indicators, unsuitable.

Of the protein metabolites, albumin concentrations were a mirror-image of body weight trends during the first year. Although these profiles did not always significantly reflect the large weight differences between the groups, levels increased with increased live weight and *vice versa*. It is concluded that albumin concentrations in growing, pregnant first-calvers are good indicators of protein status. Although total protein reflected the differences between groups more effectively, there was not much indication of nutritive differences between season within treatments. Plasma globulin concentrations seemed to be more indicative of seasonal, than nutritive trends.

Plasma urea concentrations varied tremendously within treatments, and to some degree between treatments, yet this metabolite was the best indicator of differences between the experimental animals on the 3 treatments. As shown in Table 1.1, urea levels of first-calf cows on the mixed sweetveld were twice as high than those of the animals on the sourveld, while a significant difference ($P < 0.01$) between the 2 sourveld groups was also highlighted.

As was the case with glucose concentrations, the protein parameters were also apparently influenced by lactation of the cows. Nevertheless, plasma albumin and urea concentrations could indicate differences in protein status between first-calf cows on the mixed sweetveld and those on the sourveld, but not between females on the 2 sourveld treatments.

1.6 SUMMARY

During the 1981/82 season, first-calf cows on the mixed sweetveld supplemented with an NPN lick in the winter had significantly higher mean blood (2.30 vs. 2.13 vs. 2.12 mmol/l, $P < 0.01$), plasma (3.38 vs. 3.20 vs. 3.10 mmol/l, $P < 0.01$) and red blood cell glucose (1.09 vs. 1.06 vs. 0.98 mmol/l, $P < 0.05$) concentrations than the group on the sourveld supplemented with an NPN lick in winter, and the sourveld group not supplemented. Glucose concentrations was the best parameter to indicate seasonal nutritive status, with levels decreasing during the winter, and reaching maximum concentrations during February and March (late summer). Of the 3 parameters, blood and plasma glucose concentrations were the best reflection of nutritional differences between the sourveld and mixed sweetveld.

Energy metabolites did not appear to reflect the nutritional status of the natural pastures during the 1982/83 season. It is thought that the added stress of suckling during 2 successive years may have suppressed energy levels, especially during the 1983 summer season.

Similar to energy profiles, protein metabolites were all significantly higher in first-calvers on the mixed sweetveld than those on the sourveld during the 1981/82 season. Total plasma protein reflected the higher protein content of the mixed sweetveld virtually throughout the year, mean concentrations being 72.0, 66.0 and 68 g/l ($P < 0.01$) for animals on the 3 treatments respectively.

Albumin concentrations appeared to be more sensitive to the nutritional status of the animal than total plasma protein levels. Mean concentrations of 34.0, 32.0 and 32.0 g/l ($P < 0.01$) were recorded for first-calf cows on the mixed sweetveld + N, sourveld + N and sourveld - N treatments respectively. Albumin profiles corresponded with body weight trends to a remarkable degree, especially in the 2 sourveld groups. Mean globulin concentrations of first-calf cows were 38.0 vs. 35.0 vs. 36.0 g/l ($P < 0.05$) for the 3 treatments respectively.

Urea concentrations appeared to be a good indicator of protein status of first-calf cows on the 3 treatments, concentrations being 3.75, 1.75 and 1.35 mmol/l ($P < 0.01$) respectively. Levels decreased during the period of low nutritive value of the natural grazing, and sharply increased during spring. During the following year (1982/83), differences in protein constituents were much smaller, and only albumin and urea concentrations were indicative of the higher nutritive value of the mixed sweetveld.

Although the urea lick was made available during the beginning of May, plasma urea concentrations did not significantly rise until July (winter). NPN supplementation also led to increased plasma and red blood cell glucose concentrations during 1981/82; 3.20 vs. 3.09 mmol/l ($P < 0.05$) and 1.06 vs. 0.97 mmol/l respectively for supplemented and deprived groups respectively. During the following year, cows deprived of an urea lick in winter, had significantly higher blood and plasma glucose concentrations when compared with their counterparts. Plasma urea concentrations were elevated during both years in experimental animals receiving an NPN supplementation (1.74 vs. 1.38 mmol/l, $P < 0.05$ during 1981/82, and 1.78 vs. 1.63 mmol/l during 1982/83).

The following correlation coefficients were significant during 2 consecutive years:
Blood glucose x urea ($r = 0.22$ and $r = 0.17$), blood glucose x plasma glucose ($r = 0.60$ and $r = 0.69$) and total plasma protein x globulin ($r = 0.77$ and $r = 0.95$). Albumin showed a negative relationship with globulin ($r = -0.43$ and $r = -0.12$). Live weight showed a consistent and positive relationship with albumin ($r = 0.25$ and $r = 0.23$) and urea ($r = 0.39$ and $r = 0.44$) during both seasons.

It is concluded that the experimental animals experienced nutritional stress during the winter months of 1981/82, with blood glucose concentrations decreasing by 43%, from approximately 2.8 mmol/l to 1.6 mmol/l, from mid-March to the end of July. Because blood and plasma glucose concentrations were significantly elevated in beef females on the mixed sweetveld compared to those on the sourveld, it is postulated that the former reflected an increase in the rate of gluconeogenesis, and that the metabolic demands in this group had been met. However, it is clear that the supplementation of non-protein nitrogen in the winter to beef females on the sourveld is not sufficient to provide for basic body functions such as maintenance and growth.

CHAPTER 2

INFLUENCE OF DOHNE SOURVELD AND MIXED SWEETVELD ON ADRENAL CORTEX AND THYROID ACTIVITY OF BEEF HEIFERS AND COWS

2.1 INTRODUCTION

The adrenal cortex and thyroid glands play an important role in the metabolic processes of the animal body. The activity of both glands influences protein, carbohydrate and fat metabolism to a large degree. It was documented as early as 1925, by Jackson that inadequate quantities of food, or low protein diets, cause changes in the hypophysis and other endocrine glands.

According to Guyton (1971), the best-known metabolic effect of cortisol and other glucocorticoids on metabolism is their ability to stimulate gluconeogenesis in the liver. The disturbances in carbohydrate metabolism occurring in the adrenalectomized animal are due mainly to removal of the adrenal cortex (Turner & Bagnara, 1971). According to these researchers, adrenalectomy leads to excessive oxidation of glucose and decreased gluconeogenesis from body protein.

The adrenal steroids are known to cause a reduction of protein stores in essentially all body cells except those of the liver and gastrointestinal tract (Guyton, 1971). It is believed that the reduction in protein anabolism is caused by antagonizing the effects of insulin, and by an effect on the metabolism of nucleic acids (Turner & Bagnara, 1971).

The function of the thyroid gland is to elaborate, store and discharge secretions that are concerned principally with the regulation of the metabolic rate (Turner & Bagnara, 1971). In carbohydrate metabolism, thyroxine increases the rate of monosaccharide absorption from the alimentary tract, and increases the rate of glucose utilization by the cells. In the case of protein metabolism, thyroxine increases the rates of both protein anabolism and catabolism (Guyton, 1971).

From the above-mentioned it could be concluded that changes in carbohydrate and protein metabolism may result from altered adrenal cortex and thyroid activity due to various feeding regimes. It is, therefore, the aim of this study to gain more information on the influence of various nutritional levels on the secretions of these 2 endocrine glands of free-grazing beef females.

2.2 EXPERIMENTAL PROCEDURES

Five Bonsmara first-calf cows were used in each of the following 3 experimental groups: Group 1, females were run on the Dohne Sourveld, supplemented with a bone meal and salt lick in the summer season, and an NPN lick during the winter season (sourveld + N); Group 2, females were run on the Dohne Sourveld, supplemented with bone meal and salt lick throughout the year (sourveld - N); Group 3, females were run on the mixed sweetveld, supplemented with a bone meal and salt lick in the summer, and an NPN lick in the winter (mixed sweetveld + N).

Blood samples were obtained via the jugular vein at fortnightly intervals (09:00) in heparin tubes, packed on ice, and centrifuged at 2000 G. Aliquots of plasma samples from each experimental animal were stored at -20°C for the later determinations of cortisol and thyroxine concentrations. The methods used for the analyses of the hormone concentrations were previously described (Experimental Procedures, paragraph 8 Methods and Data Collection, p 12).

2.3 RESULTS

2.3.1 Influence of pasture type on plasma cortisol concentrations

Mean plasma cortisol concentrations of first-calf cows on sourveld and mixed sweetveld are presented in Table 2.1.

Except for the 1982/83 season, hormone levels were significantly lower in females on the sourveld than those on mixed sweetveld.

During the 1979 season, there was no significant difference in the plasma cortisol concentrations between heifers and cows on the Dohne Sourveld. Rather, hormone levels were influenced to a large degree by season of the year (Figure 2.1). During 1980, no clear influence of veld type on cortisol profiles of the heifers were recorded (Figure 2.2).

Table 2.1 Mean plasma cortisol and thyroxine concentrations of Bonsmara heifers and cows on sourveld supplemented with an NPN lick, sourveld without an NPN lick, and mixed sweetveld with an NPN lick

Hormone concentrations	Treatments		
	Sourveld + N	Sourveld - N	Mixed sweetveld + N
Cortisol (ng/ml)	(mean ± SE)	(mean ± SE)	(mean ± SE)
1979	10.62 ± 0.41	-	-
1980	11.94 ± 0.53	-	13.02 ± 0.57
1981/82	8.78 [a] ± 0.39	7.32 [b] ± 0.39	9.76 [c] ± 0.39
1982/83	6.70 ± 0.23	6.80 ± 0.23	6.50 ± 0.23
Thyroxine (ng/ml)			
1979	50.08 ± 1.06	-	-
1980	53.50 ± 1.03	-	52.40 ± 1.10
1981/82	41.80 [d] ± 0.89	40.80 [e] ± 0.89	48.10 [f] ± 0.90
1982/83	39.10 [g] ± 0.71	40.80 [h] ± 0.71	46.60 [i] ± 0.71

b < a (P < 0.01)

b < c (P < 0.001)

d < f (P < 0.001)

e < f P < 0.001)

g < i (P < 0.001)

h < i (P < 0.001)

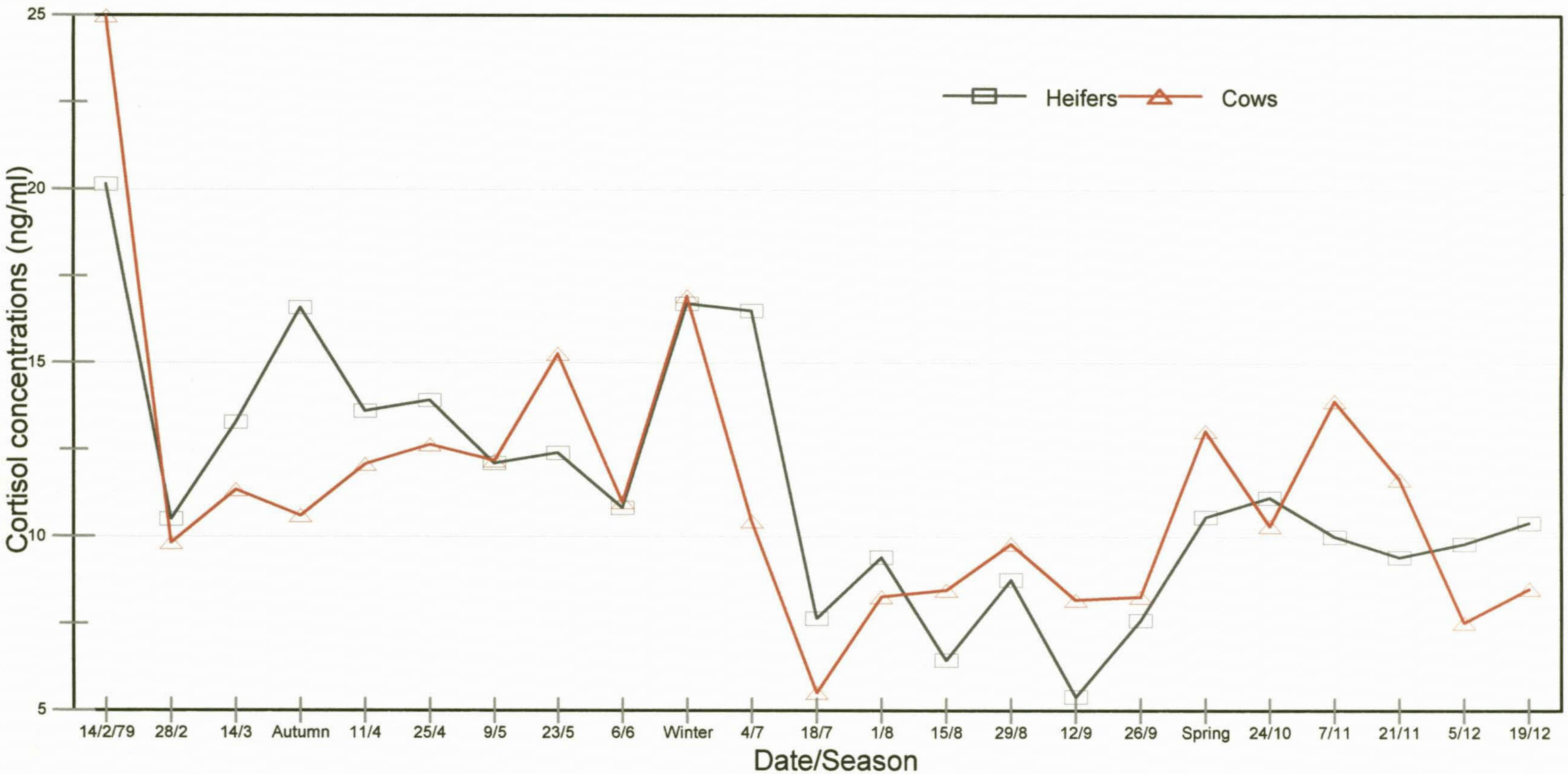


Figure 2.1 Mean plasma cortisol concentrations (ng/ml) of Bonsmara heifers and cows on the Dohne Sourveld during 1979

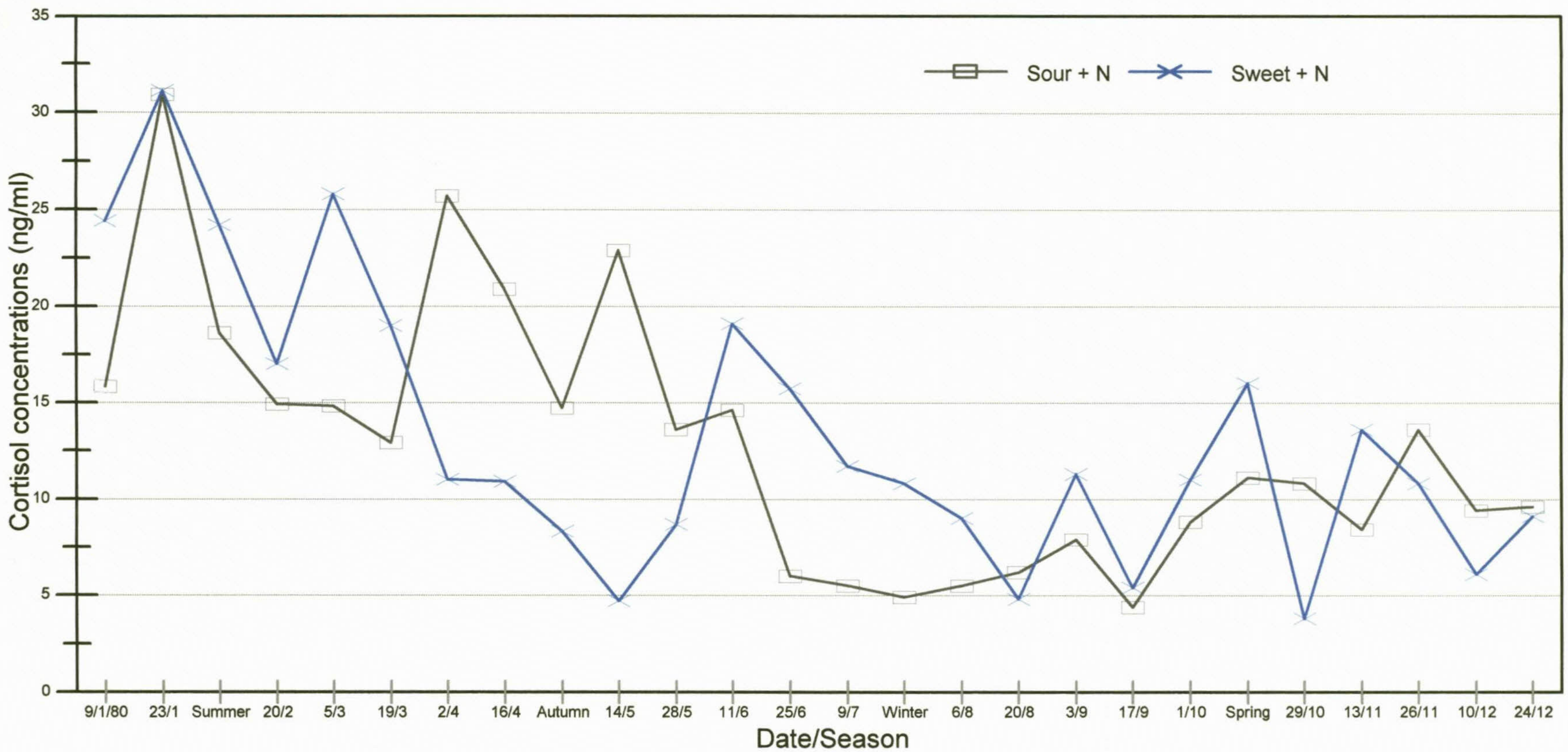


Figure 2.2 Mean plasma cortisol concentrations (ng/ml) of heifers on the Dohne Sourveld and mixed sweetveld during 1980

In the case of cows, veld type had a more pronounced influence on the plasma cortisol concentrations (Figure 2.3). Similar to the trend in heifers, higher levels were recorded in cows on mixed sweetveld than on sourveld, especially during the winter period. Again, it appears as if cortisol concentrations are rather influenced by season than veld type.

It is evident from Figure 2.4 that there was no significant difference in cortisol secretion between the 2 sourveld groups of first-calvers during 1981/82. As in previous years, plasma cortisol concentrations were significantly ($P < 0.01$) higher in females on the mixed sweetveld, compared to those on sourveld during the autumn and winter months.

During the 1982/83 season, treatments had no influence on the plasma cortisol concentrations (Figure 2.5). A characteristic of the previous seasons was that cortisol concentrations in cows on the mixed sweetveld were at a higher level throughout winter. During this season, the higher nutritive value of this type of natural grazing did not promote higher adrenal cortex activity.

2.3.2 Influence of natural pasture type on plasma thyroxine concentrations

Thyroxine (T4) concentrations did not differ significantly between females on sourveld and mixed sweetveld during 1980 (Table 2.1). Separate analyses of data revealed mean T4 levels for heifers to be 55.5 and 57.7 ng/ml, and those for cows 51.9 and 47.8 ng/ml on sourveld and mixed sweetveld respectively. Thus, results of the heifers during 1980 followed the same pattern of those during the subsequent years, with increased thyroid function resulting from increased nutritive value of the natural pasture. NPN supplementation on the sourveld did not lead to increased T4 concentrations.

During 1979, heifers on the sourveld constantly experienced higher thyroid activity compared to cows under similar conditions (Figure 2.6). There was no indication of an increased thyroid response to increased feeding levels during the summer season. During 1980, T4 concentrations in heifers were 55.3 ng/ml, compared to the 47.6 ng/ml of the cows. During this period, the superior nutritive value of the mixed sweetveld led to elevated hormone levels of the heifers towards the latter part of the season (Figure 2.7). The reverse trend was noted in the case of cows

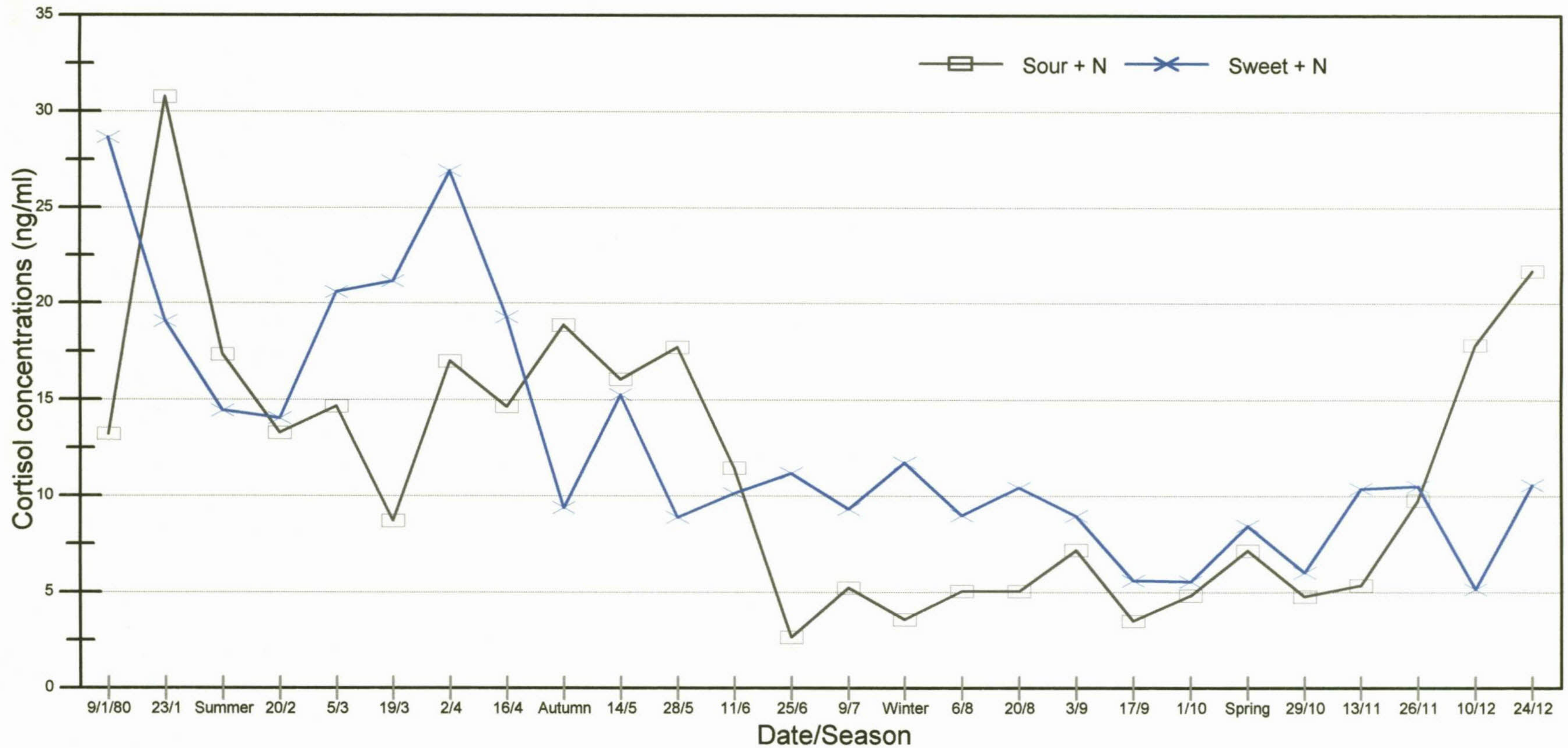


Figure 2.3 **Mean plasma cortisol concentrations (ng/ml) of cows on the Dohne Sourveld and mixed sweetveld during 1980**

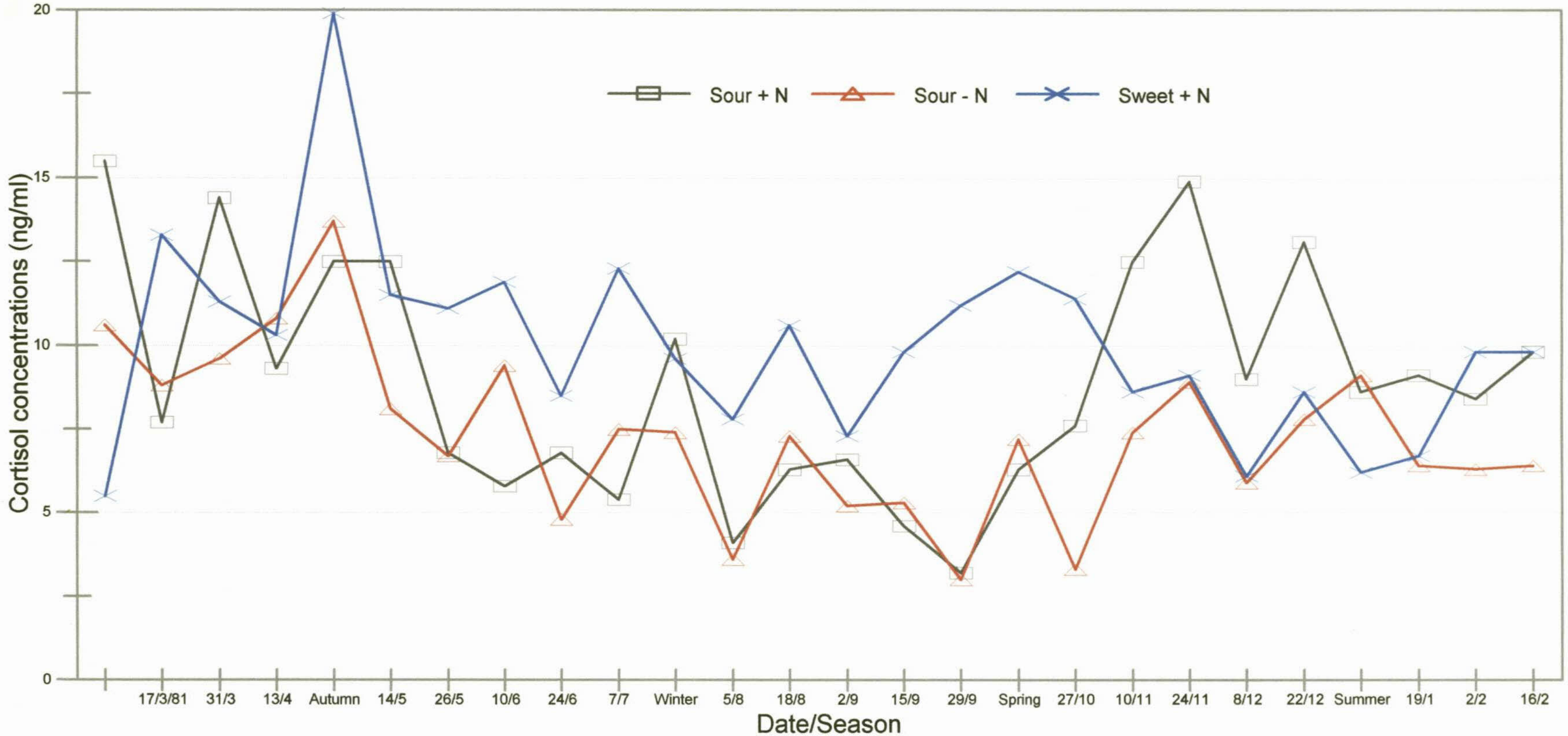


Figure 2.4 **Mean plasma cortisol profiles (ng/ml) of first-calf cows on the sourveld with NPN, sourveld without NPN and mixed sweetveld with NPN during 1981/82**

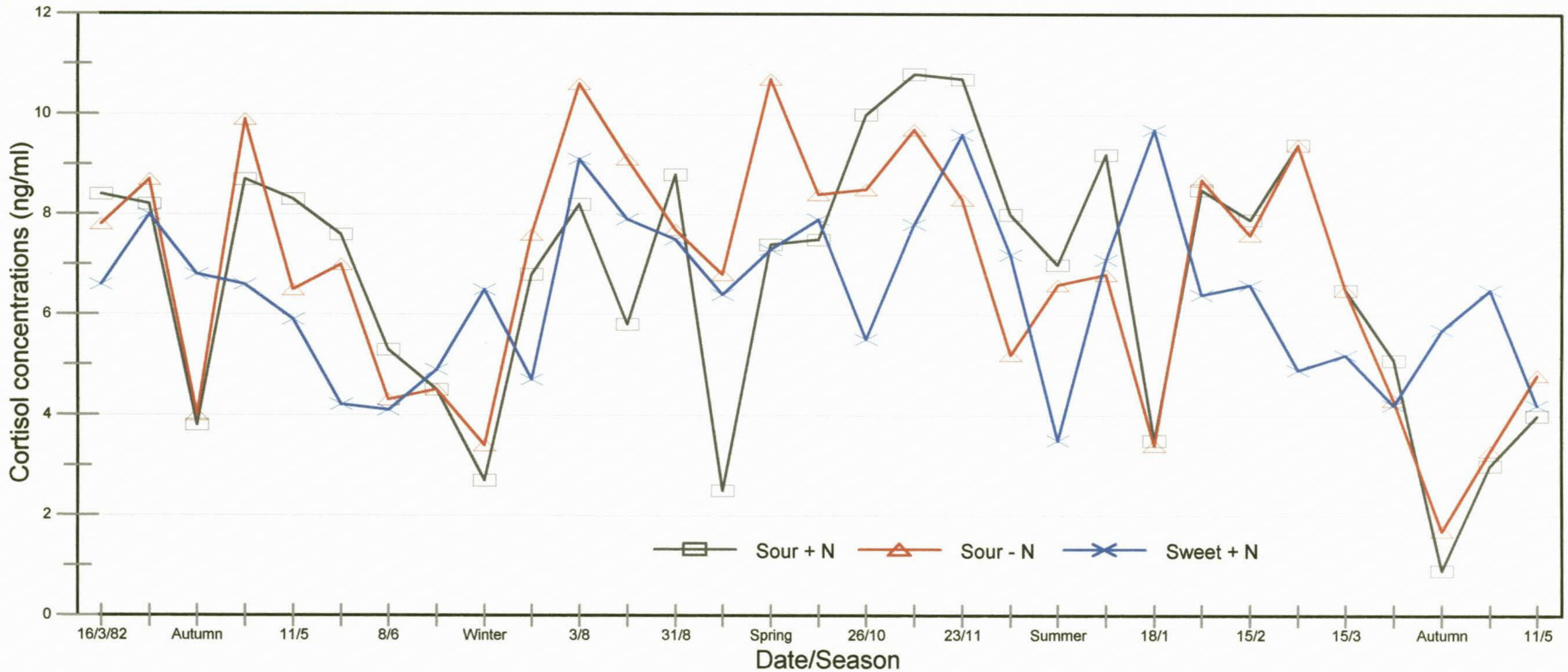


Figure 2.5 **Mean plasma cortisol profiles (ng/ml) of second-calf cows on the sourveld with NPN, sourveld without NPN and mixed sweetveld with NPN during 1982/83**

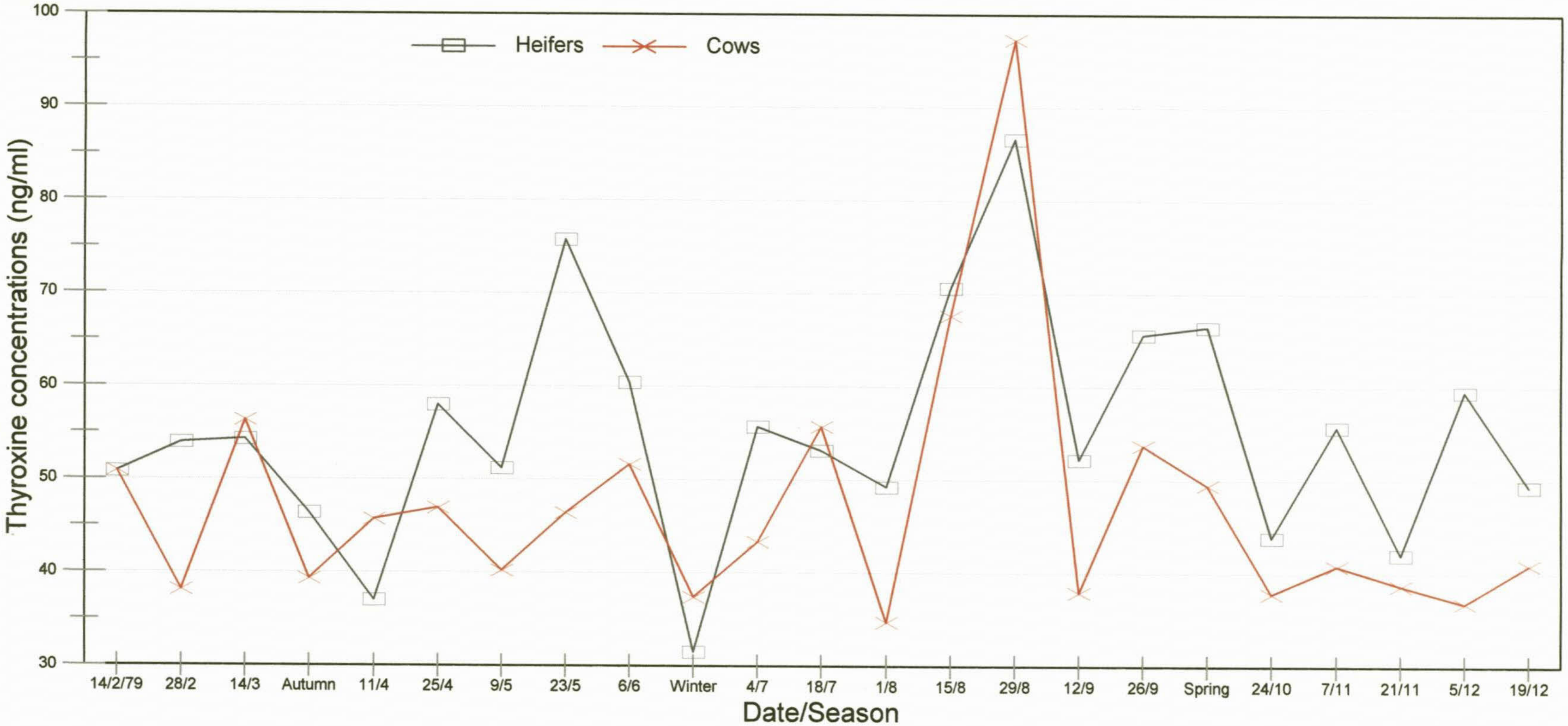


Figure 2.6 **Mean plasma thyroxine concentrations (ng/ml) of Bonsmara heifers and cows on the Dohne Sourveld during 1979**

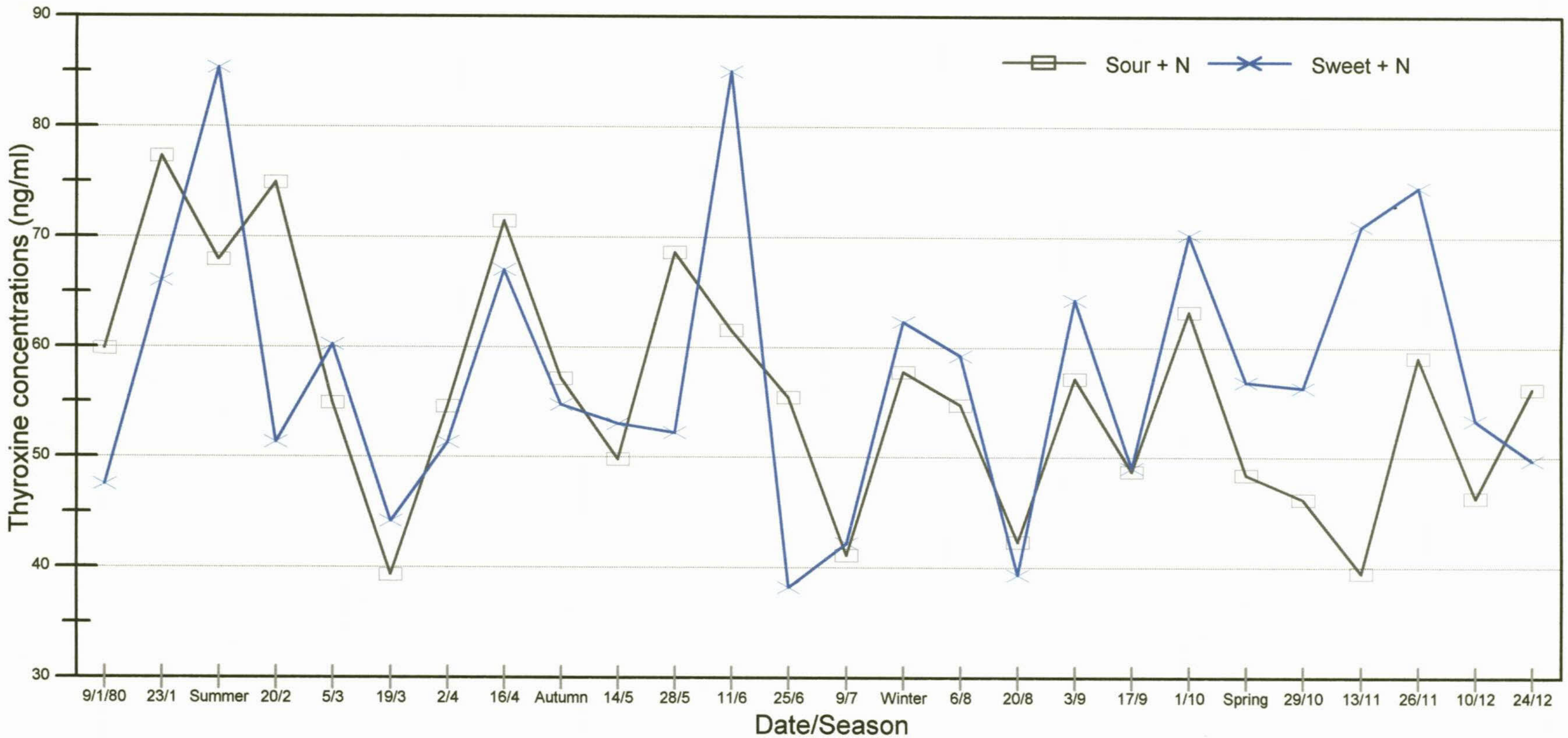


Figure 2.7 Mean plasma thyroxine concentrations (ng/ml) of heifers on the Dohne Sourveld and mixed sweetveld during 1980

(Figure 2.8).

The effect of grazing on the sourveld with NPN supplementation, sourveld without supplementation, and mixed sweetveld with NPN supplementation on plasma T4 concentrations of first-calvers is depicted in Figure 2.9. For all practical purposes, no differences in hormone levels of the 2 sourveld groups were noted. Again, increased nutritive level of the pasture promoted an increase of thyroxine levels in first-calf cows on mixed sweetveld (1981/82). During the subsequent year, as second-calf cows, the trend was repeated (Figure 2.10). It is evident from Figure 2.10 that thyroxine concentrations were not significantly influenced by seasonal variation in nutritive values of the natural pasture during any of the years. It is interesting to note that a decrease in thyroid activity was noted in sourveld and mixed sweetveld females from 1980 to 1982/83 (Table 2.1).

2.3.3 Influence of NPN supplementation on plasma cortisol and thyroxine concentrations

To determine what influence NPN supplementation had on the adrenal cortex and thyroid activity, an analysis of variance was carried out and included all 3 groups of first-calf cows. To substantiate these results, another analysis of variance was performed, using only the 2 sourveld groups of animals. The results are presented in Table 2.2.

In the first analysis (sourveld + N; sourveld - N; mixed sweetveld + N), it appeared that supplementation of NPN led to significantly ($P < 0.01$) increased cortisol and thyroxine levels during 1981/82. During the following year (1982/83), supplementation had either a reduced (thyroxine concentration) or no (cortisol concentration) influence.

Exclusion of the mixed sweetveld group from the analysis, revealed that the impact of supplementation on endocrine function was much less during 1981/82, with no influence during the subsequent year.

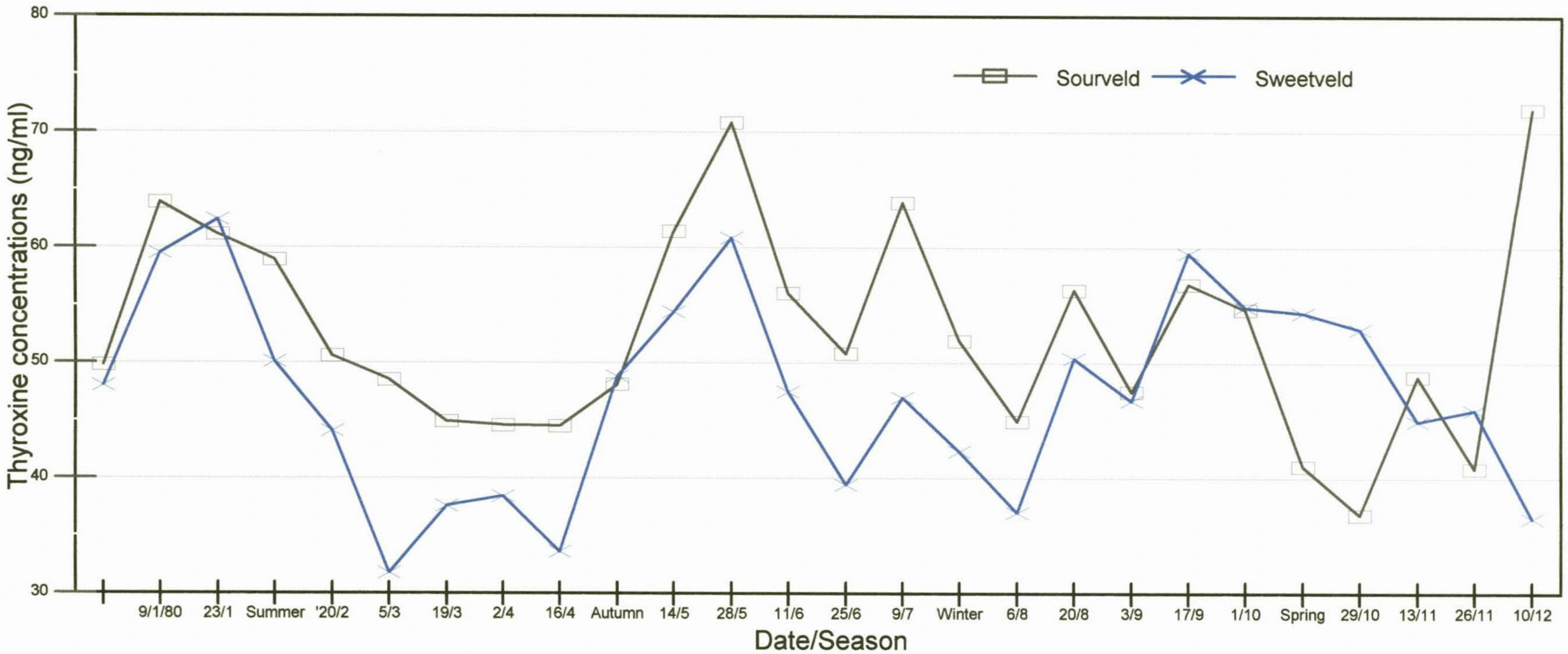


Figure 2.8 Mean plasma thyroxine concentrations (ng/ml) of cows on the Dohne Sourveld and mixed sweetveld during 1980

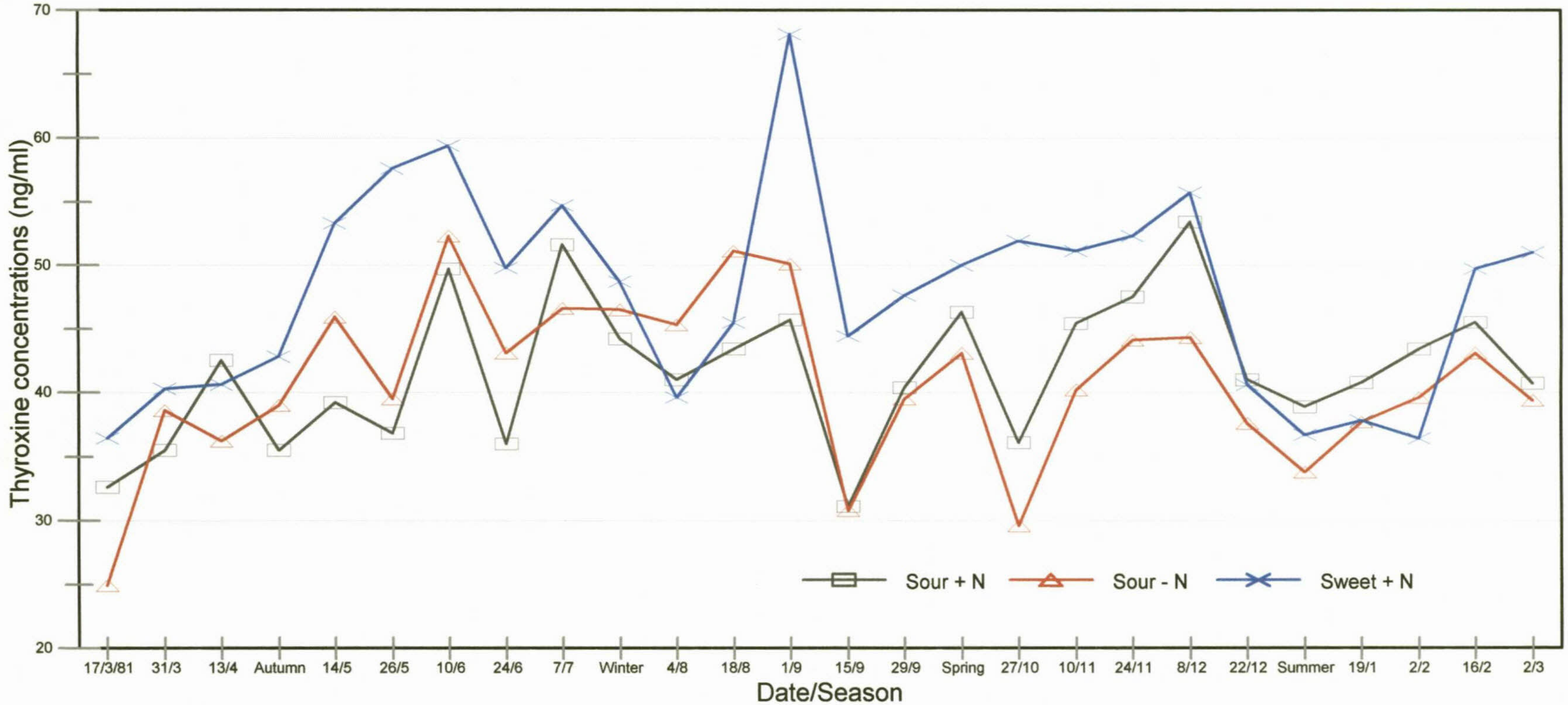


Figure 2.9 **Mean plasma thyroxine profiles (ng/ml) of first-calf cows on the sourveld with NPN, sourveld without NPN and mixed sweetveld with NPN during 1981/82**

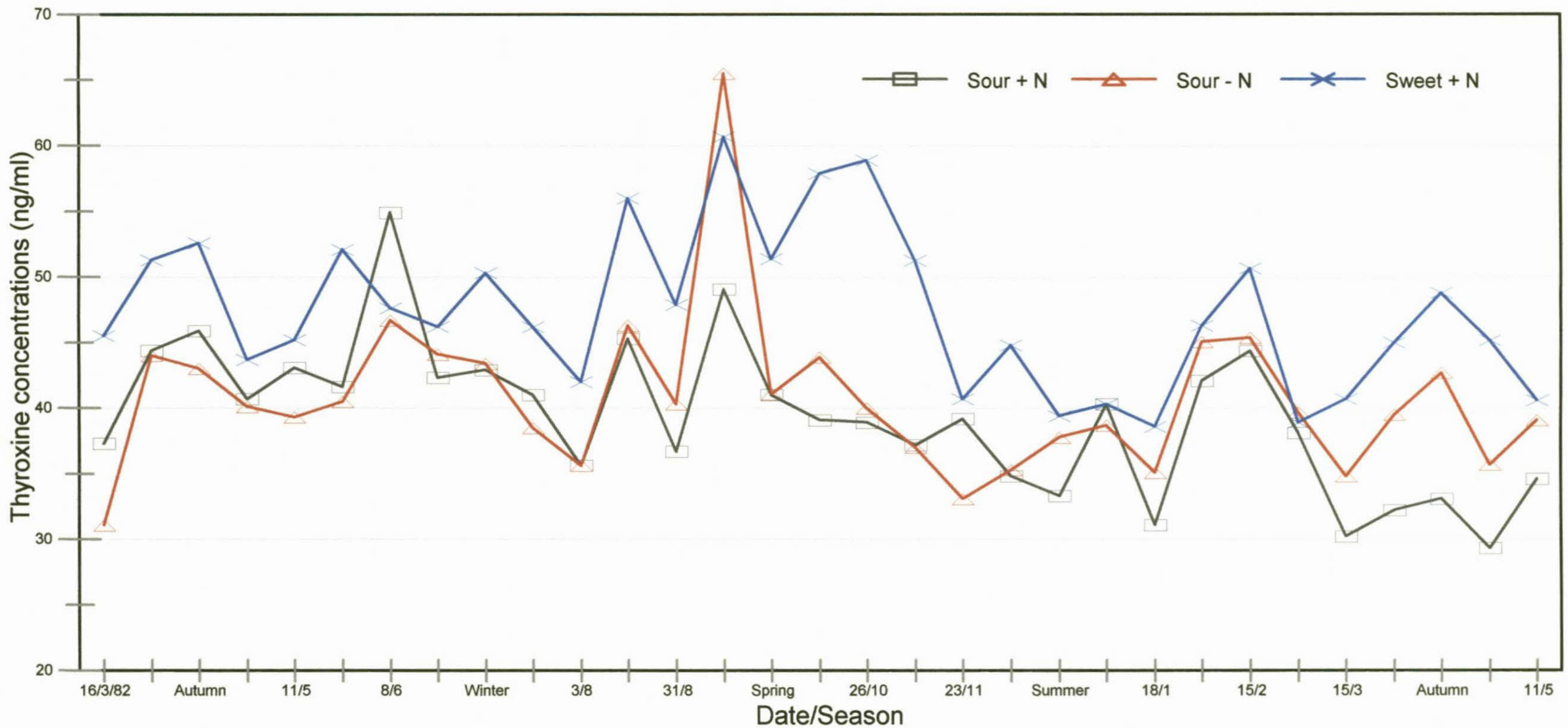


Figure 2.10 Mean plasma thyroxine profiles (ng/ml) of second-calf cows on the sourveld with NPN, sourveld without NPN and mixed sweetveld with NPN during 1982/83

Table 2.2 Influence of NPN supplementation on mean plasma cortisol and thyroxine concentrations in Bonsmara females

Hormone concentrations	Treatments			
	1981/82		1982/83	
	+ N (mean ± SE)	- N (mean ± SE)	+ N (mean ± SE)	- N (mean ± SE)
a) Inclusion of mixed sweetveld analysis:				
Cortisol concentrations (ng/ml)	9.31 [a] ± 0.26	7.32 [b] ± 0.37	6.59 ± 0.17	6.81 ± 0.24
Thyroxine concentrations (ng/ml)	44.60 [c] ± 0.63	40.80 [d] ± 0.87	42.90 ± 0.53	40.90 ± 0.75
b) Exclusion of mixed sweetveld analysis:				
Cortisol concentrations (ng/ml)	8.84 [e] ± 0.37	7.31 [f] ± 0.37	6.71 ± 0.24	6.82 ± 0.24
Thyroxine concentrations (ng/ml)	41.80 ± 0.85	40.80 ± 0.85	39.20 ± 0.71	40.8 ± 0.71

b < a ($P < 0.001$)

d < c ($P < 0.01$)

f < e ($P < 0.01$)

2.3.4 Correlation coefficients between plasma hormones, and blood and plasma metabolites

The relationships between plasma cortisol and thyroxine levels, and blood and plasma metabolites, are presented in Table 2.3.

Much variation was recorded between years. Plasma cortisol concentrations did not show a consistently strong relationship with any metabolite during both years. Plasma thyroxine levels showed a consistent and significant relationship with plasma glucose, plasma urea and body weight (Table 2.3). Correlation coefficients between plasma cortisol and T4 were weak: 0.16 during 1981/82, and 0.03 during 1982/83.

2.4 DISCUSSION

Except for the 1982/83 season, plasma cortisol concentrations of first-calf cows on the higher feeding regime (mixed sweetveld + N) were elevated when compared to those of females on the lower nutritive plane (sourveld + N) during 1980 and 1981/82 ($P < 0.01$).

It appears that plasma cortisol concentrations of mixed sweetveld females were not influenced by the nutritional status during winter of the natural pasture, as was the case with the sourveld group. However, as spring progressed, cortisol concentrations of the latter animals increased. It thus appears that adrenal cortex activity of sourveld females is influenced to a large degree by nutritional status of the pasture.

The literature reveals inconsistency regarding the influence of nutrition on glucocorticoid secretion. Mills and Jenny (1979) reported that dairy heifers on diets high in concentrates had a total glucocorticoid concentration of 14.8 ng/ml, compared to 7.9 ng/ml of control heifers, 12 hours after feeding. More recently, it was observed that when the digestible crude protein and total dietary energy ration of 1:4.5 - 5 were increased to 1:81 or 1:28, blood cortisol levels increased (Vinkler *et al.*, 1996). Undernutrition (reduction of dietary energy), on the other hand, led to a suppression of cortisol levels in beef cows (Easdon & Chesworth, 1980). Similarly, other researchers found a positive relationship between level of nutrition and glucocorticoid concentration (Sejrsen *et al.*, 1983). An increase in plasma corticoids in lambs was suggested to have been the result of anticipation of feeding (Holley *et al.*, 1975).

Table 2.3 **Correlation coefficients (r) between plasma cortisol and thyroxine concentrations, and various other blood and plasma metabolites**

Constituent	1981/82		1982/83	
	Cortisol	Thyroxine	Cortisol	Thyroxine
Blood glucose	0.0602	0.0258	0.0705	0.2637**
Plasma glucose	0.1867*	0.2133**	0.0524	0.2425**
RBC glucose	0.1752*	0.2450**	-0.0084	0.0747
Plasma protein	0.1059	0.0861	0.0866	0.1254**
Plasma albumin	0.1169	0.1395	0.0409	0.2552**
Plasma globulin	0.0413	-0.0070	0.0711	0.0630
Plasma urea	0.2121**	0.2960**	-0.0628	0.3136**
Body weight	0.2836**	0.4428**	0.0042	0.2691**

* P < 0.05

** P < 0.01

*** P < 0.001

Weights of adrenal glands in gravid sows fed on a high, medium and low plane of nutrition were 5.8, 4.42 and 3.11g respectively (Holness & Smith, 1974). Similar results were reported earlier where adrenal gland weights became progressively less as the plane of nutrition was reduced (Holness & Smith, 1970). However, plasma cortisol concentrations were not significantly affected by energy or fat intake in high yielding dairy cows (Blum *et al.*, 1985). In a study, McCann and Hansel (1986) found that fasting did not influence cortisol concentrations in heifers. Similar results, where nutrition did not affect adrenal function, were reported in cows (Hudson *et al.*, 1975; Breves *et al.*, 1980; Hall *et al.*, 1991), heifers (Kazmer *et al.*, 1983), sheep (Bassett, 1974; Oda & Sasaki, 1996) and weaner lambs (Holcombe *et al.*, 1992).

In contrast to the previous quotes, there is evidence that limiting feed intake of ruminants increases the plasma corticosteroids (Dewit, 1973). In weanling thoroughbred horses, serum cortisol concentrations were higher during fasting, and decreased after feeding (Glade *et al.*, 1984). In lactating cows, cortisol concentrations also decreased after feeding (Blum *et al.*, 1983). Trenkle and Topel (1978) found that glucocorticoid concentrations were highest in steers on restricted feeding.

According to Trenkle (1978), the influence of nutrition on glucocorticoids has neither been studied specifically, nor clarified. It has been shown that factors other than nutrition influence adrenal cortex activity. Diurnal patterns of plasma corticoid concentrations have been observed in cattle (MacAdam & Eberhart, 1972; Wagner & Oxenreider, 1972) and sheep (McNatty *et al.*, 1972; McNatty & Young, 1973). The influence of stress almost certainly altered glucocorticoid concentrations. In the past, attention has been given to the involvement of the pituitary-adrenal axis in stress adaptation of cattle (Whipp & Lyon, 1970; Heitzman *et al.*, 1970). When lambs were transferred from mixed feeding to grass pastures alone, animals showed anxiety and signs of the adaptation syndrome. Dar'enko (1979) found that adrenal weights increased by 132%, and hypertrophy of the adrenal cortex occurred. Plasma glucocorticoid concentrations of ruminants are also increased by many routine husbandry or laboratory procedures (Reid & Mills, 1962; Bassett & Hinks, 1969; Kilgour & De Langen, 1970; Willett & Erb, 1972).

Environmental temperature, and thus also season, is known to influence adrenal cortex activity (Thompson *et al.*, 1963; Christison *et al.*, 1970; Alvarez & Johnson, 1973; Lee *et al.*, 1976). It is thus clear that, although the adrenal gland is an important regulator of the metabolic processes, factors mentioned above also influence this endocrine gland to a large degree. One could postulate that if the influence of feeding is excessive, it may act on the endocrine system to override the diurnal patterns of cortisol secretion, and this effect may be detectable in the blood plasma. However, the question is to what degree will factors such as temperature, season and lactation influence the adrenal cortex, and override its metabolic function.

An aspect that should be researched further, is the duration of the stimulus or stimuli acting upon the adrenal gland to bring forth cortisol secretion. In a trial by Blum *et al.* (1983), 39 blood samples were taken from lactating cows over a period of 24 hours, at 15 to 120 minute intervals. These workers found the concentrations to decrease after feeding. On the other hand, cortisol concentrations increased in a very short time (within minutes) in calves following the rapid drinking of four pints of milk (Bloom *et al.*, 1975). Secretion of glucocorticoids could be increased twentyfold within minutes by physiological stress (Guyton, 1971). Lindsay (1978) postulated the possibility of nervous responses being related to the frequency of feeding. Present results were obtained from a long-term study involving the sampling of blood fortnightly over a period of 4.5 years. Although the effects were not always dramatic, plasma cortisol concentrations varied to some extent. An increase in levels was obtained in relation to the increased nutritional status of the natural pasture. The exception was, of course, results obtained during the 1982/83 season.

These results are in line with the general view that glucocorticoids stimulate gluconeogenesis. During the 1981/82 season, first-calf cows on the mixed sweetveld had significantly ($P < 0.01$) higher adrenal cortex activity than females in the 2 sourveld groups throughout most of the year (Figure 2.4). Increased plasma cortisol concentrations resulting from increased feeding regimes, enhanced stimulation of gluconeogenesis. This was probably accompanied by a reduction in glucose utilization rate and subsequently, increased blood glucose concentrations, as shown in Figure 1.5.

During 1982/83, increased nutritional status did not increase cortisol concentrations (Figure 2.5), and neither did it influence blood glucose levels to a large extent (Figure 1.8). It is postulated that other processes in the body, such as the increased influence of lactation and pregnancy, may override the homeostatic functioning of the adrenal gland on metabolism. It should be kept in mind that the sour grassveld has a relatively high protein content and is succulent during the early summer, especially during a normal rainfall season such as 1980. In the case of both heifers and cows during this year, not much difference was noted in cortisol concentrations in summer between heifers on the mixed sweetveld and sourveld. During the winter season, when the nutritive value of mixed sweetveld does not fall to the level of that of the sourveld, animals on the mixed sweetveld had higher cortisol concentrations, and one would expect metabolic processes not to be affected to the same degree as in sourveld females. However, it was previously mentioned that the mixed sweetveld is unable to maintain the body weight of the Bonsmara cows during the second reproductive year (1982/83). This was attributed to the increased nutritional requirements of and stress placed on cows during calving, lactation and rebreeding. It is, therefore, concluded that the higher nutritive value of the mixed sweetveld was not reflected by the adrenal cortex activity as a result of these processes. It is postulated that, because reproductive performance of the females in the 2 sourveld groups were lower, these processes did not have as great an influence on cortisol concentrations.

In conclusion, with regard to adrenal function, these results confirm the observations of Lindsay (1978) that, in obtaining profiles of blood metabolites or hormones for purposes such as in this study, it is not desirable to take blood samples within a short period before or after feeding. This may avoid the effects of anticipation by animals that they are going to be fed, and the resultant rise in plasma corticoids as experienced by Holly *et al.* (1975).

Present results indicate increased thyroid activity associated with improved feeding, and are mostly in accordance with results obtained by other researchers. Plasma thyroxine of Hereford steers rose with increased dietary protein, irrespective of the source of protein (Hammond *et al.*, 1984). In their study, Coggins and Field (1978) obtained thyroxine concentrations of 57.3, 68.5 and 83.7 ng/ml during pre-feeding of lactating beef cows on a low, medium and high plane of nutrition. After feeding, thyroxine concentrations of respectively 72.3, 75.2 and 83.8 ng/ml were recorded. Results obtained with young Kholmogor bulls indicated plasma thyroxine levels increasing after feeding (period 1). However, when the crude protein content was increased by 27.1% (period 2), levels of the hormone decreased (Komkova,1984). It should be noted, however, that before feeding, thyroxine levels were higher in period 2 compared to period 1. In a study, Blum *et al.* (1983) reported an increase in thyroxine concentration in lactating cows after concentrates were fed.

Where energy intakes matched requirements, T4 levels were lower, compared to cows given energy *ad lib.* (Kunz & Blum, 1981). Similar results were obtained in Hungary (Pethes *et al.*, 1985). Cows fed according to NRC recommendations for TDN, had thyroxine concentrations of 69 ng/ml 20 days before parturition, whilst those on a diet of 15 to 21% higher energy, had concentrations of 71.4 ng/ml. Fourteen days before parturition, levels were 38.0 and 52.6 ng/ml, and 20.5 and 26.9 ng/ml at parturition, respectively. It is clear that pregnancy and parturition influenced thyroid activity, but the differences between groups resulting from feeding were still significant. Elhassan (1985) observed that T4 levels decreased slightly during energy restrictions in both fertile and infertile heifers. In calves, it was found that plasma thyroxine decreased on day 1 of fasting, whilst in 50% fed calves, there was a significant ($P < 0.05$) increase in levels on day 3 of fasting (Ergun & Ozsar, 1988). Similar results, where fasting or feed restrictions led to decreased T4 concentrations, were observed in Holstein-Friesian breeding bulls (Janan *et al.*, 1995) and beef heifers (Yambayamba *et al.*, 1996).

In lactating dairy cows, results indicate that an energy deficiency may provoke a decrease in thyroid secretion (Bertoni *et al.*, 1983). Lundgren and Johnson (1964) observed that limiting feed intake resulted in lower clearance rates of (^{131}I) thyroxine from plasma.

In gravid pigs, the weights of thyroid glands were 4.83, 4.72 and 3.05 g respectively for animals on a high, medium and low plane of nutrition (Holness & Smith, 1974). This is in accordance with earlier results, where it was found that the weight of the thyroid glands were progressively reduced, as the plane of nutrition was decreased (Holness & Smith, 1970).

In trials with grazing lambs, Godden and Weeks (1981) found a steady decrease in thyroxine concentrations from 2 to 12 hours after feeding. Where the level of feed intake in beef steers kept outdoors was increased to twice maintenance, plasma T4 concentrations were reduced (Christopherson *et al.*, 1979). The same researchers found the level of feed intake to have no effect on plasma T4 concentrations for steers kept in a heated barn. In studies with crossbred Chianina crossbred steers, Hayden *et al.* (1993) also found decreased T4 concentrations during refeeding. When comparing 3 diets, animals on the protein deficient diet produced the highest values for plasma thyroxine (Muller, 1973). McNiven (1984) reported that feeding did not influence serum thyroxine concentrations of sheep, but free T4 levels were significantly lower for both thin and fat sheep at fasting. It appears that dietary fibre content had little influence on plasma thyroxine secretions (Magdub *et al.*, 1981). Nutritional differences also did not affect plasma thyroxine levels in buffalo heifers (Sengar *et al.*, 1995) and sheep (Oda & Sasaki, 1996).

It is known that several physiological and environmental factors influence thyroid function. Of interest are the results of Christopherson *et al.* (1979) who, as previously mentioned, found plasma concentrations of T4 to be higher in beef steers kept outdoors compared to those kept indoors. Housing altered the influence of feed intake in relation to thyroid activity. In a trial where the influence of penning on thyroid activity was studied, thyroxine concentrations increased by 214 and 196% in Afrikaner *(Sanga)* and Bonsmara (intermediate) breeds when moved from free-ranging to pen conditions (Erasmus & Krause, 1983). It has thus been established that a seemingly unimportant aspect such as type of housing can provoke a massive outpouring of thyroid hormone. It is postulated that this, and other factors, may override the influence of long-term feeding of free-grazing animals.

Other factors that influence thyroid activity are breed (Blincoe, 1958; Howes, 1964; Cowley *et al.*, 1971), season of the year (Lodge *et al.*, 1957; Premachandra *et al.*, 1958), pregnancy (Fisher *et al.*, 1978) and lactation (Vanjonack & Johnson, 1975). It is known that environmental temperature also interacts with nutritional status to influence the thyroid gland. It was found that in pigs, both a cold environment and high energy intake increased the rate of degradation of thyroxine (Macari *et al.*, 1983). In a study on the influence of energy intake and environmental temperature on the thyroid gland, Dauncey *et al.* (1984) reported height and area of the epithelium surrounding the thyroid follicles to be significantly increased by a high energy intake. It was postulated that energy intake appears to have a greater influence on thyroid histology than environmental temperature. Present results appear to verify this finding.

As was the case with cortisol, any of the above-mentioned factors or combinations thereof, may alter thyroid function. This is also probably the reason why inconsistent results were obtained regarding the influence of feeding on thyroxine concentrations. In the present study, plasma thyroxine levels of experimental animals on the mixed sweetveld were not consistently higher than those on the sourveld (Figure 2.7). In the case of cows, thyroxine levels were constantly higher in animals under sourveld conditions (Figure 2.8). Two factors that may have influenced these responses, are age, and the influence of lactation. During the 1981/82 season and the subsequent season, the enhanced thyroxine concentrations of first-calvers on the mixed sweetveld is evident (Figures 2.9 and 2.10). It appears that during this period, neither physiological processes such as pregnancy and lactation, nor environmental factors, had a major influence over and above that of feeding level.

According to Guyton (1971), thyroxine has 2 specific effects on carbohydrate metabolism. In the first instance, it increases the rate of glucose absorption. Secondly, it increases the rate of glucose utilization by the cells. In protein metabolism, thyroxine concentration increases the rate of both protein anabolism and catabolism (Turner & Bagnara, 1971). It is difficult to determine whether the increased thyroid activity of females on the mixed sweetveld is due to an increased energy or protein intake. During 1981/82, both thyroxine and blood metabolite concentrations were elevated in first-calf cows on the high feeding regime. During 1982/83, however, thyroxine levels generally remained elevated, but blood metabolite concentrations were lower when compared to the sourveld - N group. It is postulated that, because of the increased feeding requirements of the animals during this year due to pregnancy and lactation, thyroxine concentrations led to oxidation of carbohydrates and protein. In their study, Pethes *et al.* (1985) explained the low T4 levels in the lower energy intake group by the sensitive mechanism of peripheral thyroid hormone metabolism. Considerable evidence exists that thyroid hormones can be altered by fasting without alternation of the basal TSH or thyrotropin-releasing hormone response (Chopra *et al.*, 1975; Burman *et al.*, 1980).

The higher plasma cortisol and thyroxine concentrations could largely be attributed to the influence of the higher nutritive level of the mixed sweetveld, and not to the influence of NPN supplementation (Table 2.2). When the animals in this group were excluded in the statistical analysis, it appeared that NPN supplementation led to a slight increase in adrenal cortex and thyroid activity. This tendency was not observed during a subsequent year, and it is concluded that NPN supplementation does not have a major influence on these glands. No evidence to refute this aspect could be found in the literature. In diets based on either soya bean meal or urea as protein sources, or a protein deficient diet, it was observed that the latter gave highest plasma thyroxine concentration (Muller, 1973).

Results contained in Table 2.3 reveal plasma cortisol concentrations to have no consistent and strong relationship with various blood and plasma constituents studied. Breves *et al.* (1980) found a positive and significant correlation of r = 0.177 between blood glucose and cortisol concentrations in pregnant dairy cows.

The relatively strong relationship between cortisol concentrations and body weight during the first year of the present study was not repeated during 1982/83. Whilst some workers found a high correlation between body weight and weight of the adrenals (Curl *et al.*, 1968), others found the correlations to be negligible (Arave *et al.*, 1977). In Shorthorn, Afrikaner and Bonsmara heifers, a positive relationship was generally found during the winter, and negative relationship during summer on sourveld (Erasmus, 1978).

Plasma thyroxine concentration showed a stronger and more consistent correlation with metabolites, notably with plasma glucose and urea. Because the thyroid gland is recognized as a regulator of metabolic processes, it is thought that some relationship between its concentration and growth rate might be anticipated (Post, 1963). The correlation coefficients of r = 0.44 and r = 0.27 obtained in the present study are not as high as the r = 0.81 reported for growing lambs (Singh *et al.*, 1956). In beef cattle, Curl *et al.* (1968) found the correlation coefficient between body weight and thyroid weight of beef cattle to be r = 0.921, while Van der Westhuysen (1973) reported that elevated thyroid activity was associated with improved growth in beef cattle. However, the weak relationships between growth and thyroid function found by Luitingh (1962), Erasmus (1978) and others indicate that considerable variation exists, and that this hormone is probably of little use for predicting productive capacities of animals.

The consistent and significant ($P < 0.01$) correlation coefficients between plasma glucose and thyroxine could not substantiate results by McNiven (1984), who recorded coefficients of $r = -0.166$ for thin sheep, and $r = 0.193$ for fat sheep.

2.5 CONCLUSIONS

It appears that in first-calf cows, a higher feeding regime leads to an increase in adrenal cortex activity. However, as no significant difference in hormone levels were noted following this period, this phenomenon is ascribed to the incidence of increased nutritional requirements relating to processes such as calving, lactation and rebreeding. In addition, as there was no significant elevation of cortisol concentrations in the sourveld groups of females, it is concluded that these animals were not under stress due to undernutrition.

Regarding the influence of sourveld and mixed sweetveld on thyroxine secretions, the small difference noted between the 2 groups during 1980 is attributed to the contribution made by the cows. However, when the data are analysed separately, it was found that thyroxine concentrations were 55.5 and 57.7 ng/ml for heifers on the sourveld and mixed sweetveld, respectively. Although this difference was not great, it is in accordance with the results obtained during the subsequent years. Thus, it would seem that the activity of the thyroid gland is a more sensitive barometer of nutritional status of the natural pasture than cortisol concentrations. Unlike the latter levels, thyroxine values in females on the mixed sweetveld were significantly ($P < 0.001$) higher than those of females on the sourveld during 1981/82 and 1982/83. This indicates that thyroxine profiles are strongly related to the metabolic processes of the body and, therefore, apparently override processes such as pregnancy, calving and lactation which may also interact with the activity of the thyroid gland. It is thus not surprising that plasma thyroxine concentrations had a consistent and significantly ($P < 0.01$) positive relationship with live weight of the experimental animals. However, this relationship with live weight, as was the case with cortisol concentrations, was not strong. It is concluded that these hormones are apparently not of any use in the prediction of the productive capacities of beef females. It appears that non-protein nitrogen supplementation does not alter adrenal cortex and thyroid activity.

2.6 SUMMARY

First-calf cows on the sourveld had significantly ($P < 0.01$) lower cortisol concentrations than their counterparts on mixed sweetveld during the 1980 period (11.94 vs. 13.02 ng/ml for the females on the sourveld + N and mixed sweetveld + N respectively), and 1981/82 period (8.78 and 7.32 vs. 9.76 ng/ml for females on the sourveld + N, sourveld - N and mixed sweetveld + N respectively). During the subsequent year, virtually no differences in cortisol levels were observed (6.70 and 6.80 vs. 6.50 ng/ml for animals in the sourveld + N, sourveld - N and mixed sweetveld + N groups respectively).

Regarding plasma thyroxine concentrations, no significant difference in hormone levels was noted between females on the sourveld and mixed sweetveld during the 1980 period (53.50 vs. 52.40 ng/ml for females on the sourveld + N and mixed sweetveld + N respectively). During the following 2 seasons, T4 levels were significantly ($P < 0.001$) elevated in the experimental animals on the mixed sweetveld (41.80 and 40.80 vs. 48.10 ng/ml during 1981/82, and 39.10 and 40.80 vs. 46.60 ng/ml during 1982/83 for females in the sourveld + N, sourveld - N and mixed sweetveld + N groups respectively). It is concluded that nutritional status of the natural pasture significantly alters the thyroid activity in Bonsmara females, and is therefore strongly related to metabolic processes of the body. It appears that NPN supplementation to beef females on the sourveld does not influence plasma cortisol and thyroxine secretions to a large degree. Plasma glucose ($r = 0.21$ [$P < 0.01$] and $r = 0.24$ [$P < 0.01$]), plasma urea ($r = 0.21$ [$P < 0.01$] and $r = 0.31$ [$P < 0.01$]) and body weight ($r = 0.28$ [$P < 0.01$] and $r = 0.27$ [$P < 0.01$]) showed positive and significant correlation coefficients with plasma T4 concentrations during both 1981/82 and 1982/83 seasons.

It is concluded that the activity of the thyroid gland is a more sensitive barometer of nutritional status of the natural pasture than cortisol concentrations. As there was no significant elevation of cortisol concentrations in the sourveld group of females, it is concluded that these animals were not under stress due to undernutrition. Thyroxine and cortisol concentrations are apparently not of any use in the prediction of the productive capacities of beef females.

CHAPTER 3

INFLUENCE OF PREGNANCY, LACTATION AND REBREEDING ON METABOLIC PROFILES, ADRENAL CORTEX AND THYROID ACTIVITY OF BONSMARA FEMALES ON DOHNE SOURVELD AND MIXED SWEETVELD

3.1 INTRODUCTION

The ruminant synthesizes most of its required glucose, as little or no glucose is absorbed from the gastrointestinal tract (Reid, 1968). According to Prior and Christenson (1978), glucose is quantitatively less important in the metabolism of ruminants than in non-ruminants, but the significance of gluconeogenesis and glucose metabolism increases during pregnancy. An increase in gluconeogenesis in ruminants during pregnancy was established earlier (Bergman, 1963; Ford, 1963; Steel & Leng, 1968). It is well-known that the nutritional requirements of the dam increases as pregnancy progresses. It is, however, only in the last third of pregnancy (from the 6th month onwards in cattle) that special provision in the diet has to be made for the growth of the foetus (McDonald *et.al.*, 1973).

The foetal demand for energy in the pregnant ewe is supplied by maternal blood glucose, derived mainly from propionate and amino acids from ingested nutrients (Russel *et. al.*, 1967). According to Prior and Christenson (1978), glucose appears to be a primary source of energy for the foetus. As pregnancy progresses, the nutrient requirements of the foetus for glucose increases. In the case of undernourishment, when the level of nutrient intake does not meet the nutrient requirements, normal blood glucose levels cannot be maintained. The result is that body fat reserves are mobilized. Russel *et al.* (1967) stressed that undernourishment can and does occur as a result of increasing foetal demand for glucose. Further research indicated that the uterus and its contents in the pregnant ewe, utilize most of the maternal glucose (Reid, 1968). It has been estimated that total glucose uptake by the uterus and its contents accounts for approximately 70% of the glucose metabolism in the pregnant ewe (Setchell *et al.*, 1972).

It is not only energy that is of importance during pregnancy. According to Guyton (1971), the supplementary food needed for the foetus and foetal membranes includes dietary quantities of various minerals, vitamins and proteins. Lactation is also known to influence blood glucose concentrations in cattle (Shaw, 1943), sheep (Bergman & Hogue, 1967) and goats (Annison & Linzell, 1964). The importance of this process is indicated by Bines and Hart (1978); the cow partitions dietary energy between the production of milk and the maintenance of body tissues.

It has been established that proteins are also influenced by pregnancy, lactation and fertility. A negative relationship has been reported between serum albumin concentrations and the number of services required for conception (Rowlands *et al.*, 1977). Several researchers noted decreased serum albumin concentrations around the time of calving, and following parturition (Little, 1974; Szulc, 1975). More recently, it was established that ewes lambing with a single lamb 9 months earlier, had lower serum concentrations of glucose and albumin than ewes that had not lambed (Woollians *et al.*, 1984). During pregnancy, secretion of many hormones are increased, including thyroxine and adrenocortical hormones. Hormones are the primary physiological factors that stimulate mammary growth and initiate and maintain lactation (Tucker, 1981). Milking and suckling influence the adrenal physiology in lactating cows, causing rapid increases in corticoid concentrations (Wagner, 1969). However, conflicting results have also been reported. Small doses of glucocorticoids did not affect milk yield (Head *et al.*, 1976), while in another trial, milk yield was increased (Swanson & Lind, 1976).

In addition to environmental factors, the physiological status such as pregnancy and milk production influences thyroid function, and is of greater importance than was recognised earlier (Pethes *et al.*, 1985). The importance of the thyroid gland during lactation was emphasized when its removal led to reduced milk yield (Graham, 1934). Since then more studies followed, showing the importance of thyroid hormone in relation to lactation. According to Tucker (1981), thyroid hormone may play an important role in the secretion of milk, postpartum.

Unlike dairy cattle, beef heifers and cows in South Africa are kept mainly on natural pasture throughout the year, supplemented with non-protein nitrogen in some regions during the winter. It is, therefore, expected that long-term nutritional stress, coupled with additional processes such as pregnancy and lactation, will have an adverse influence on reproductive efficiency. Because of the low nutritive value of the natural pasture during the winter and early spring, a temporary non-infectious infertility may be expected, especially during seasons of below-normal rainfall. It is well-known that animal production is handicapped during this period in the sourveld areas (Chapter 1). It was, therefore, attempted to measure blood and hormonal parameters during pregnancy and lactation in order to determine what additional influence pregnancy and lactation have on the metabolism of the animal. This study was thus aimed at determining whether the homeostatic mechanisms of beef cattle are able to regulate various blood metabolites during pregnancy and lactation.

In conclusion, it was also attempted to link metabolic and hormonal profiles to fertility. It was reported that cows with low plasma glucose concentrations were much less likely to conceive than those with a normal level (McClure, 1968). Blowey and Parker (1978) observed that Friesian cattle which conceived to first service, had higher plasma glucose concentrations than animals which did not conceive. However, this tendency could not always be substantiated (Blowey *et al.*, 1973).

3.2 EXPERIMENTAL PROCEDURES

Five Bonsmara first-calf cows were used in each of the following 3 experimental groups: Group 1, females were run on the Dohne Sourveld, supplemented with a bone meal and salt lick in the summer season, and an NPN lick during the winter season (sourveld + N); Group 2, females were run on the Dohne Sourveld, supplemented with bone meal and salt lick throughout the year (sourveld - N); Group 3, females were run on the mixed sweetveld, supplemented with a bone meal and salt lick in the summer, and an NPN lick in the winter (mixed sweetveld + N).

Blood samples (10 ml) were obtained via the jugular vein at fortnightly intervals (09:00) in heparin tubes, packed on ice, and immediately analysed for blood and plasma glucose, total plasma protein, plasma albumin and plasma urea concentrations. Aliquots of plasma samples from each experimental animal were stored at -20°C for the later determinations of cortisol and thyroxine concentrations. The methods used for the analyses of the mentioned blood and plasma metabolites and hormone concentrations, were previously described (Experimental Procedures, paragraph 8 Methods and Data Collection, pp 11 to 12).

3.3 RESULTS

3.3.1 Influence of pregnancy on energy metabolites

During the 1981/82 season, pregnancy had a significant ($P < 0.001$) influence on the mean blood glucose concentrations of all 3 groups of first-calf cows. Pregnant females had a mean concentration of 2.1 mmol/l, compared to 2.27 mmol/l of open females.

The influence of pregnancy on blood and plasma metabolites of the experimental animals on the 3 treatments during the first year, is set out in Table 3.1. Pregnancy did not have a consistent influence on blood and plasma glucose concentration of animals on sourveld and mixed sweetveld. However, there was a tendency for blood glucose concentrations to be lower in pregnant females in all 3 treatments (Figure 3.1). This trend was especially evident in the sourveld + N group. In the sourveld - N group, a short decline in blood glucose concentrations occurred in both pregnant and open first-calvers during the winter season. In the mixed sweetveld group of animals, glucose concentrations in pregnant and non-pregnant first-calf cows increased during this period, as the season progressed.

During the following year, pregnancy did not have a consistent influence on blood glucose concentrations (Table 3.2). This trend is reflected in Figure 3.2. In the mixed sweetveld group of animals, all the second-calf cows were pregnant, and could thus not be compared to the non-pregnant animals. The marked increase in blood glucose concentration, 10 to 12 weeks before parturition, occurred in pregnant and open females in all 3 treatments.

No significant differences in mean plasma glucose concentration were observed in all 3 groups of females during the first year (1981/82). Levels were 3.23 and 3.20 mmol/l for the pregnant and open first-calf cows, respectively. It is evident that pregnancy did not have a consistent effect on plasma glucose concentrations (Table 3.1). From Figure 3.3 it is evident that non-pregnant females had higher plasma glucose levels than pregnant animals, especially in the 2 sourveld groups. In the mixed sweetveld group, more variation existed between animals, and little influence of pregnancy could be found.

Table 3.1 Effect of pregnancy on blood and plasma metabolites (mean ± SE) during the 1981/82 season in Bonsmara first-calf cows maintained on 3 different feeding regimes

Constituent	Sourveld + N		Sourveld - N		Sweetveld + N	
	Pregnant ± SE	Open ± SE	Pregnant ± SE	Open ± SE	Pregnant ± SE	Open ± SE
Blood glucose (mmol/l)	2.06[a] ± 0.03	2.25[b] ± 0.04	2.16 ± 0.04	2.13 ± 0.04	2.27 ± 0.04	2.28 ± 0.05
Plasma glucose (mmol/l)	3.20 ± 0.06	3.25 ± 0.06	3.26[e] ± 0.06	2.98[f] ± 0.06	3.50[m] ± 0.05	3.21[n] ± 0.07
Red blood cell glucose (mmol/l)	1.13[c] ± 0.04	1.00[d] ± 0.04	1.10[g] ± 0.04	0.85[h] ± 0.04	1.23[o] ± 0.03	0.93[p] ± 0.05
Total plasma protein (g/l)	65 ± 0.92	68 ± 1.00	70[i] ± 0.81	66[j] ± 0.78	71 ± 0.55	72 ± 0.75
Plasma albumin (g/l)	32 ± 0.44	32 ± 0.48	33[k] ± 0.49	31[l] ± 0.47	34 ± 0.44	34 ± 0.06
Plasma globulin (g/l)	35 ± 0.75	35 ± 0.81	37 ± 0.91	36 ± 0.87	38 ± 0.62	38 ± 0.85
Plasma urea (mmol/l)	1.80 ± 0.18	1.70 ± 0.20	1.47 ± 0.12	1.25 ± 0.11	3.80 ± 0.17	3.76 ± 0.23

a < b, 0.01
d < c, 0.05
f < e, 0.01
g < h, 0.01
j < i, 0.05
l < k, 0.05
n < m, 0.01
p < o, 0.001

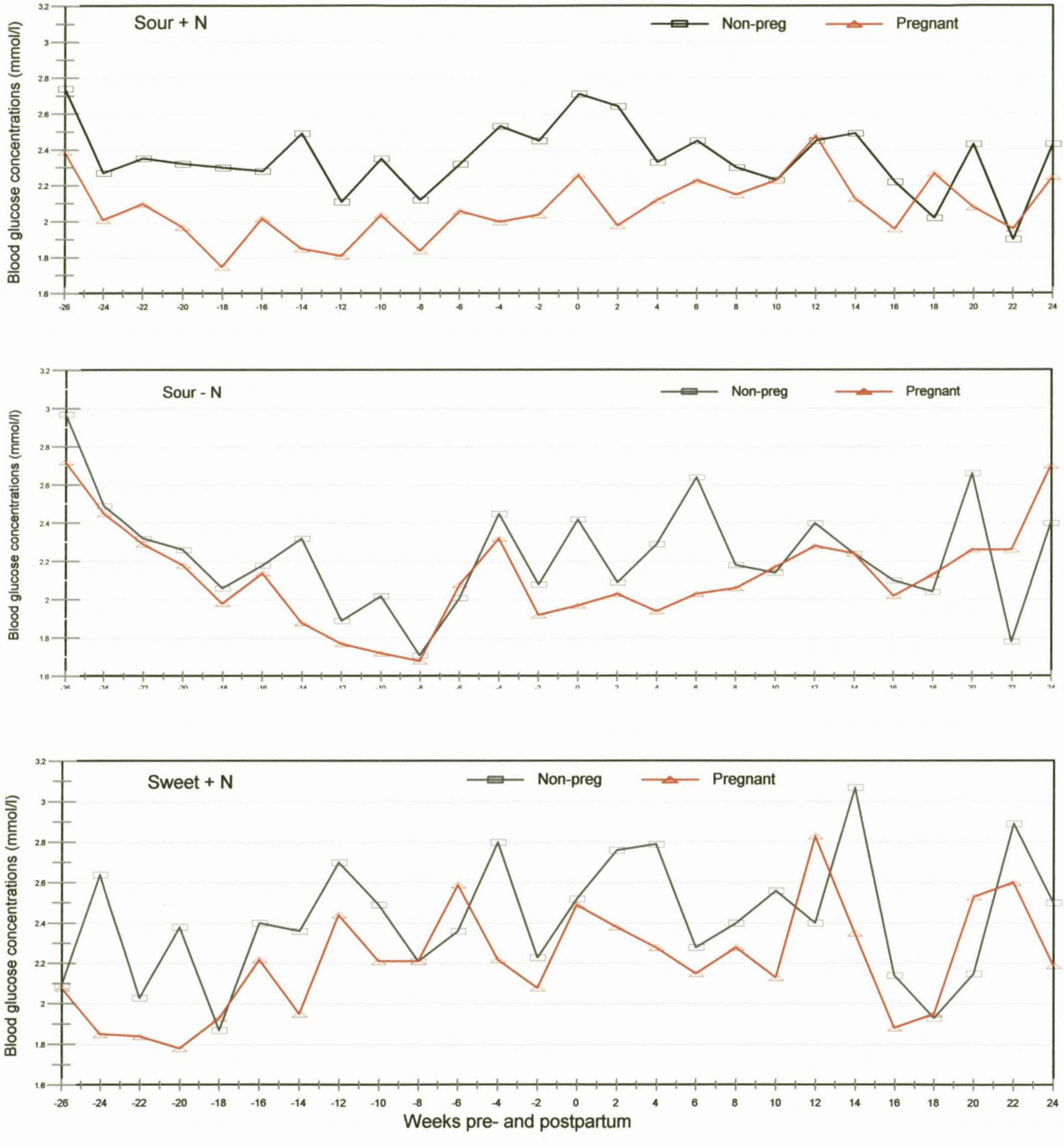


Figure 3.1 Mean blood glucose profiles (mmol/l) of first-calf cows in 3 treatment groups during gestation and lactation (1981/82)

Table 3.2 Effect of pregnancy on blood and plasma metabolites (mean ± SE) during the 1982/83 season in Bonsmara second-calf cows maintained on 3 different feeding regimes

Constituent	Sourveld + N		Sourveld - N		Sweetveld + N	
	Pregnant ± SE	Open ± SE	Pregnant ± SE	Open ± SE	Pregnant ± SE	Open ± SE
Blood glucose (mmol/l)	1.90[a] ± 0.03	2.16[b] ± 0.04	2.21 ± 0.04	2.18 ± 0.04	2.15 ± 0.04	-
Plasma glucose (mmol/l)	3.09[c] ± 0.05	3.26[d] ± 0.05	3.37 ± 0.06	3.25 ± 0.05	3.31 ± 0.05	-
Red blood cell glucose (mmol/l)	1.20 ± 0.04	1.10 ± 0.04	1.16 ± 0.04	1.08 ± 0.04	1.16 ± 0.04	-
Total plasma protein (g/l)	73 ± 1.20	74 ± 1.30	76 ± 1.40	75 ± 1.20	78 ± 1.30	-
Plasma albumin (g/l)	31[e] ± 0.40	29[f] ± 0.50	32[g] ± 0.40	30[h] ± 0.40	32 ± 0.30	-
Plasma globulin (g/l)	42 ± 1.20	44 ± 1.30	44 ± 1.30	45 ± 1.20	46 ± 1.30	-
Plasma urea (mmol/l)	1.83 ± 0.09	1.73 ± 0.10	1.57 ± 0.11	1.71 ± 0.09	3.35 ± 0.13	-

a < b, 0.001

c < d, 0.05

f < e, 0.05

h < g, 0.05

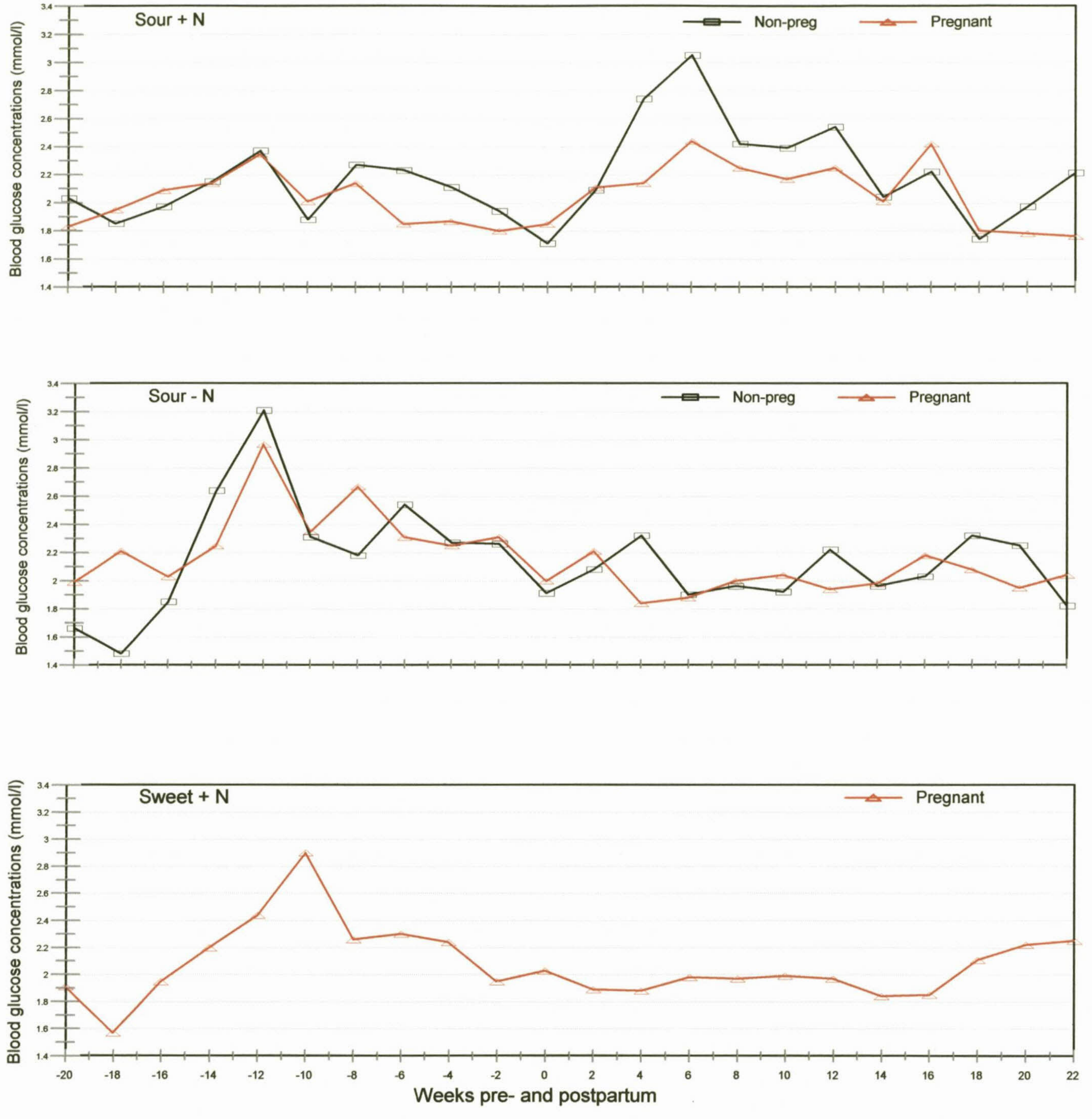


Figure 3.2 Mean blood glucose profiles (mmol/l) of second-calf cows in 3 treatment groups during gestation and lactation (1982/83)

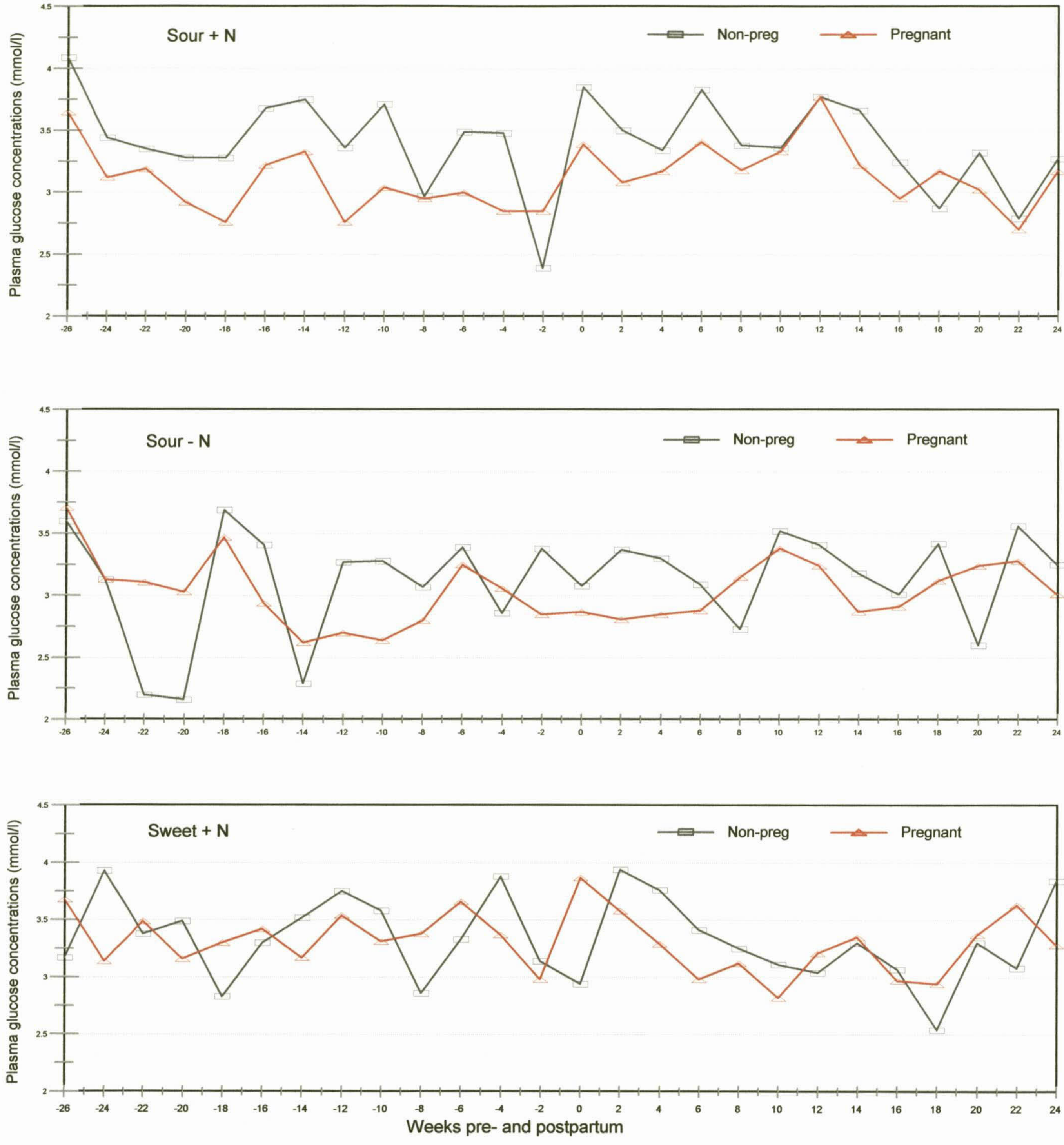


Figure 3.3 Mean plasma glucose profiles (mmol/l) of first-calf cows in 3 treatment groups during gestation and lactation (1981/82)

Results also varied during the 1982/83 season, with pregnancy not having a major influence on the plasma glucose levels (Table 3.2). Concentrations between pregnant and non-pregnant first-calf cows varied in a similar manner, indicating little influence of pregnancy on this parameter (Figure 3.4).

Pregnant females had significantly ($P < 0.001$) higher mean red blood cell (RBC) glucose concentrations than their non-pregnant counterparts (mean of 1.13 vs. 0.95 mmol/l) during 1981/82. When looking at the different treatment groups, the significant and consistent effect of pregnancy on RBC glucose levels is evident (Table 3.1). However, the significantly higher levels of pregnant animals are not reflected in Figure 3.5. The reason for this anomaly is that in the statistical analysis, data were computered from mating onwards, whilst data used to construct graphs, featured the last 28 weeks of pregnancy. Another factor which may have influenced the results, is the fact that after the cows had calved, they were coded as non-pregnant, although the process of lactation may have influenced the profiles. Nevertheless, it is clear from Figure 3.5 that pregnancy did not influence the RBC glucose concentrations to any large degree. This is confirmed by results obtained during the following season (1982/83), as indicated in Table 3.2. Very little variation in glucose levels was observed between pregnant and non-pregnant cows (Figure 3.6).

3.3.2 <u>Influence of pregnancy on protein metabolites</u>

Pregnancy did not have a significant influence on total plasma protein levels during 1981/82. Mean plasma protein concentrations were 68 and 69 g/l for pregnant and non-pregnant first-calvers, respectively. The difference between pregnant and non-pregnant females within treatment groups was small during 1981/82 (Table 3.1).

Of the 3 treatments, it would appear as if pregnancy influenced total plasma protein concentration of the animals on the mixed sweetveld, especially towards the end of pregnancy (Figure 3.7). The lower protein levels of pregnant first-calf cows are in accordance with the blood and plasma glucose concentrations.

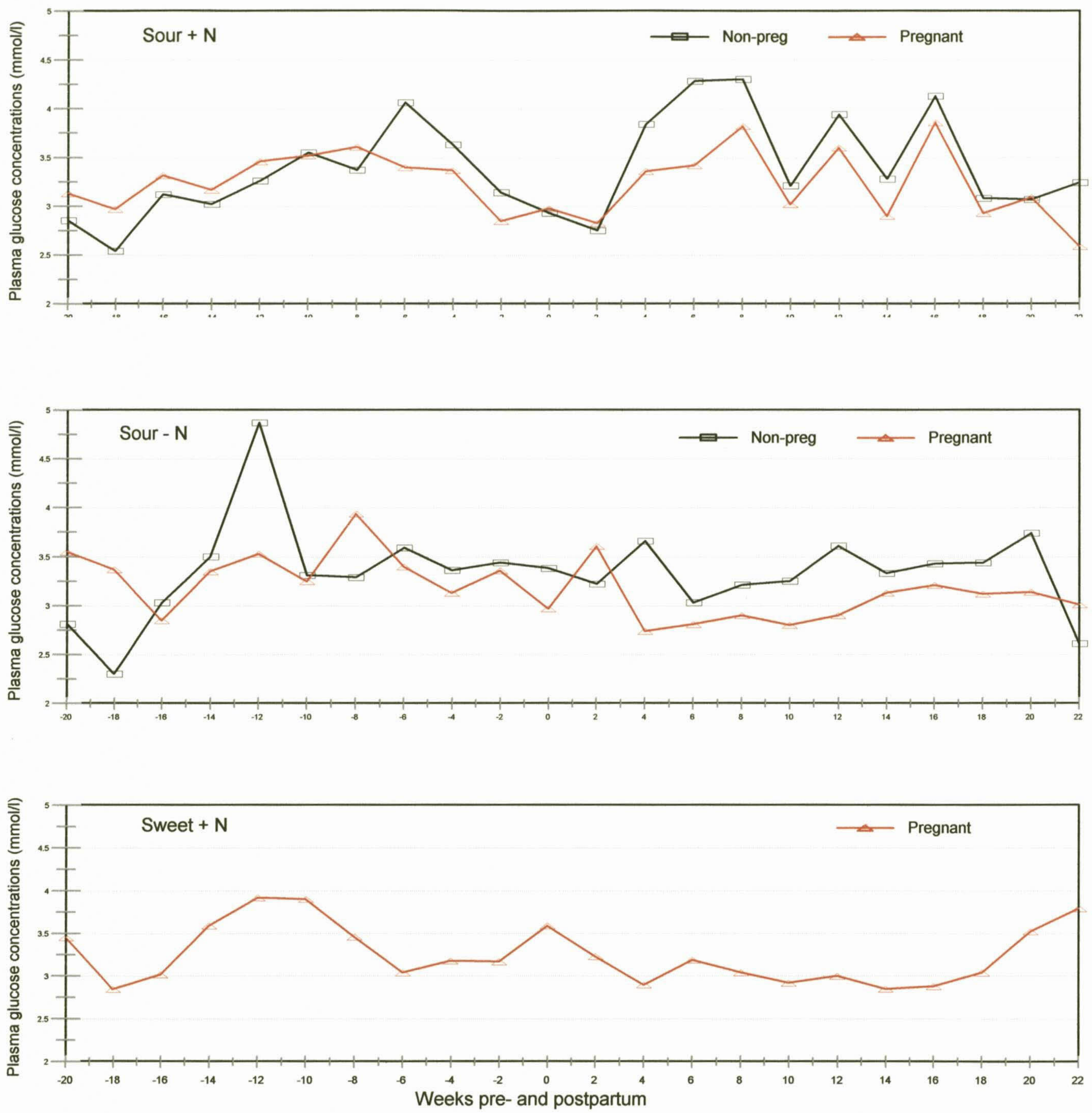


Figure 3.4 Mean plasma glucose profiles (mmol/l) of second-calf cows in 3 treatment groups during gestation and lactation (1982/83)

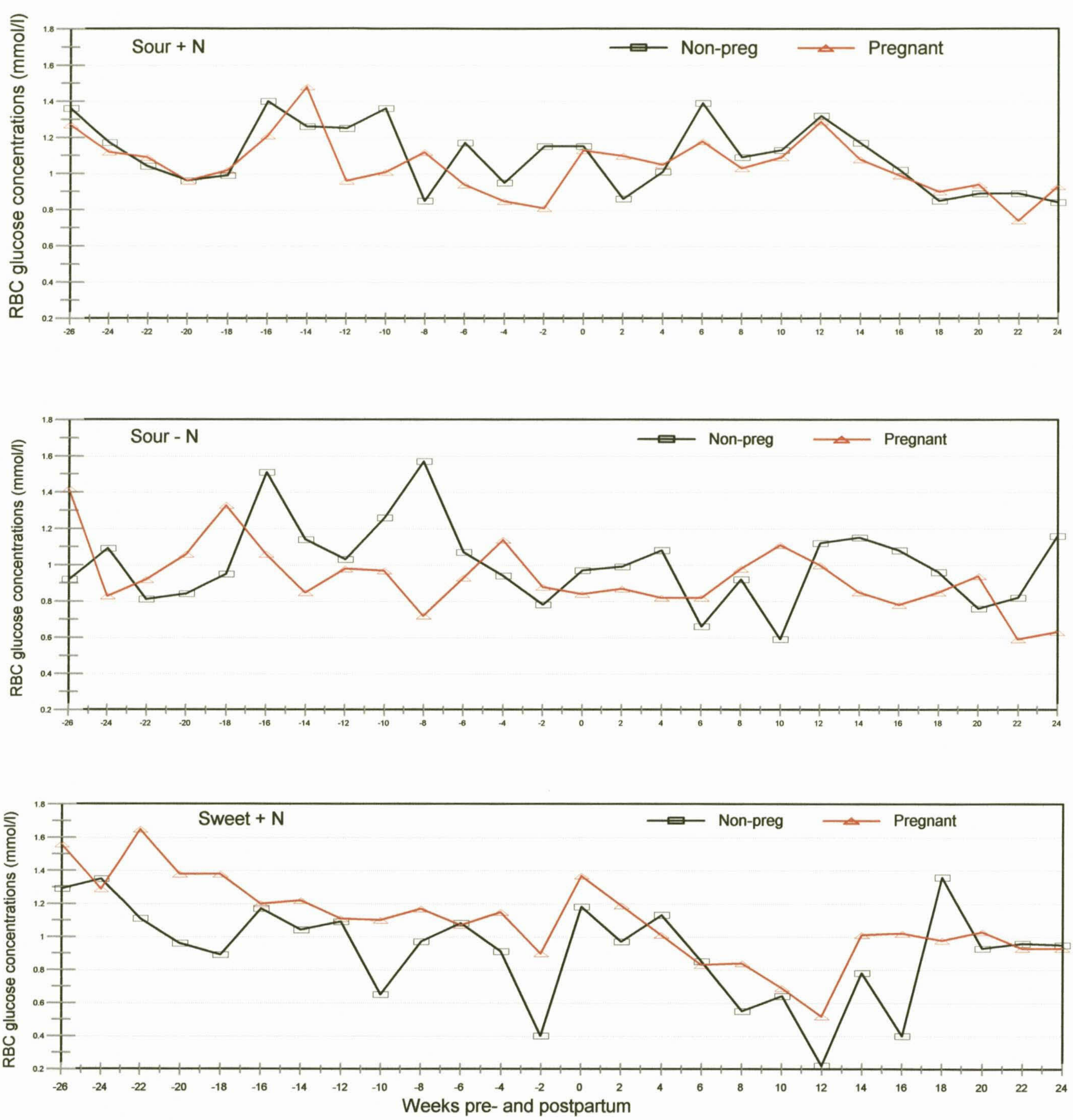


Figure 3.5 Mean red blood cell glucose profiles (mmol/l) of first-calf cows in 3 treatment groups during gestation and lactation (1981/82)

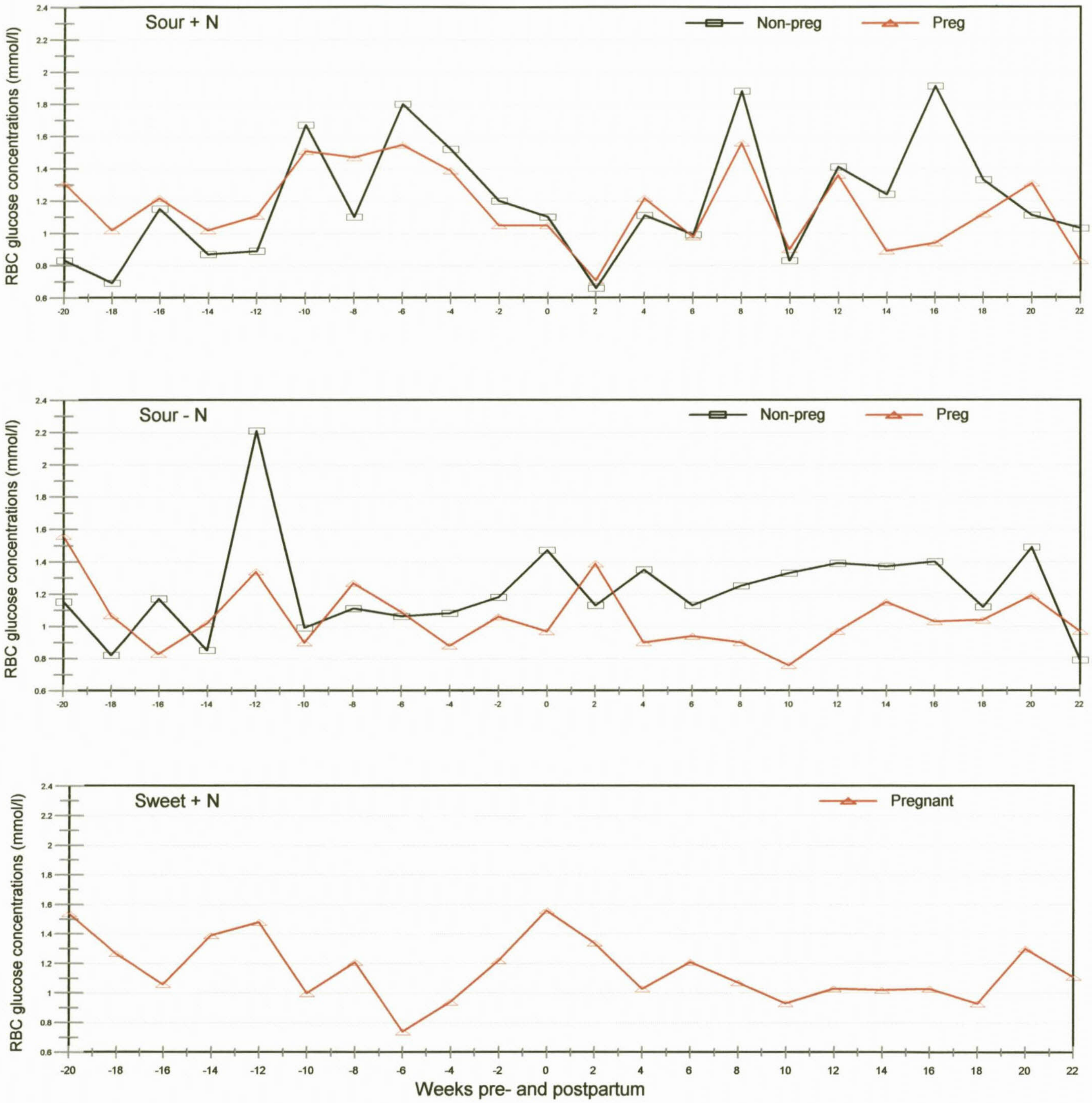


Figure 3.6 Mean red blood cell glucose profiles (mmol/l) of second-calf cows in 3 treatment groups during gestation and lactation (1982/83)

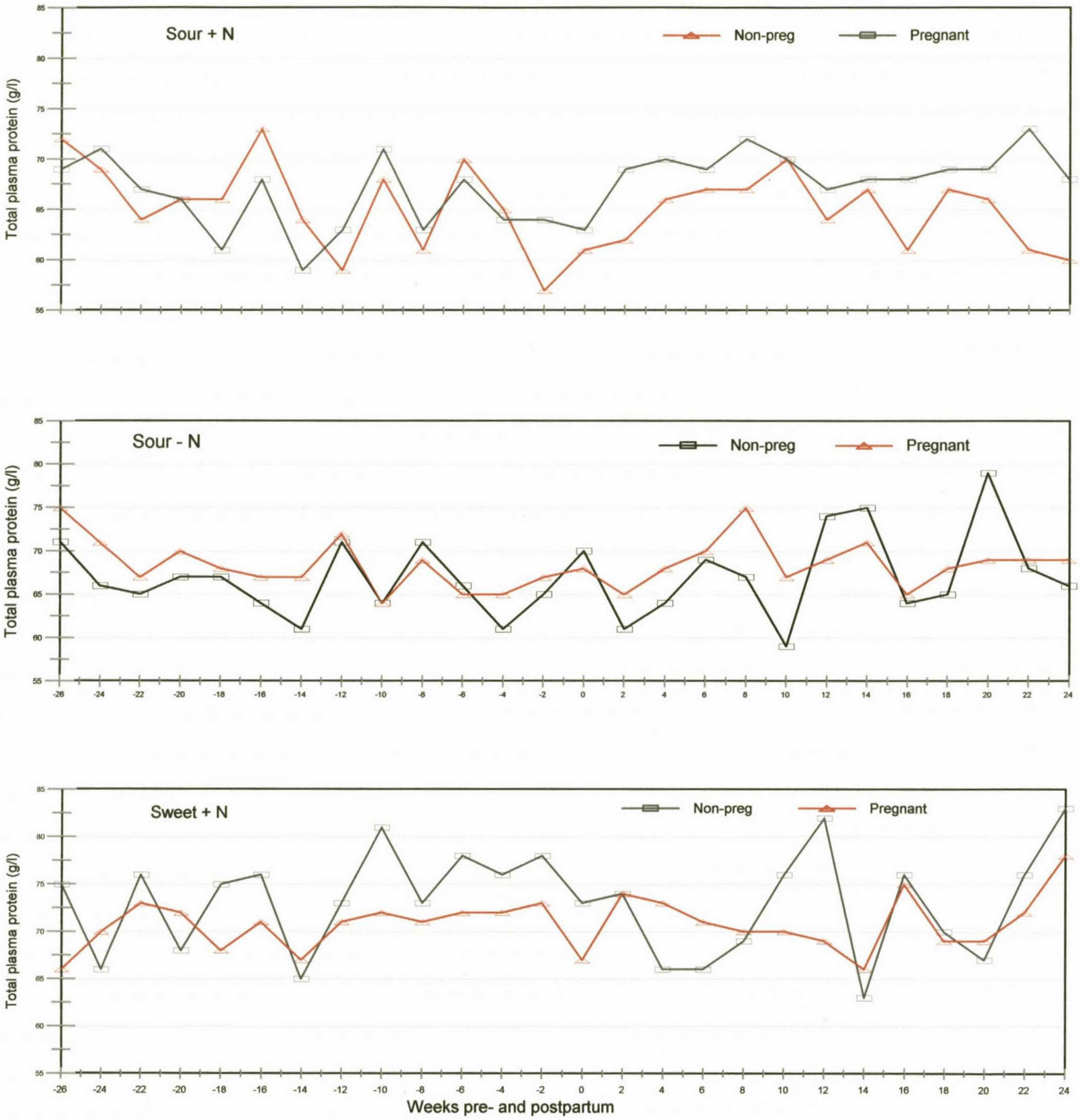


Figure 3.7 Mean total plasma protein profiles (g/l) of first-calf cows in 3 treatment groups during gestation and lactation (1981/83)

During the 1982/83 season, pregnancy also had little effect on plasma protein levels (Table 3.2). An interesting feature was the exact opposite way in which plasma protein levels of pregnant and non-pregnant cows reacted during the observation period (Figure 3.8). In the case of cows on the mixed sweetveld, total plasma protein concentrations decreased as pregnancy and lactation progressed.

Pregnancy had no significant effect on plasma albumin concentrations during the first year (1981/82). Concentrations for pregnant and non-pregnant first-calvers were 32.6 and 32.4 g/l, respectively. Similar results were obtained for both pregnant and non-pregnant animals within treatment groups (Table 3.1). Plasma albumin profiles, as depicted in Figure 3.9, revealed a decrease in concentration over time. Although plasma albumin concentrations were significantly ($P < 0.05$) higher in pregnant than open females during the 1982/83 season (Table 3.2), the effect of pregnancy was not marked, as results illustrate in Figure 3.10

No significant differences (mean 36.0 and 36.6 g/l) for plasma globulin levels were recorded in all 3 groups of pregnant and non-pregnant first-calf cows during 1981/82. There was also no significant difference in plasma globulin concentrations within groups (Table 3.1). The plasma globulin levels of pregnant and non-pregnant animals increased over time, indicating a stronger environmental influence on this metabolite (Figure 3.11). The influence of pregnancy was also not significant during 1982/83 (Table 3.2). However, there was a strong tendency for plasma globulin concentration to decrease over time in females on the sourveld - N and mixed sweetveld + N. Levels in animals on the sourveld + N treatment showed the opposite tendency (Figure 3.12).

As in the case of the other protein metabolites, differences in plasma urea concentrations between pregnant and non-pregnant first-calf cows were not significant (2.25 vs. 2.31 mmol/l). This is confirmed by separate statistical analysis for the 3 groups (Table 3.1). This parameter was largely influenced by feeding regime, with mixed sweetveld animals having the highest, and sourveld - N females having the lowest concentrations. The influence of pregnancy on plasma urea concentrations during 1981/82 is graphically illustrated in Figure 3.13. Similar results were ob-

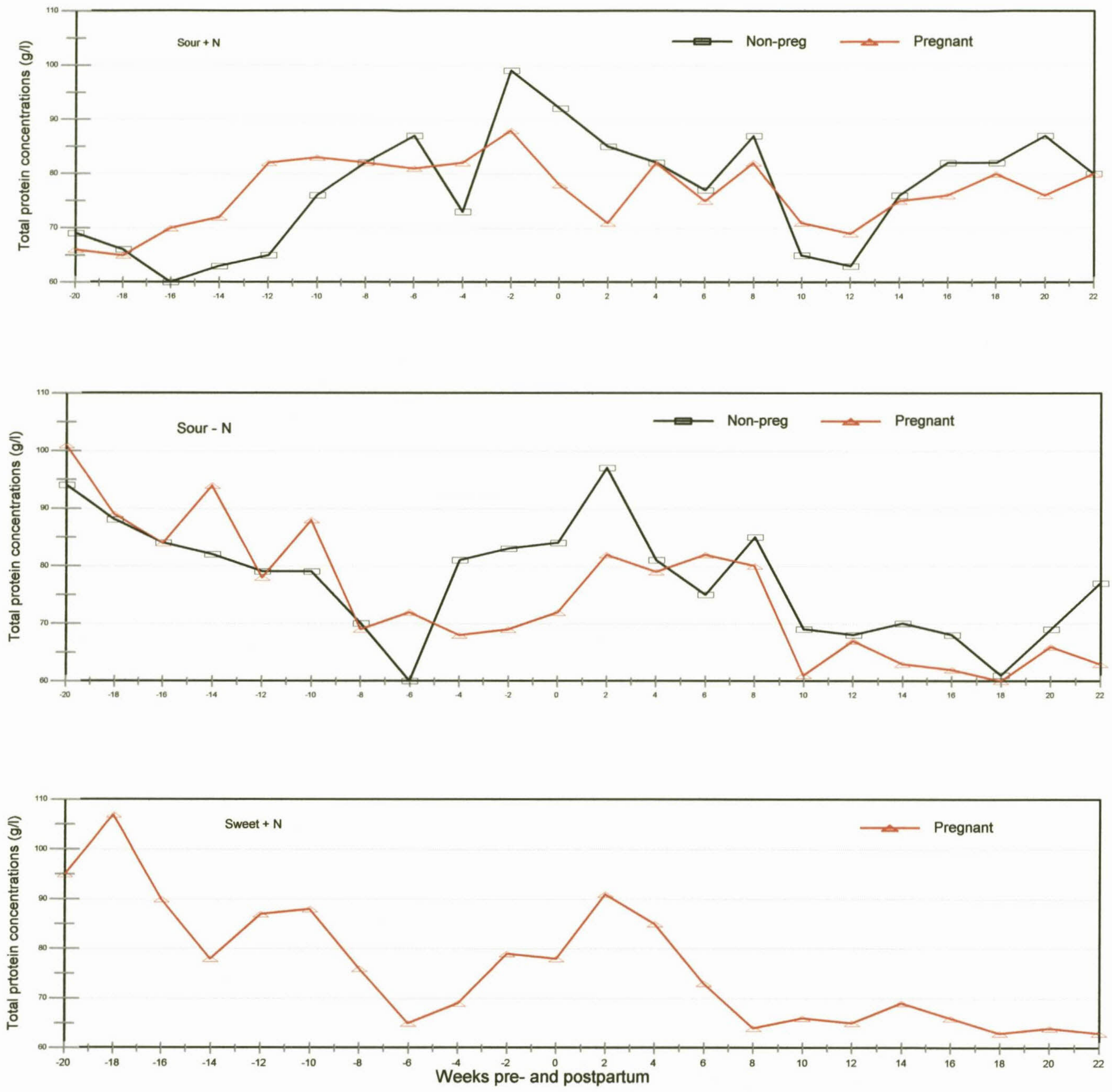


Figure 3.8 Mean total plasma protein profiles (g/l) of second-calf cows in 3 treatment groups during gestation and lactation (1982/83)

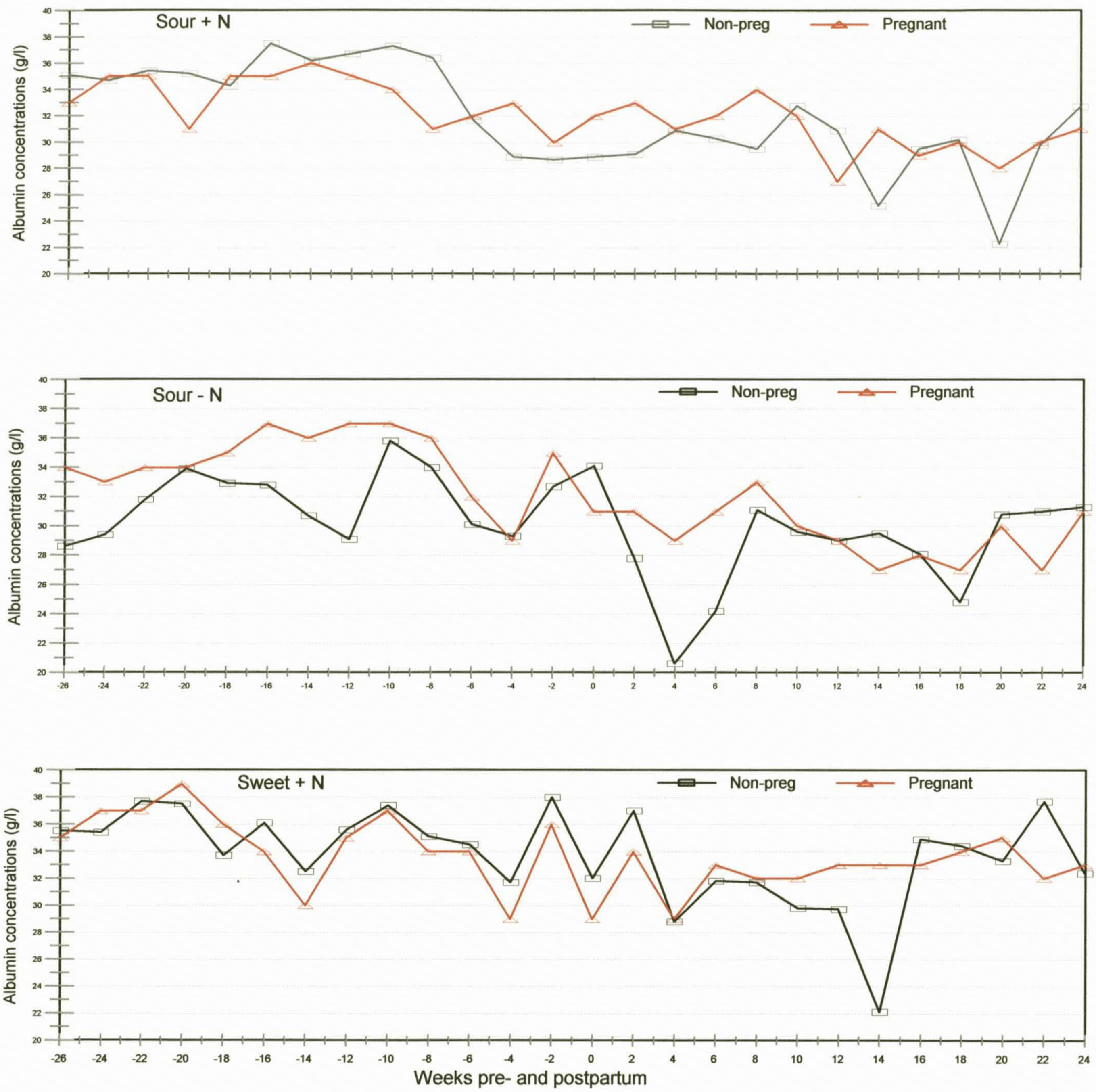


Figure 3.9 Mean plasma albumin profiles (g/l) of first-calf cows in 3 treatment groups during gestation and lactation (1981/82)

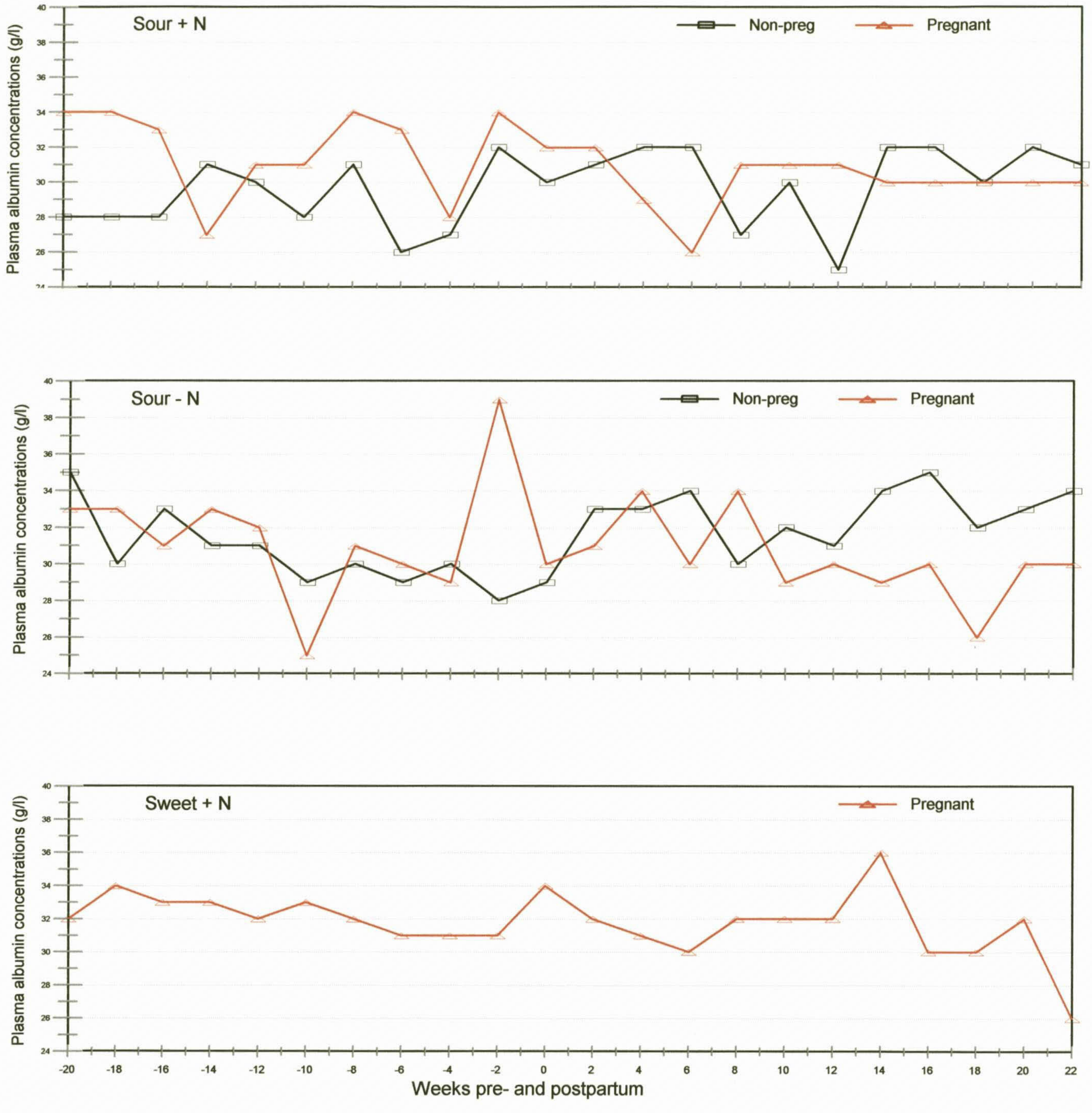


Figure 3.10 Mean plasma albumin profiles (g/l) of second-calf cows in 3 treatment groups during gestation and lactation (1982/83)

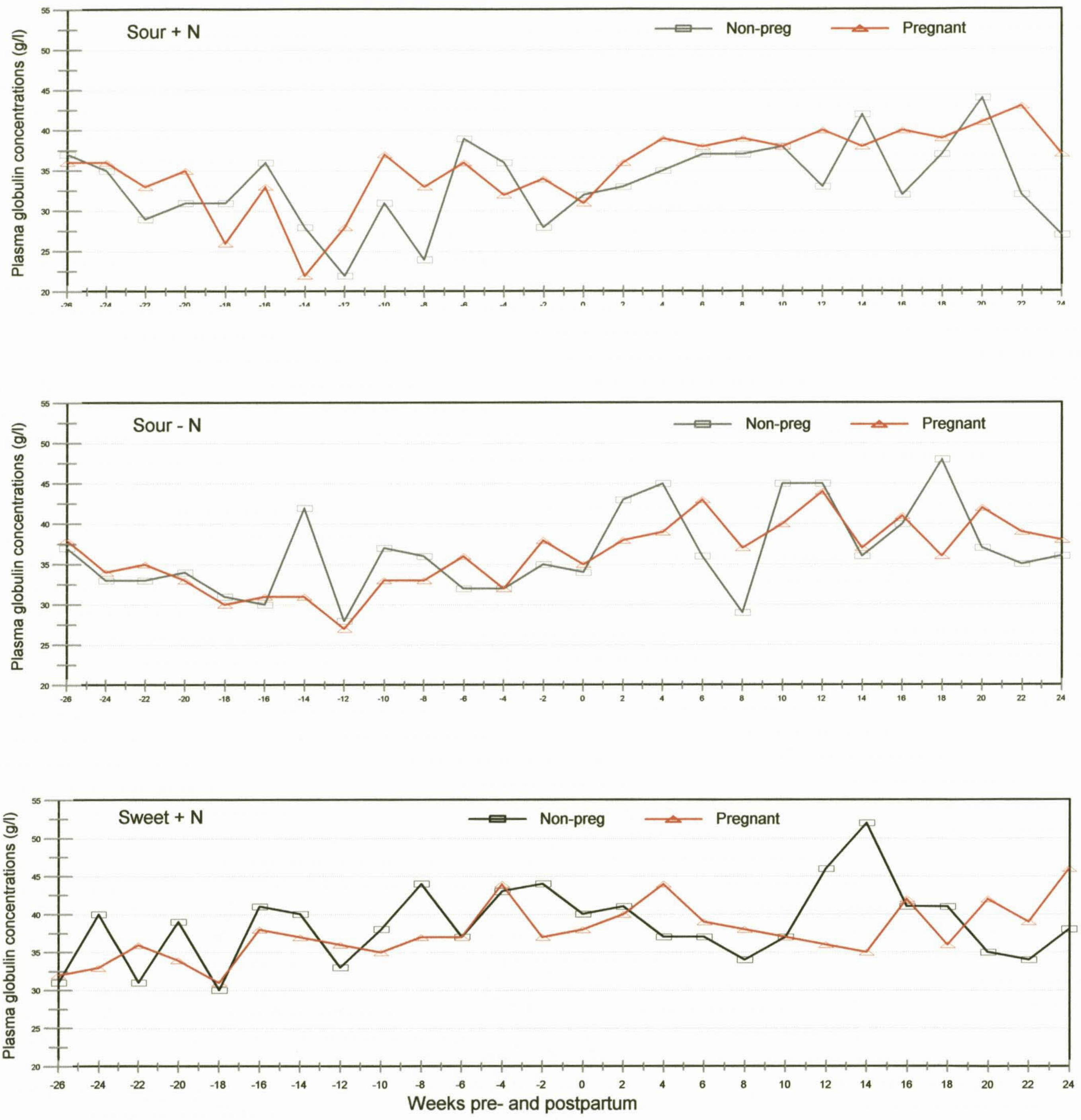


Figure 3.11 Mean plasma globulin profiles (g/l) of first-calf cows in 3 treatment groups during gestation and lactation (1981/82)

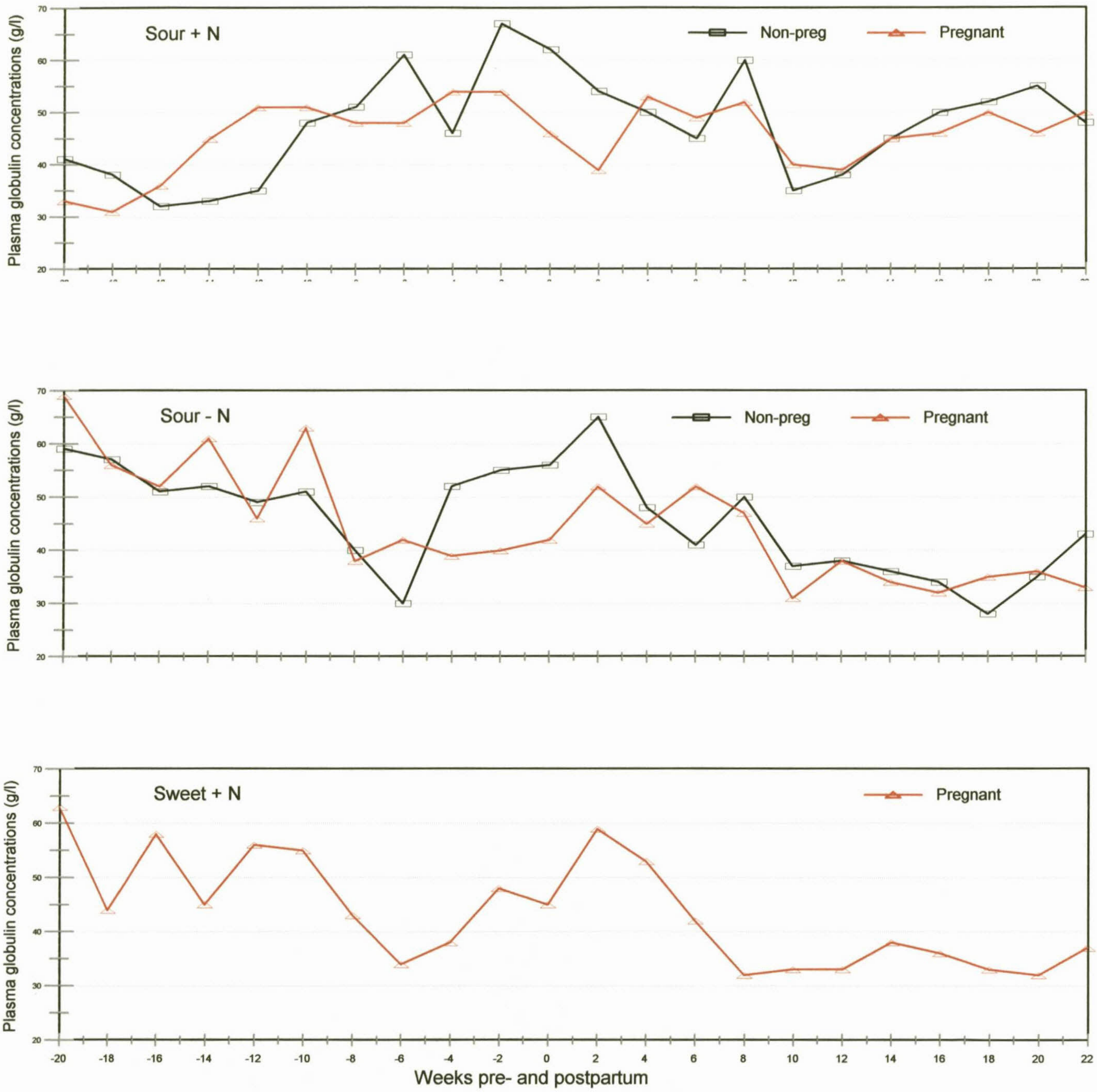


Figure 3.12 Mean plasma globulin profiles (g/l) of second-calf cows in 3 treatment groups during gestation and lactation (1982/83)

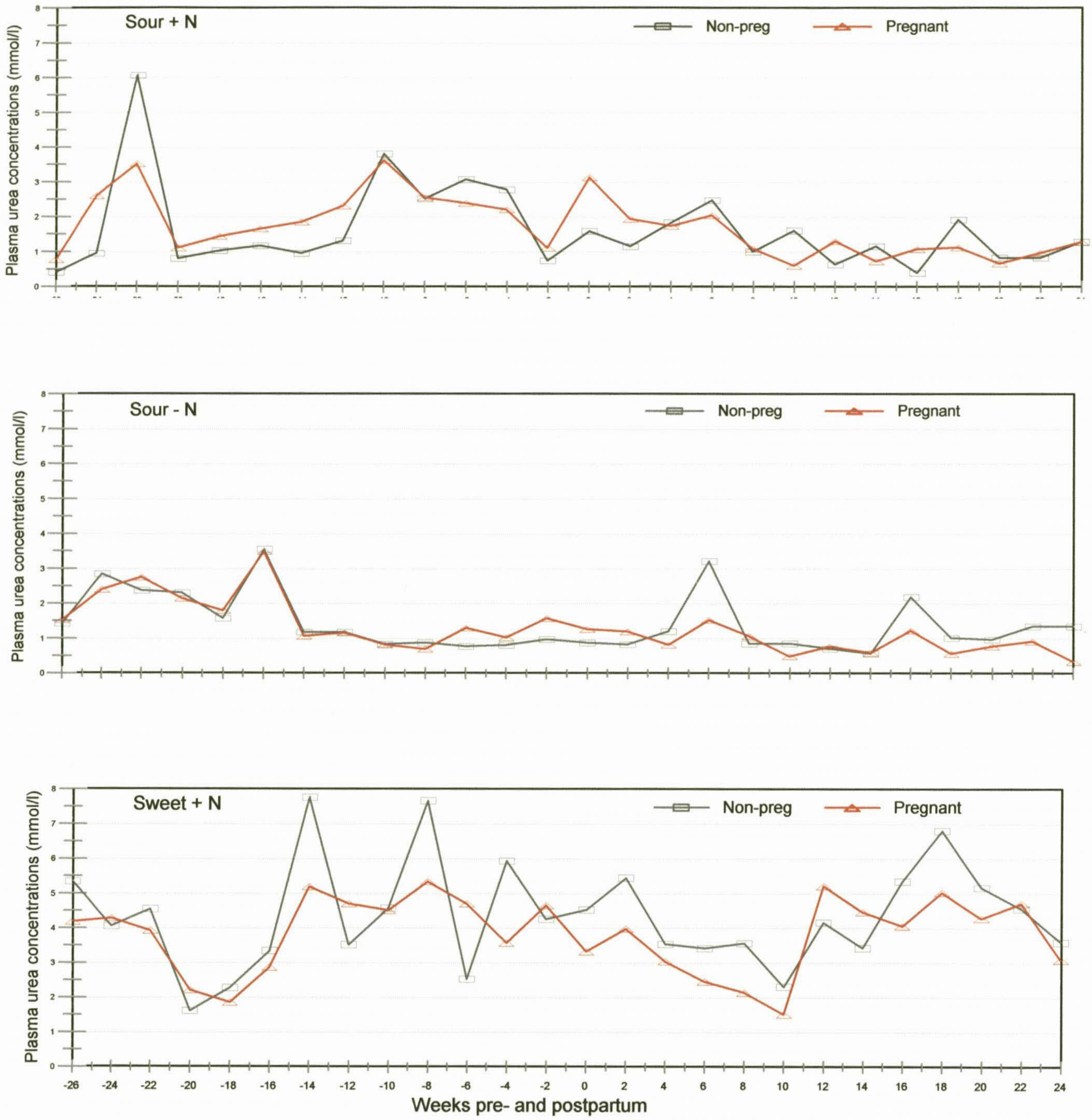


Figure 3.13 Mean plasma urea profiles (mmol/l) of first-calf cows in 3 treatment groups during gestation and lactation (1981/82)

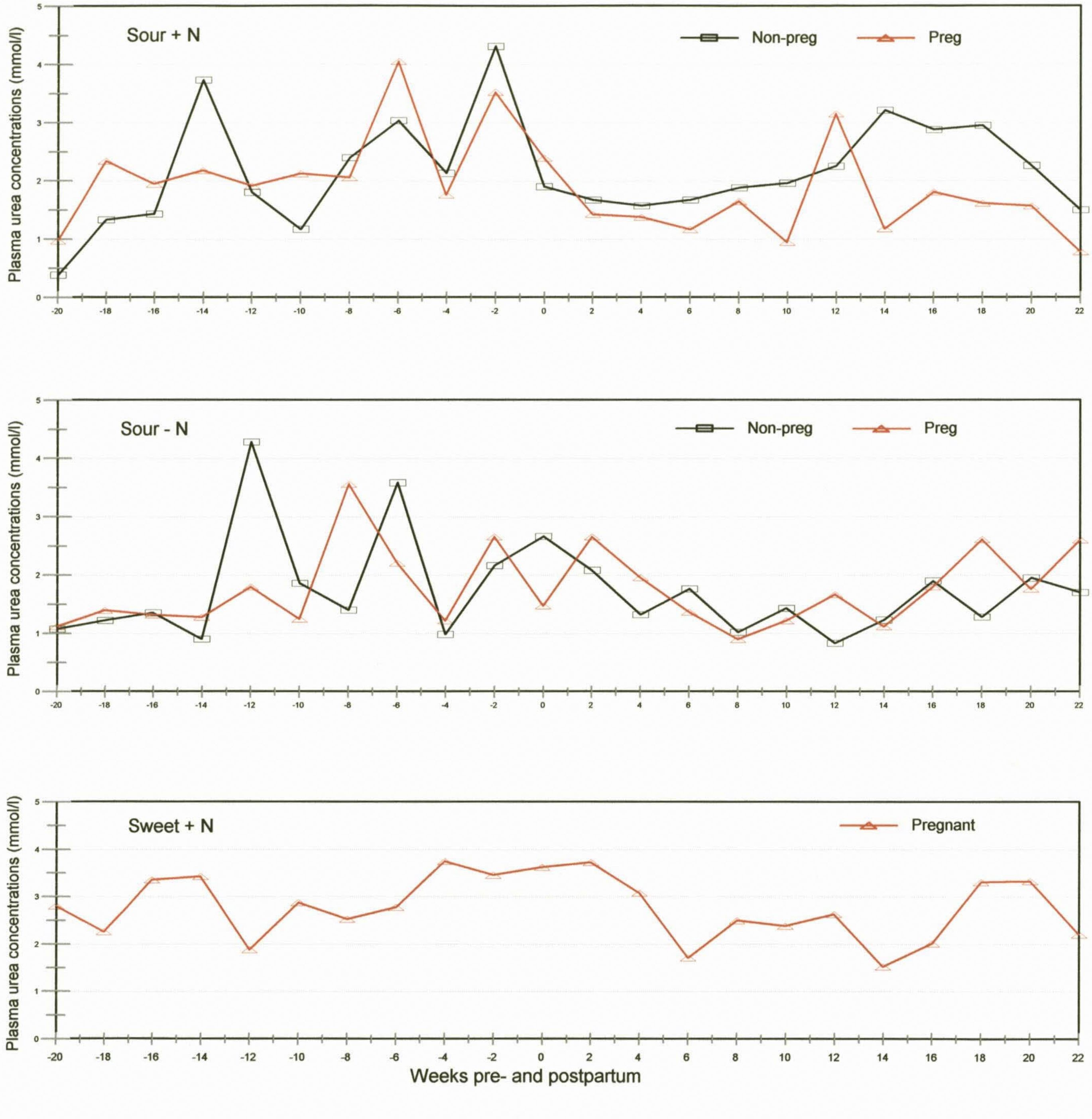


Figure 3.14 Mean plasma urea profiles (mmol/l) of second-calf cows in 3 treatment groups during gestation and lactation (1982/83)

tained during the following season (Table 3.2 and Figure 3.14). Although no significant differences were recorded between pregnant and non-pregnant females, the plasma urea concentrations were generally lower in the first group of experimental animals (Figure 3.13).

3.3.3 Influence of pregnancy on hormonal secretions

3.3.3.1 Adrenal cortex activity

During 1979, pregnancy had a significant ($P < 0.05$) effect on plasma cortisol concentration in cows and heifers (Table 3.3). Plasma cortisol level of the pregnant heifers averaged 11.8 ng/ml, compared to 9.4 ng/ml in the non-pregnant heifers. In cows, the values were 10.0 and 8.9 ng/ml, respectively. From Figure 3.15 it is evident that, although pregnancy resulted in elevated plasma cortisol concentrations in heifers, this tendency was not consistent. It would seem that the levels tend to decrease towards the end of pregnancy, when compared with open heifers.

During 1980, all the heifers in both groups that were inseminated, conceived. The non-pregnant data shown in Table 3.3 refers to hormonal concentrations of heifers and cows before mating.

Pregnant heifers on the sourveld had significantly ($P < 0.001$) lower plasma cortisol concentrations than during non-pregnancy (Table 3.3). This difference was also notable in heifers on the mixed sweetveld, although not significant.

From Figure 3.16 it can be seen that plasma cortisol concentrations tended to decline as pregnancy progressed. This was especially evident in heifers on mixed sweetveld. There also appeared to be less variation in cortisol activity in heifers on the mixed sweetveld compared to those on the sourveld. Hormonal differences between heifers and cows during pregnancy were not significant.

During the 1981/82 season, pregnancy led to significantly higher cortisol concentrations in first-calf cows in 2 out of the 3 treatments (Table 3.3). An interesting phenomenon

Table 3.3 Influence of pregnancy on mean (± SE) plasma cortisol and thyroxine concentrations (ng/ml) during the 1979 to 1983 seasons in Bonsmara females maintained on 3 feeding regimes

Season	Sourveld + N		Sourveld - N		Sweetveld + N	
	Pregnant ± SE	Open ± SE	Pregnant ± SE	Open ± SE	Pregnant ± SE	Open ± SE
1979 season:						
Cortisol	11.6[a]	9.6[b]				
(ng/ml)	± 0.41	± 0.71				
Thyroxine	50.1	50.1				
(ng/ml)	± 1.04	± 1.84				
1980 season:						
Cortisol	9.4[c]	18.2[d]			11.8	14.6
(ng/ml)	± 0.58	± 0.92			± 0.89	± 1.11
Thyroxine	52.8[e]	57.6[f]			53.3	52.0
(ng/ml)	± 1.13	± 1.77			± 1.52	±1.91
1981/82 season:						
Cortisol	9.8[g]	7.7[h]	7.1	7.6	10.9[o]	8.5[p]
(ng/ml)	± 0.60	± 0.70	± 0.50	± 0.50	± 0.45	± 0.66
Thyroxine	40.0[i]	43.8[j]	37.0[k]	44.7[l]	49.8[q]	44.2[r]
(ng/ml)	± 0.98	± 1.14	± 1.56	± 1.56	± 1.05	± 1.52
1982/83 season:						
Cortisol	6.7	6.8	6.9	6.8	6.3	-
(ng/ml)	± 0.30	± 0.35	± 0.36	± 0.31	± 0.31	
Thyroxine	39.3	39.4	43.4[m]	38.5[n]	49.4	-
(ng/ml)	± 0.96	± 1.13	± 0.99	± 0.85	± 0.99	

b < a, 0.05   e < f, 0.05   i < j, 0.05   n < m, 0.01   r < q, 0.01

c < d, 0.001   h < g, 0.05   k < l, 0.01   p < o, 0.01

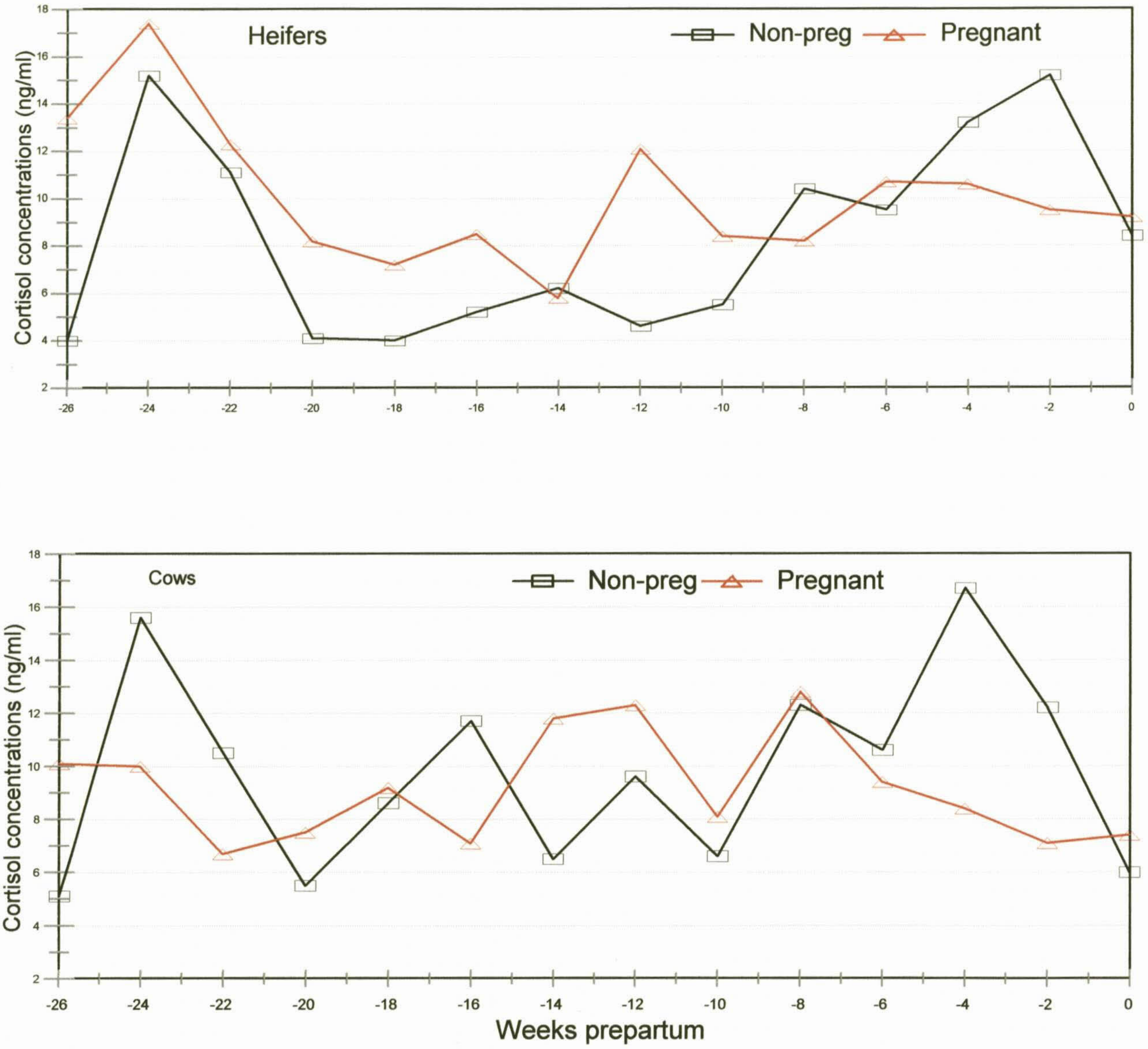


Figure 3.15 Mean plasma cortisol profiles (ng/ml) of pregnant and open heifers and cows on the Dohne Sourveld during gestation (1979)

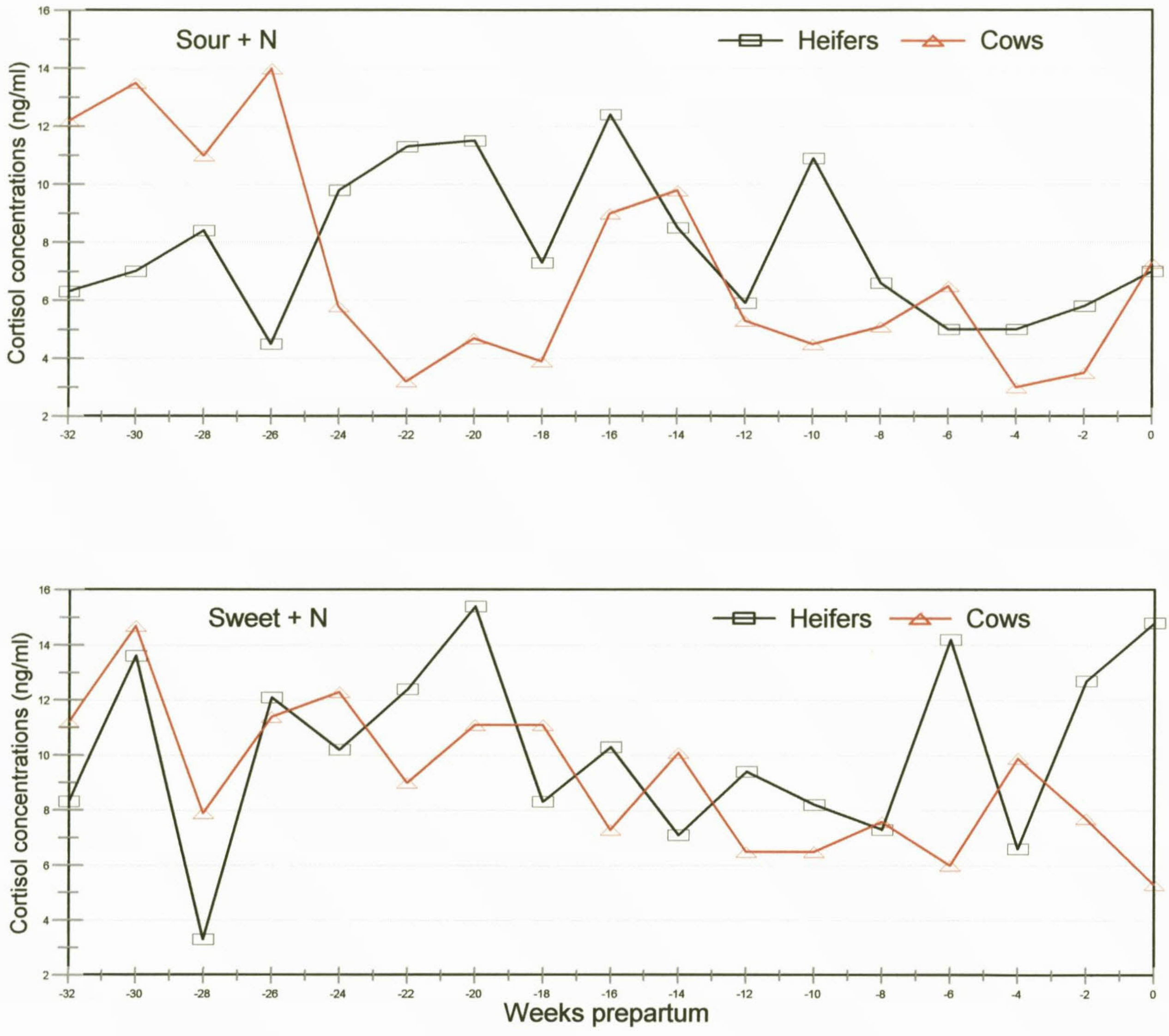


Figure 3.16 Mean plasma cortisol profiles (ng/ml) of heifers and cows in the sourveld + N and mixed sweetveld + N treatment groups during gestation (1980)

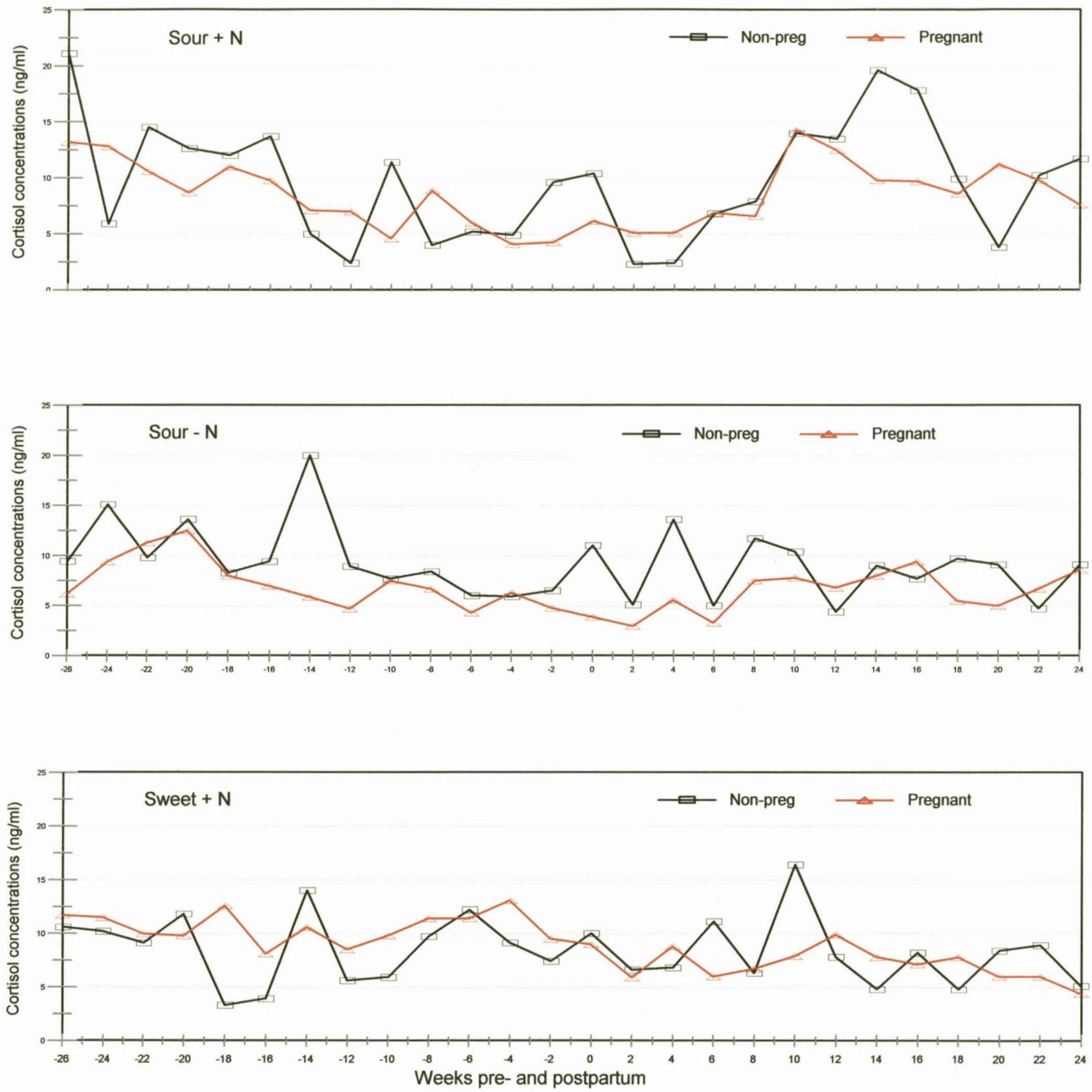


Figure 3.17 Mean plasma cortisol concentrations (ng/ml) of first-calf cows in 3 treatment groups during gestation and lactation (1981/82)

regarding the influence of pregnancy on plasma cortisol concentrations, was the hormonal reaction to the feeding regimes (Figure 3.17). Firstly, the cortisol profiles of the pregnant females were generally lower than those of the non-pregnant counterparts in the 2 sourveld groups. This trend was not evident in animals on mixed sweetveld. Secondly, plasma cortisol profiles of pregnant females on sourveld decreased steadily during the course of pregnancy. This was less obvious in the mixed sweetveld group. Thirdly, it appears as if plasma cortisol concentrations in the first-calvers of all 3 treatment groups were less subject to variation compared to the non-pregnant counterparts.

During the 1982/83 period, pregnancy had virtually no effect on the adrenal cortex activity (Table 3.3). The plasma cortisol profiles of pregnant cows in the 2 sourveld groups showed a decreasing tendency during pregnancy (Figure 3.18).

3.3.3.2 <u>Thyroid activity</u>

Pregnancy had no effect on females kept on sourveld during the 1979 season (Table 3.3). Plasma T4 concentrations of pregnant and open heifers were 53.6 ng/ml and 57.7 ng/ml respectively, and those of cows 47.2 ng/ml and 42.7 ng/ml, respectively. In the case of the pregnant heifers, plasma T4 concentrations stabilised between 50 and 60 ng/ml. Considerably more variation occurred in the open heifers. The suppressed plasma thyroxine concentrations of the pregnant heifers are evident in Figure 3.19.

Cows generally had lower plasma T4 concentrations. An inverse trend was recorded to that in heifers, namely that pregnant cows had higher plasma T4 levels than that in non-pregnant cows. Plasma T4 concentrations increased from 45 ng/ml at 6 weeks prior to calving, to 73 ng/ml at parturition.

Plasma thyroxine concentrations did not always vary consistently before and after pregnancy in females on the sourveld and mixed sweetveld during 1980 (Table 3.3). From Figure 3.20 it would appear as if T4 levels of heifers and cows on the sourveld declined over the pregnant period, whilst the open animals on the mixed sweetveld were able to maintain their thyroxine concentrations.

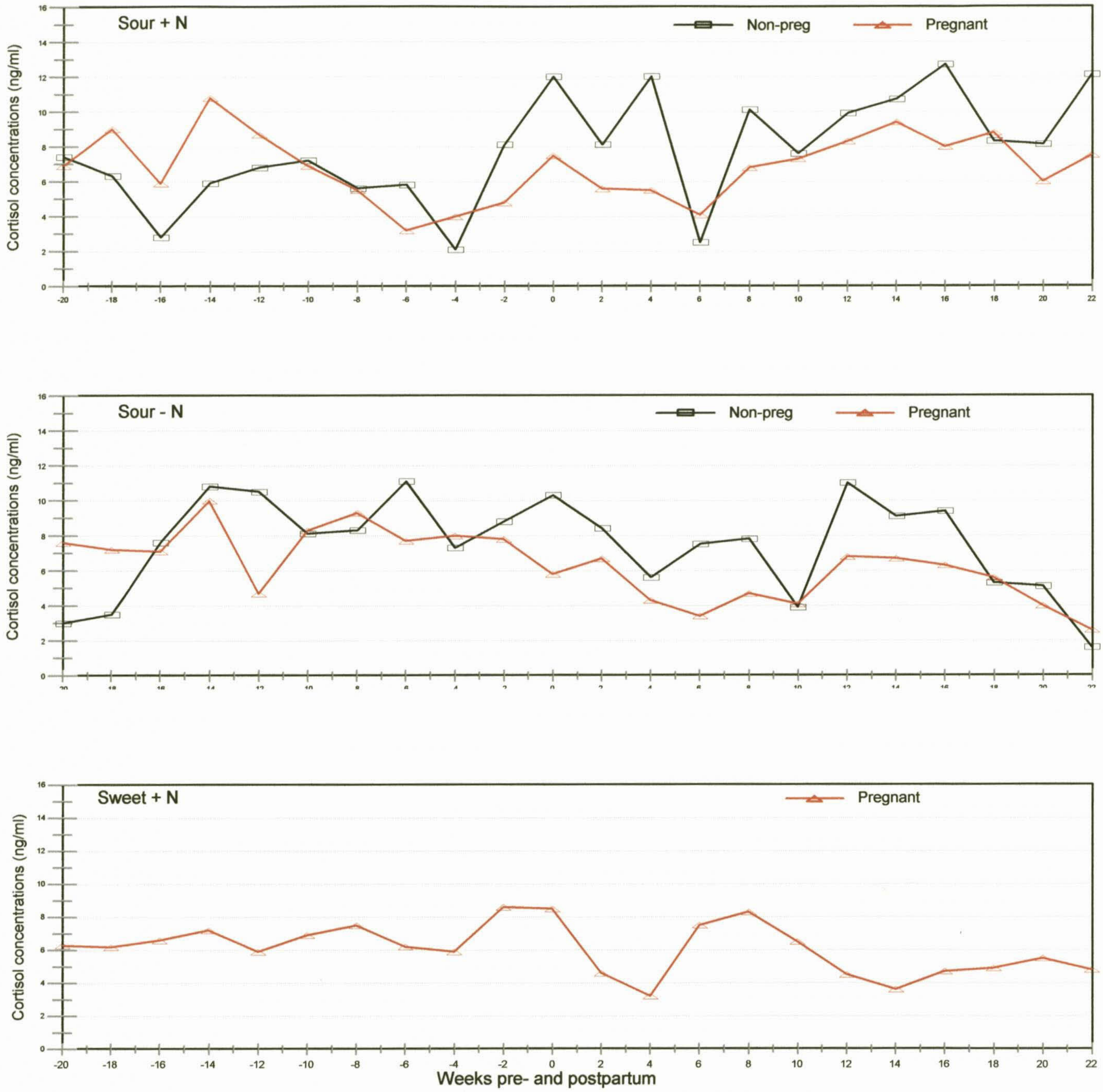


Figure 3.18 Mean plasma cortisol profiles (ng/ml) of second-calf cows in 3 treatment groups during gestation and lactation (1982/83)

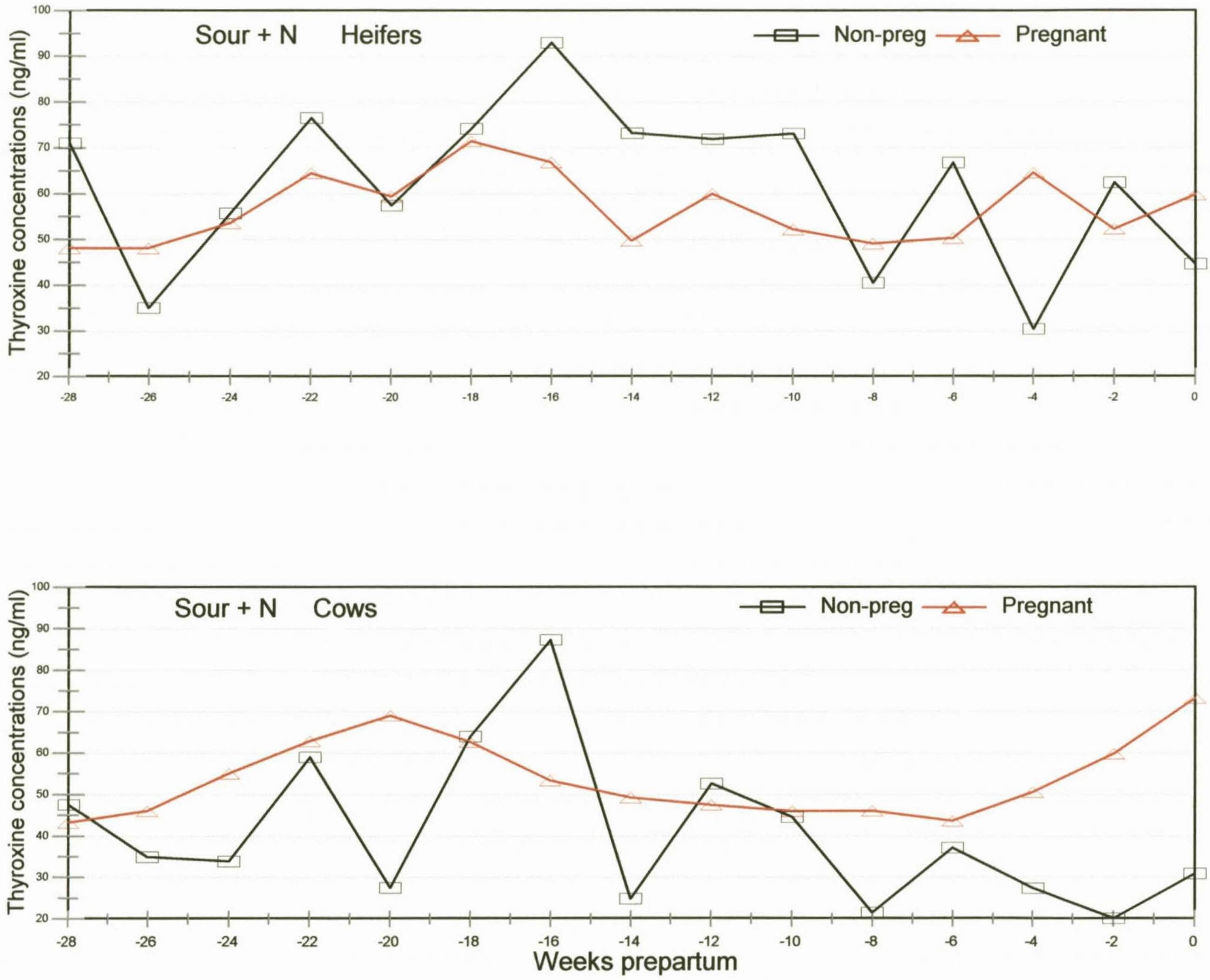


Figure 3.19 Mean plasma thyroxine profiles (ng/ml) of pregnant and open heifers and cows on the Dohne Sourveld during gestation (1979)

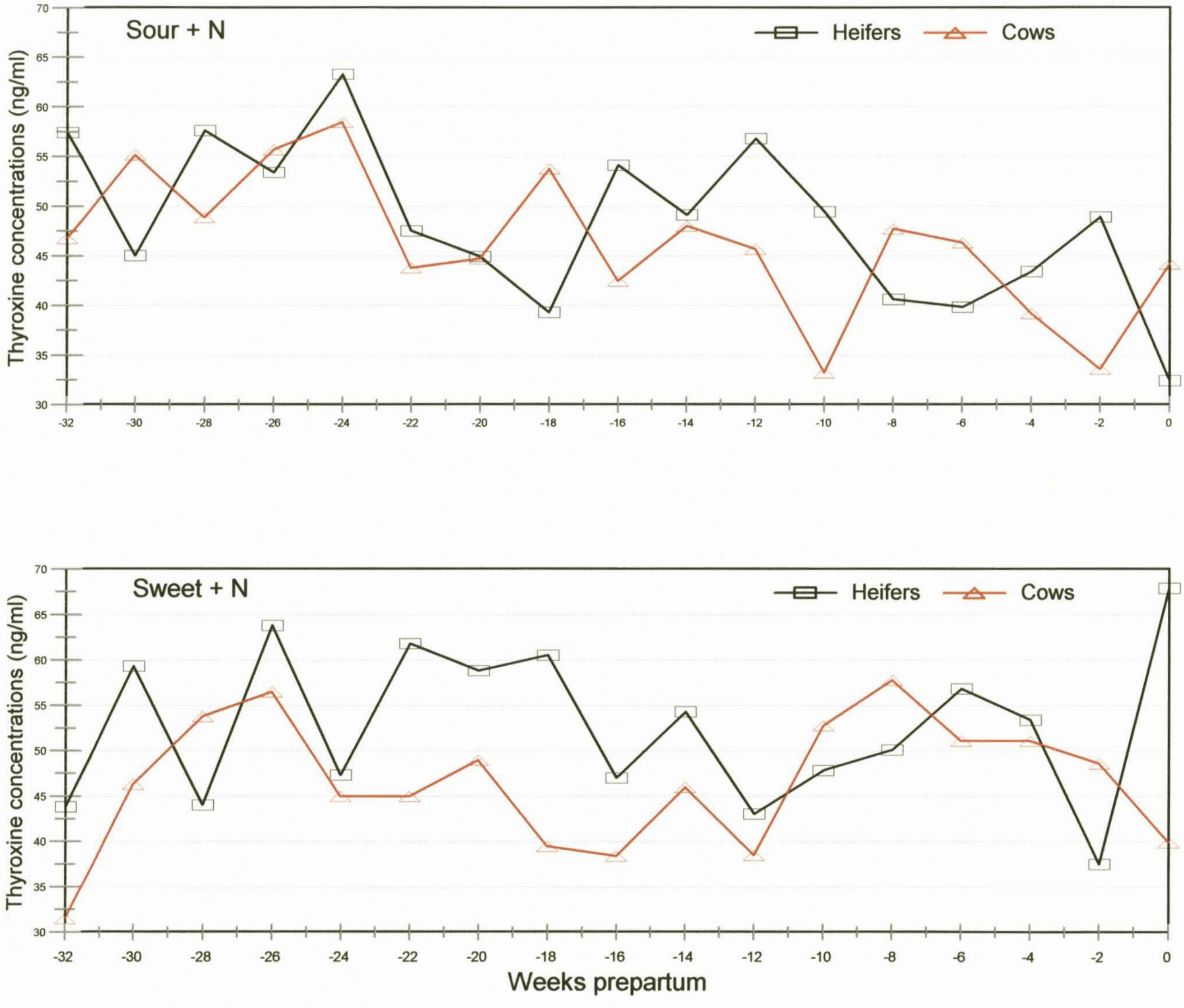


Figure 3.20 Mean plasma thyroxine profiles (ng/ml) of heifers and cows in the sourveld + N and mixed sweetveld + N treatment groups during gestation (1980)

During the 1981/82 period, pregnancy influenced thyroid activity to a certain degree. Pregnant first-calf cows showed significantly depressed thyroxine levels, compared to the non-pregnant animals in both sourveld groups (Table 3.3). Mixed sweetveld females reacted the opposite way around. According to Figure 3.21, the only clear effect of pregnancy on plasma T4 concentration, was in the group of females on the sourveld, deprived of an NPN lick. It would appear as if T4 levels were suppressed as pregnancy progressed.

During the following season (1982/83), pregnancy led to increased plasma T4 concentrations of second-calf cows in 2 out of the 3 treatment groups (Table 3.3). The strongest influence of pregnancy was on the sourveld - N group of females (Figure 3.22), which led to significantly ($P < 0.01$) elevated plasma thyroxine concentrations.

3.3.4 Influence of lactation on energy metabolites

In an analysis of variance, lactation was shown not to have a significant effect on blood glucose concentrations (Table 3.4), although it would appear as if the levels in lactating cows were constantly lower than those in dry cows for at least the first 10 weeks after calving (Figure 3.1). After that, no clear differences between groups were noticeable. During the 1982/83 season, it appeared as if non-lactating cows tended to have a higher blood glucose concentration (Table 3.5). This is illustrated in the glucose levels recorded in the sourveld + N group in Figure 3.2. No difference in blood glucose concentrate was noted in the sourveld - N group.

Plasma glucose concentrations also appeared to be influenced to a lesser extent by lactation. Although no great differences were recorded, levels in lactating cows were consistently lower when compared to those of their non-lactating counterparts in all 3 groups (Table 3.4). As in the case of blood glucose, plasma glucose levels were lower for 8 to 10 weeks after parturition, whereafter insignificant differences were noted (Figure 3.3). Similar results were observed during the following year (1982/83) (Table 3.5). However, the plasma glucose levels of pregnant cows were suppressed for a longer period, up to 5 months (Figure 3.4). In the mixed sweetveld group, it appears as if plasma glucose concentrations decreased during lactation, but increased by 14 weeks after parturition.

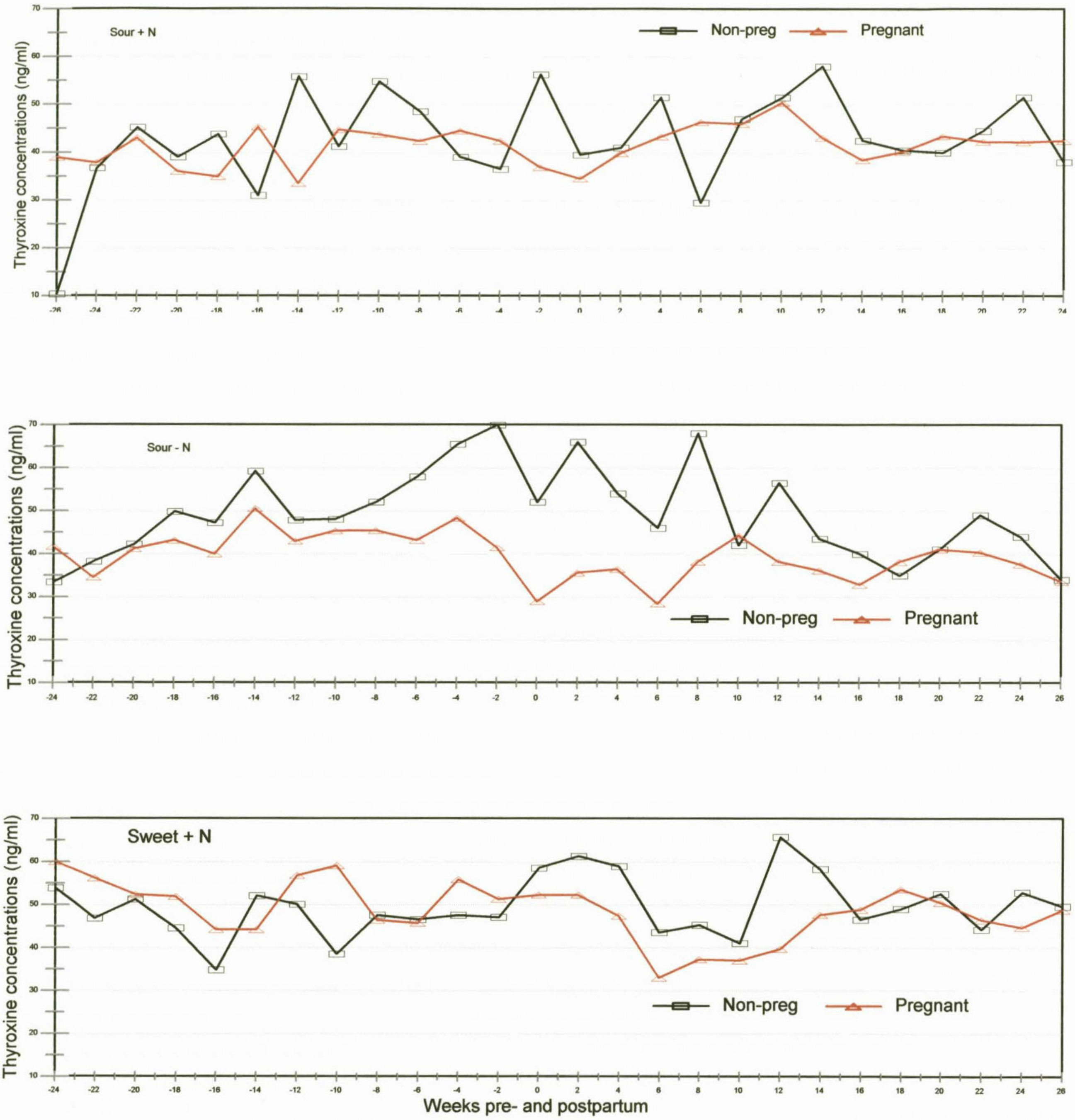


Figure 3.21 Mean plasma thyroxine profiles (ng/ml) of first-calf cows in 3 treatment groups during gestation and lactation (1981/82)

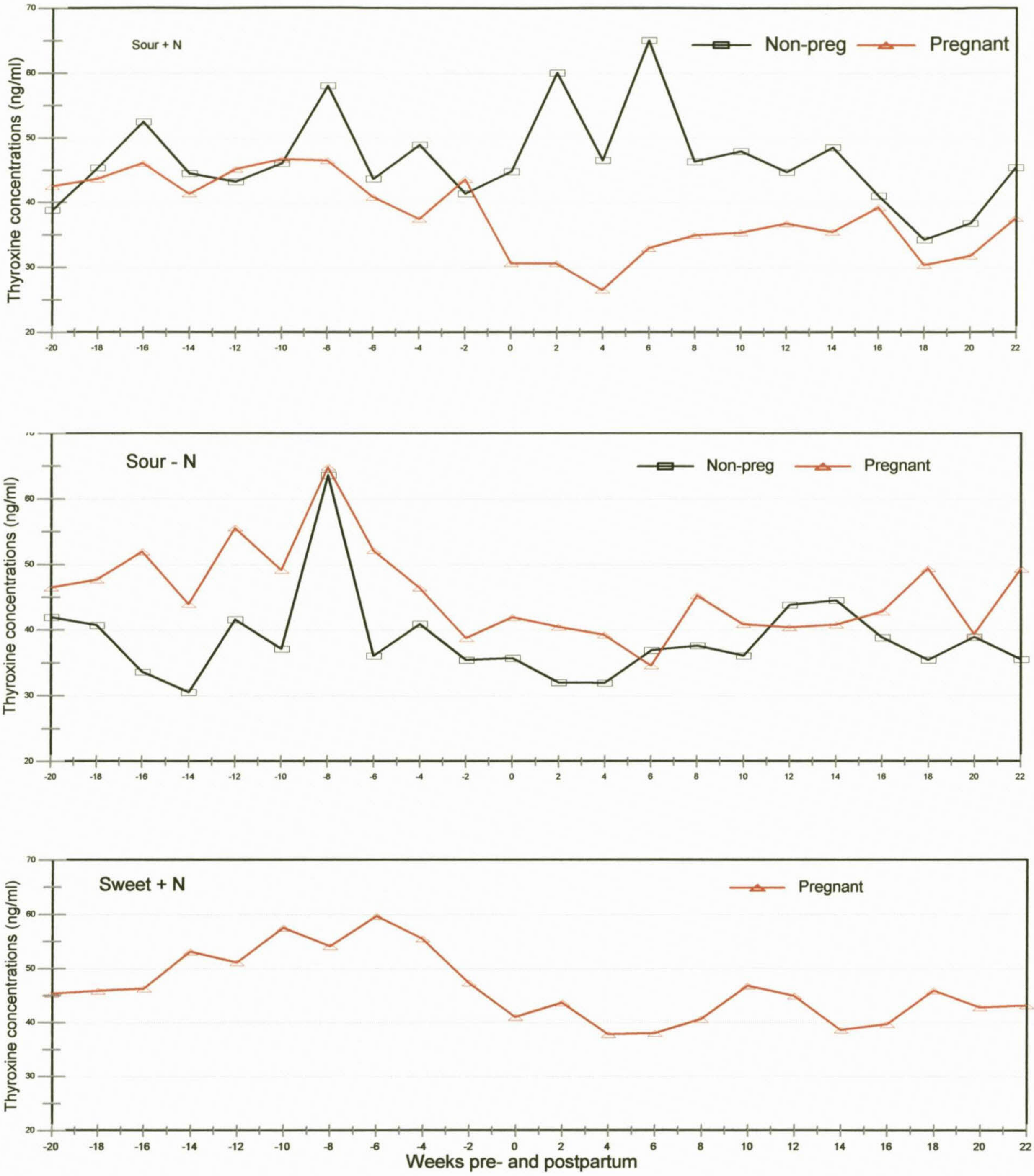


Figure 3.22 Mean plasma thyroxine profiles (ng/ml) of second-calf cows in 3 treatment groups during gestation and lactation (1982/83)

Table 3.4 Influence of lactation on mean (± SE) blood and plasma metabolites, and cortisol and thyroxine concentrations in first-calf cows on 3 feeding regimes during the 1981/82 season

Constituent	Sourveld + N		Sourveld - N		Sweetveld + N	
	Lactating ± SE	Dry ± SE	Lactating ± SE	Dry ± SE	Lactating ± SE	Dry ± SE
Blood glucose (mmol/l)	2.09 ± 0.05	2.20 ± 0.03	2.11 ± 0.06	2.16 ± 0.04	2.30 ± 0.08	2.31 ± 0.04
Plasma glucose (mmol/l)	3.15 ± 0.07	3.26 ± 0.05	3.04 ± 0.09	3.15 ± 0.06	3.22 ± 0.09	3.43 ± 0.05
Red blood cell glucose (mmol/l)	1.06 ± 0.05	1.07 ± 0.03	0.93 ± 0.06	0.99 ± 0.04	0.93^{o} ± 0.06	1.12^{p} ± 0.03
Total plasma protein (g/l)	69^{a} ± 1.13	65^{b} ± 0.81	69 ± 1.10	68 ± 0.68	70 ± 1.06	72 ± 0.53
Plasma albumin (g/l)	31^{c} ± 0.57	33^{d} ± 0.41	31 ± 0.69	32 ± 0.43	32^{q} ± 0.89	34^{r} ± 0.44
Plasma globulin (g/l)	39^{e} ± 0.96	33^{f} ± 0.69	38 ± 1.31	36 ± 0.85	38 ± 1.26	38 ± 0.63
Plasma urea (mmol/l)	1.28^{g} ± 0.23	1.96^{h} ± 0.17	1.02^{i} ± 0.15	1.53^{j} ± 0.10	3.05^{s} ± 0.33	3.98^{t} ± 0.16
Plasma cortisol (ng/ml)	10.7 ± 0.82	9.1 ± 0.59	5.2^{k} ± 0.64	8.4^{l} ± 0.42	8.1^{u} ± 0.85	10.2^{v} ± 0.47
Plasma thyroxine (ng/ml)	41.9 ± 1.38	41.6 ± 0.98	33.3^{m} ± 2.10	44.8^{n} ± 1.37	42.6^{w} ± 1.97	48.4^{x} ± 1.09

b < a, 0.01 f < e, 0.001 i < j, 0.01 m < n, 0.01 q < r, 0.05 u < v, 0.05

c < d, 0.05 g < h, 0.05 k < l, 0.01 o < p, 0.05 s < t, 0.05 w < x, 0.05

Table 3.5 Influence of lactation on mean (± SE) blood and plasma metabolites, and cortisol and thyroxine concentrations in second-calf cows on 3 feeding regimes during the 1982/83 season

Constituents	Sourveld + N		Sourveld - N		Sweetveld + N	
	Lactating ± SE	Dry ± SE	Lactating ± SE	Dry ± SE	Lactating ± SE	Dry ± SE
Blood glucose (mmol/l)	1.95[a] ± 0.04	2.10[b] ± 0.03	2.04[o] ± 0.07	2.23[p] ± 0.03	2.07 ± 0.04	-
Plasma glucose (mmol/l)	3.02[c] ± 0.05	3.32[d] ± 0.05	3.08[q] ± 0.09	3.36[r] ± 0.04	3.18 ± 0.05	-
Red blood cell glucose (mmol/l)	1.06[e] ± 0.04	1.22[f] ± 0.04	1.03 ± 0.07	1.14 ± 0.03	1.11 ± 0.04	-
Total plasma protein (g/l)	70[g] ± 1.30	76[h] ± 1.20	69[s] ± 0.22	77[t] ± 1.10	70 ± 1.14	-
Plasma albumin (g/l)	30 ± 0.50	30 ± 0.40	31 ± 0.70	31 ± 0.30	32 ± 0.30	-
Plasma globulin (g/l)	40[i] ± 1.40	46[j] ± 1.20	38[u] ± 0.22	46[v] ± 1.00	39 ± 1.40	-
Plasma urea (mmol/l)	1.50[k] ± 0.11	2.03[l] ± 0.10	1.65 ± 0.18	1.63 ± 0.08	3.23 ± 0.14	-
Plasma cortisol (ng/ml)	6.55 ± 0.36	6.89 ± 0.35	5.47[w] ± 0.26	7.05[x] ± 0.26	5.45 ± 0.31	-
Plasma thyroxine (ng/ml)	35.8[m] ± 1.02	42.8[n] ± 0.99	44.2[y] ± 1.75	40.5[z] ± 0.71	44.5 ± 1.05	-

a < b, 0.01 g < h, 0.01 m < n, 0.001 s < t, 0.01 z < y, 0.05

c < d, 0.001 i < j, 0.01 o < p, 0.05 u < v, 0.05

e < f, 0.01 k < l, 0.001 q < r, 0.05 w < x, 0.05

Lactation did not have a constant and significant influence on RBC glucose levels during the first year (1981/82) (Table 3.4), although there was a tendency for lactating cows to have lower levels. Although this tendency was illustrated in the sourveld + N group, no clear relationship between the lactating cows and RBC glucose levels in the other 2 groups were recorded (Figure 3.5). During the following year, the effect of lactation seemed to be more pronounced in the 2 sourveld groups (Figure 3.6), especially in cows on the sourveld - N treatment.

3.3.5 <u>Influence of lactation on plasma protein metabolites</u>

Lactation did not have a significant effect on total plasma protein during the 1981/82 season (Table 3.4). This is reflected in Figure 3.7. During the following year, non-lactating animals had significantly higher levels, compared to the lactating females in all 3 treatments (Table 3.5). However, as can be seen in Figure 3.8, results were not consistent. Protein levels tended to decrease as lactation progressed, especially in the sourveld - N and mixed sweetveld groups.

The influence of lactation on albumin levels was not significant during the 1981/82 period (Table 3.4). This is substantiated by the graphic results presented in Figure 3.9. Similar results were obtained during the 1982/83 period (Table 3.5 and Figure 3.10).

During the first year, lactation did not have any specific effect on plasma globulin levels (Table 3.4). Cows on the sourveld showed a persistent increase in levels as pregnancy progressed through to 5 months after lactation (Figure 3.11). During the 1982/83 season, lactation had a more pronounced effect on the plasma globulin levels (Table 3.5), with lactating cows having significantly lower concentrations (Figure 3.12).

From Table 3.4, it is evident that lactation had a considerable effect on the plasma urea concentration. As was often the case with previous metabolites, plasma urea concentrations were suppressed in the lactating cows of all 3 treatment groups. As results in Figure 3.13 indicate, this influence was not consistent in all 3 treatment groups. This was due to the occasional plasma urea increase in dry cows. There was a tendency for urea concentrations to decrease during lactation in the 2 sourveld groups, whilst an increase in levels were observed in cows kept on the mixed sweetveld. During the 1982/83 period, results concerning urea were variable (Table 3.5).

Nevertheless, urea concentrations tended to decrease during lactation, especially during the early lactation period (Figure 3.14).

3.3.6 Influence of lactation on hormonal secretions

3.3.6.1 Plasma cortisol concentrations

The plasma cortisol concentrations of lactating cows were not similarly influenced by the 3 treatments (Table 3.4). However, it would appear as if lactation suppresses adrenal cortex activity (Figure 3.17). Cortisol levels also tended to increase in the cows on sourveld after parturition. During the 1982/83 season, lactation had a more pronounced, suppressing effect on plasma cortisol concentrations (Table 3.5). During both years, lactation seemed to have little effect on the hormonal secretions of cows on sourveld, supplemented with an NPN lick. From Figure 3.18 it is evident that lactating cows had lower plasma cortisol levels, compared to their non-pregnant counterparts in the different treatment groups. It is clear that cortisol concentrations in the 2 sourveld groups reacted in different ways.

3.3.6.2 Plasma thyroxine concentrations

As was the case in plasma cortisol concentrations, lactation suppressed plasma T4 levels more in first-calf cows on sourveld - N and mixed sweetveld, than in the sourveld + N group, during 1981/82 (Table 3.4). It is apparent that the larger variation in plasma thyroxine levels of the dry cows on the sourveld + N treatment clouded the statistical analysis (Figure 3.21). The greater effect of lactation on plasma T4 concentrations of first-calf cows in the sourveld - N and mixed sweetveld groups is evident from Figure 3.21. In the case of the sourveld - N group, the suppressed thyroid activity originated earlier, during the latter half of pregnancy. It appears as if the effect of lactation was removed 14 weeks after partition.

In cows on mixed sweetveld, plasma T4 levels segregated just before parturition, and remained low for approximately 14 weeks thereafter. In all 3 treatment groups, levels decreased to a basal level at or just after calving.

During 1982/83, cows on the sourveld - N produced strange results (Table 3.5), and in contrast with the cows in the other groups, lactating females had significantly higher T4 concentrations than dry females. In spite of this anomaly, results clearly indicate a decrease in plasma T4 concentrations during pregnancy, reaching minimum concentrations after calving, and increasing during lactation (Figure 3.22). In the case of females on sourveld + N, the significant ($P < 0.001$) difference between lactating and non-lactating cows decreased over time. Little difference existed 14 weeks after parturition. Thyroid activity was constantly higher during pregnancy and lactation in the sourveld - N cows.

3.3.7 Metabolites and hormones in relation to fertility

3.3.7.1 Energy profiles relating to fertility

During 1981/82, energy, protein and hormonal profiles were monitored 10 weeks prior to conception in all 3 groups of females. For this analysis, treatment data were pooled. The profiles determined during the 1982/83 period for the same cows after parturition up to the time of conception, are presented in Table 3.6.

Blood glucose concentrations of the cows during the 2 seasons are depicted in Figure 3.23. During the first year (1981/82), the females that conceived had declining blood glucose concentrations up to 2 weeks before mating and conceiving. This is in direct contrast with females that did not conceive. The blood glucose levels of the fertile cows were consistently higher than those of the infertile cows, just after parturition.

During the 1982/83 season, the blood glucose concentrations of the fertile cows were also higher than those of the infertile cows, although this difference was not as large as during the previous season.

The plasma glucose concentrations in both groups of females during 1981/82 is reflected in the blood glucose levels (Figure 3.24). Plasma glucose levels were higher in fertile females during both seasons, especially just after parturition. As was the case with blood glucose concentrations, differences were smaller during the 1982/83 season.

Table 3.6 Mean (± SE) blood and plasma metabolite differences between fertile and infertile cows during 2 seasons

Constituent	1981/82 Season		1982/83 Season	
	Fertile	Infertile	Fertile	Infertile
Blood glucose (mmol/l)	$2.34^{a} \pm 0.04$	$2.10^{b} \pm 0.07$	2.13 ± 0.05	2.11 ± 0.05
Plasma glucose (mmol/l)	$3.32^{c} \pm 0.06$	$2.95^{d} \pm 0.09$	3.29 ± 0.07	3.17 ± 0.08
Red Blood Cell glucose (mmol/l)	0.99 ± 0.04	0.85 ± 0.07	1.16 ± 0.06	1.06 ± 0.06
Plasma total protein (g/l)	68 ± 0.80	71 ± 1.30	75 ± 1.70	75 ± 1.80
Plasma albumin (g/l)	31 ± 0.50	32 ± 0.70	30 ± 0.50	31 ± 0.60
Plasma globulin (g/l)	37 ± 0.90	39 ± 1.30	45 ± 1.80	45 ± 1.90
Plasma urea (mmol/l)	1.76 ± 0.15	1.86 ± 0.22	1.80 ± 0.90	2.06 ± 0.16

b < a, 0.05

d < c, 0.05

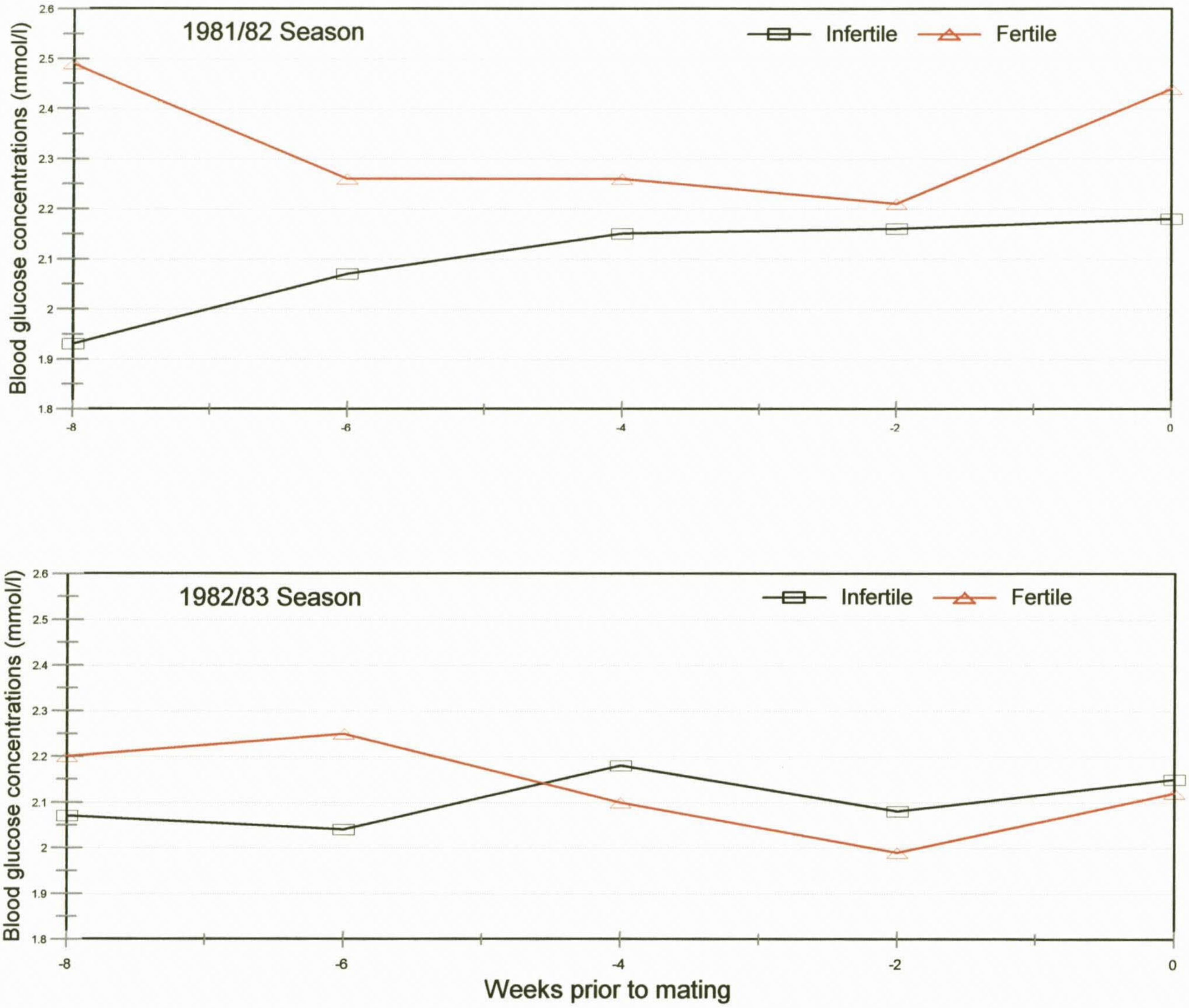
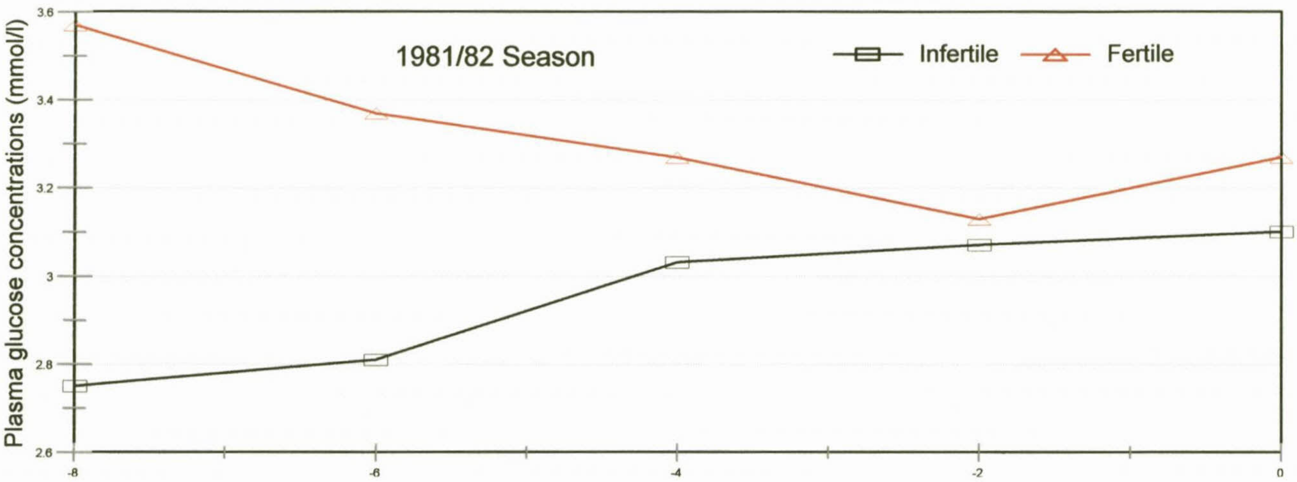


Figure 3.23 Mean blood glucose concentrations (mmol/l) of fertile and infertile cows from 8 weeks prior to the 1981/82 and 1982/83 mating seasons



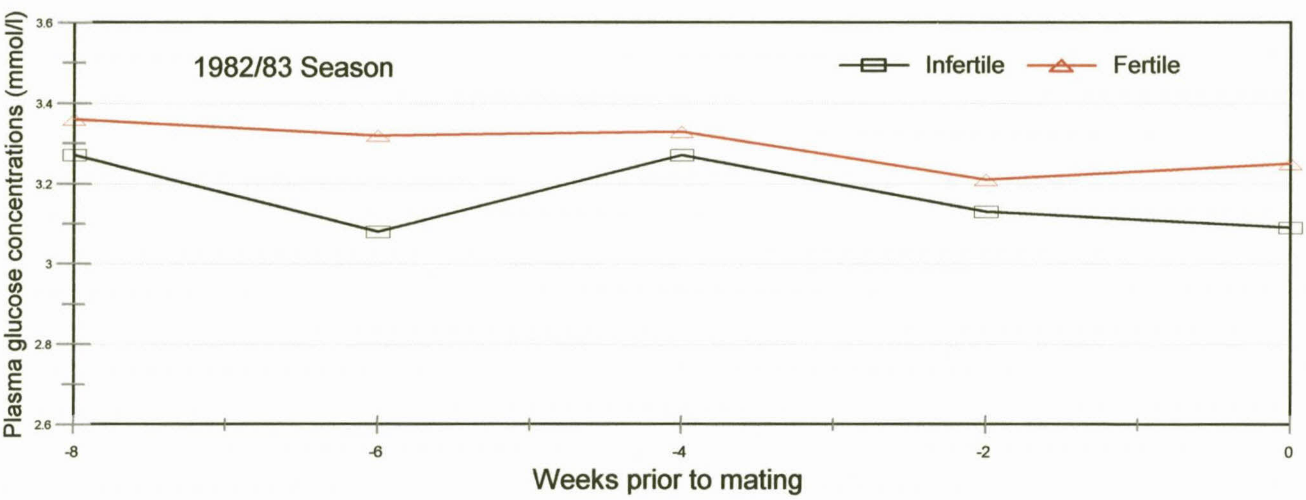


Figure 3.24 Mean plasma glucose concentrations (mmol/l) of fertile and infertile cows from 8 weeks prior to the 1981/82 and 1982/83 mating seasons

Red blood cell glucose concentrations decreased in the fertile first-calf cows during the first year, and in both the fertile and infertile groups during the 1982/83 season (Figure 3.25). These RBC glucose levels were higher during the 1982/83 season.

Regarding the energy metabolites, it is thus clear that females that conceived had consistently higher plasma glucose concentrations than animals that did not conceive (Table 3.6).

3.3.7.2 Protein profiles relating to fertility

Total plasma protein concentrations were much lower in females that conceived during the 1981/82 season, viz. 68 and 71 g/l respectively (Figure 3.26). During the second year, the levels of both groups decreased prior to mating, and no difference between the fertile and infertile females were recorded, both having a mean plasma protein concentration of 75 g/l.

Fertile females had lower plasma albumin concentrations prior to, and at mating than infertile females during both breeding seasons (Figure 3.27). It is evident that much less variation occurred in this metabolite in animals that conceived.

Plasma globulin concentrations reacted similar to that of the total plasma protein (Figure 3.28), with concentrations of fertile females being lower during the first breeding season, and higher during the second breeding season, when compared to animals that did not conceive.

Plasma urea levels of fertile animals reacted inversely during the 2 years. During the 1981/82 season, a decrease in urea concentrations was followed by an increase 2 weeks before mating. On the other hand, plasma urea concentration showed an increase, followed by a decrease prior to mating (Figure 3.29). During both seasons, plasma urea concentrations of animals that conceived were slightly lower, than that of females not conceiving (1.76 vs. 1.86 mmol/l for 1981/82, and 1.80 vs. 2.06 mmol/l for 1982/83 respectively). It would thus appear as if protein profiles were either sustained, or lower

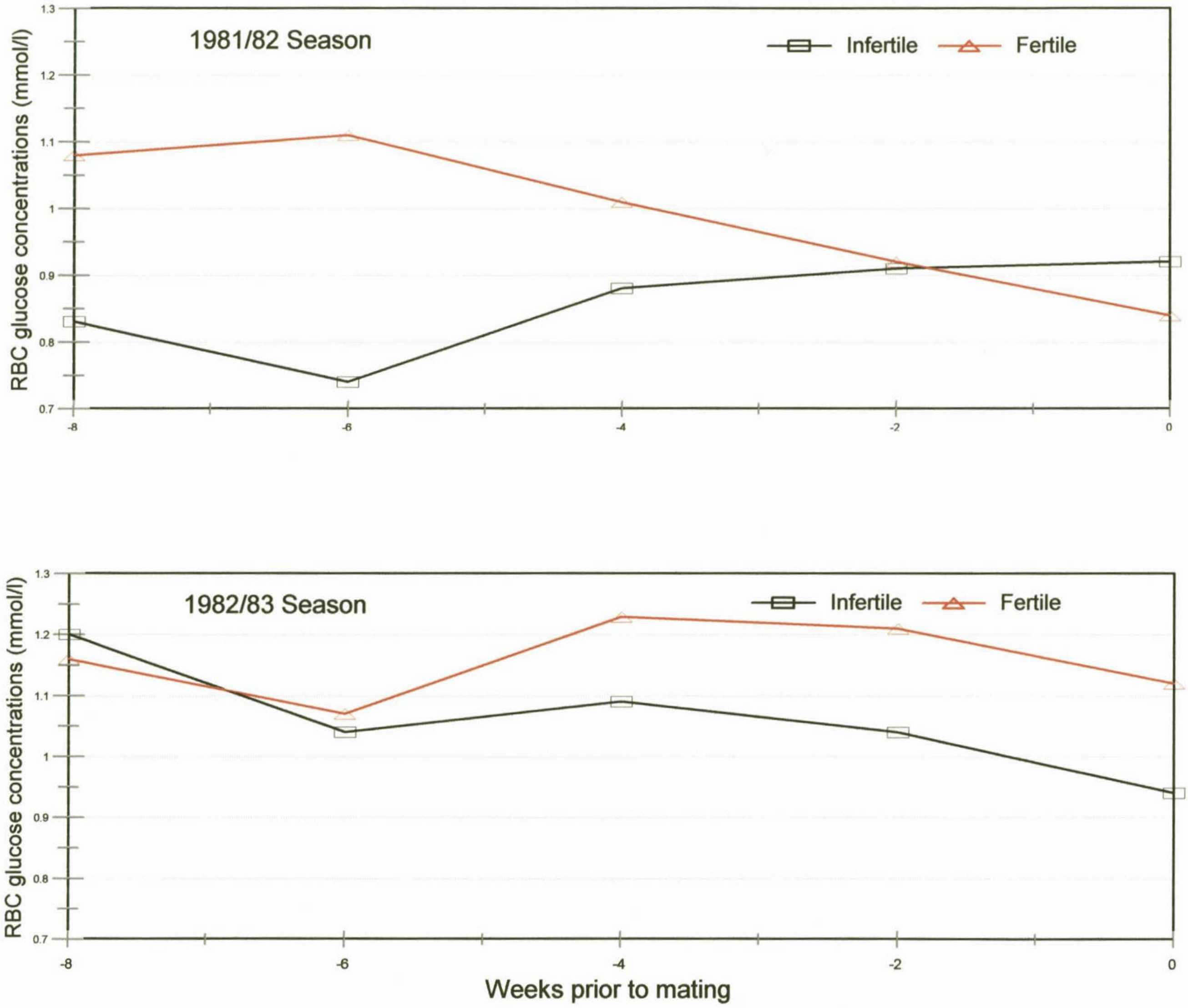


Figure 3.25 Mean red blood cell glucose concentrations (mmol/l) of fertile and infertile cows from 8 weeks prior to the 1981/82 and 1982/83 mating seasons

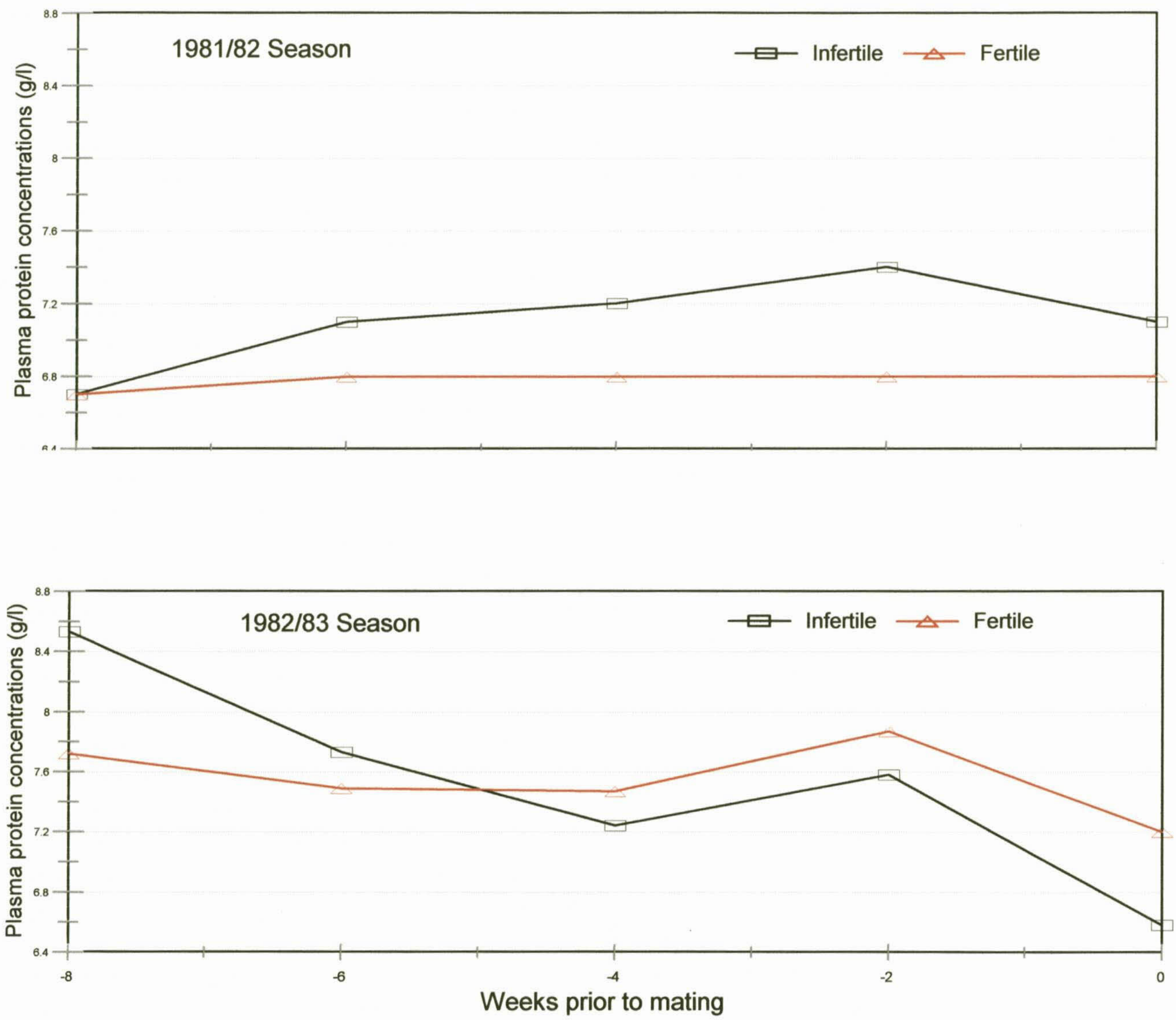


Figure 3.26 Mean plasma protein concentrations (g/l) of fertile and infertile cows from 8 weeks prior to the 1981/82 mating seasons

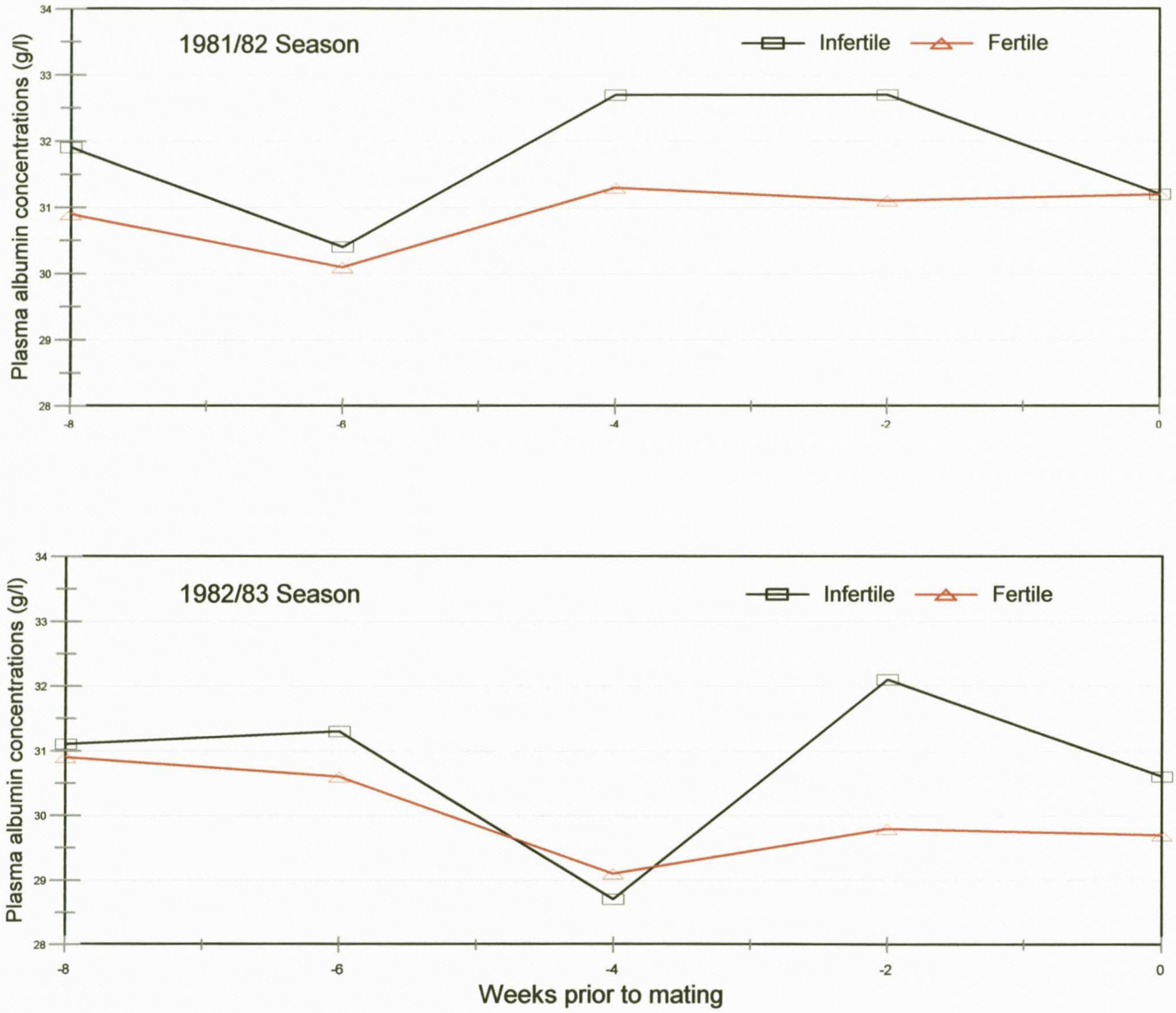


Figure 3.27 Mean plasma albumin concentrations (g/l) of fertile and infertile cows from 8 weeks prior to the 1981/82 and 1982/83 mating seasons

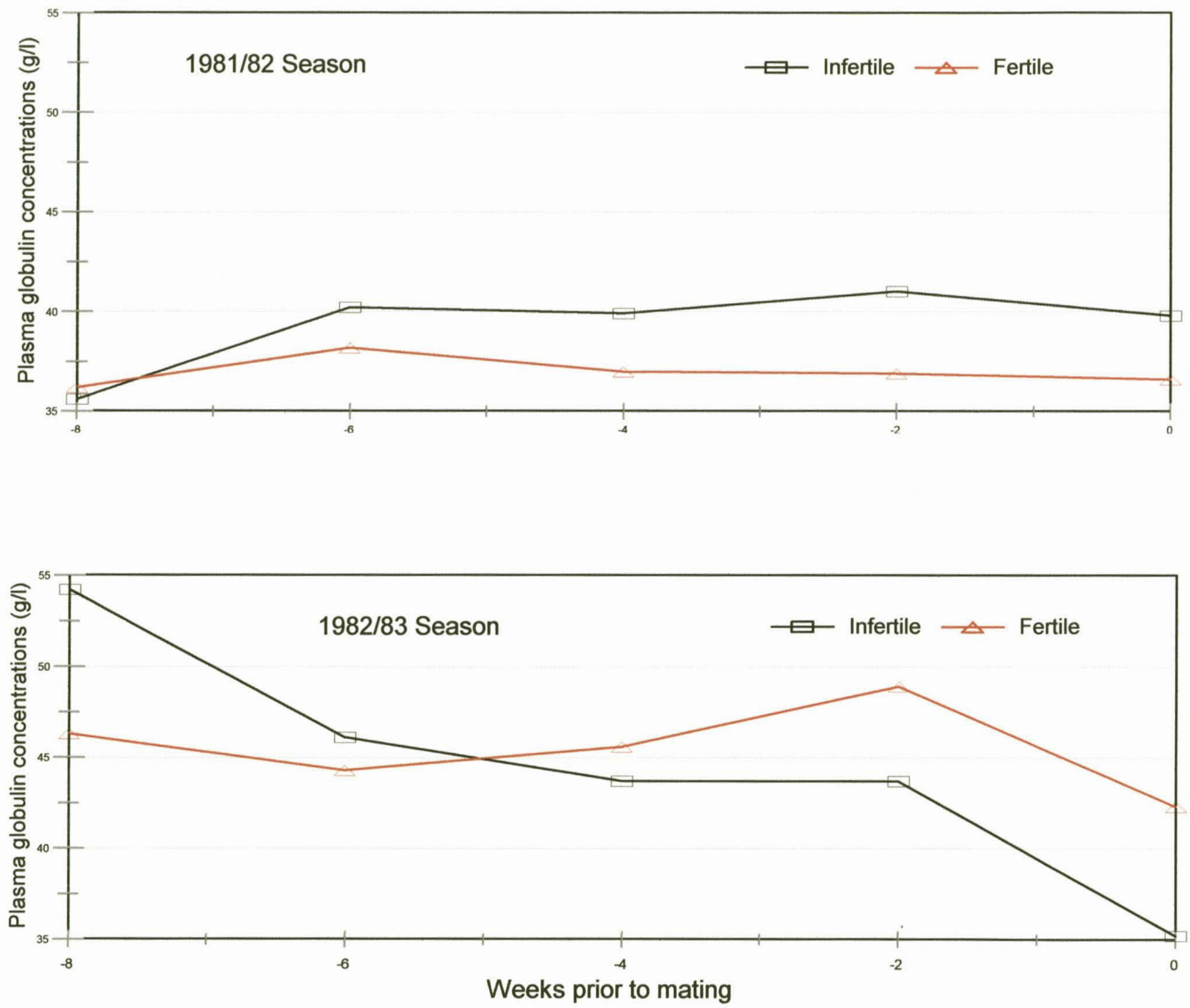


Figure 3.28 Mean plasma globulin concentrations (g/l) of fertile and infertile cows from 8 weeks prior to the 1981/82 and 1982/83 mating seasons

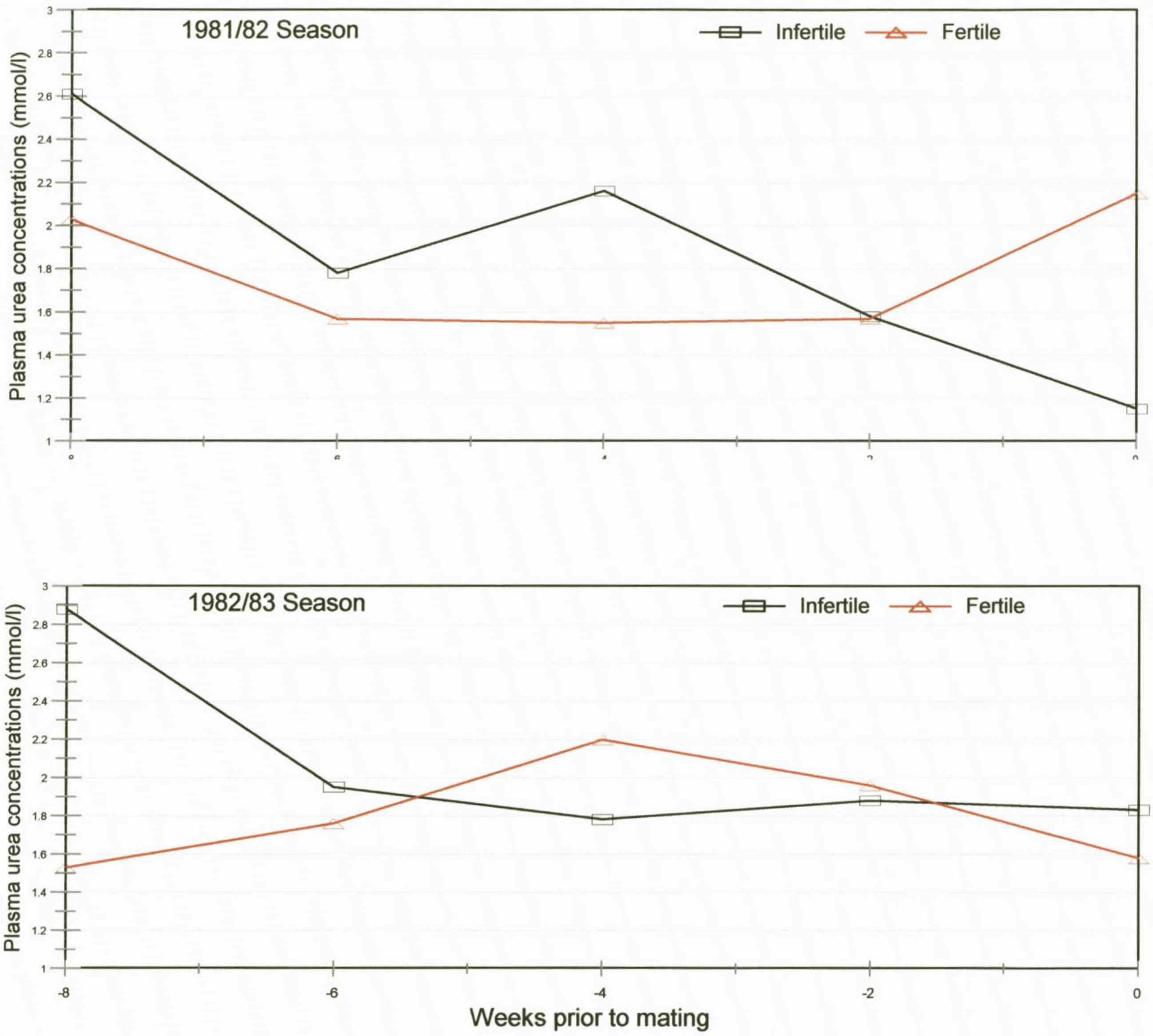


Figure 3.29 Mean plasma urea concentrations (mmol/l) of fertile and infertile cows from 8 weeks prior to the 1981/82 and 1982/83 mating seasons

in females that conceived (Table 3.6).

3.3.7.3 Hormonal profiles relating to fertility

Plasma cortisol concentrations were higher in first-calf cows that conceived, compared to infertile heifers, during both seasons (Table 3.7). From Figure 3.30 it is evident that plasma cortisol levels in both groups followed a similar pattern just prior to mating. The only difference between the groups was the large difference 6 and 8 weeks before mating.

Fertile females had plasma cortisol concentrations that were 100% (1981/82) and 56.6% (1982/83) higher than their infertile counterparts 8 weeks prior to mating.

Plasma thyroxine levels were also higher in the fertile females (Table 3.7). In this parameter, T4 profiles were constantly elevated in heifers that conceived (Figure 3.31), fertile females having 31.3% (1981/82) and 9.9% (1982/83) higher plasma T4 concentrations than the infertile animals. As was the case with plasma cortisol concentrations, the T4 levels differed more at 6 or 8 weeks prior to mating, than at mating.

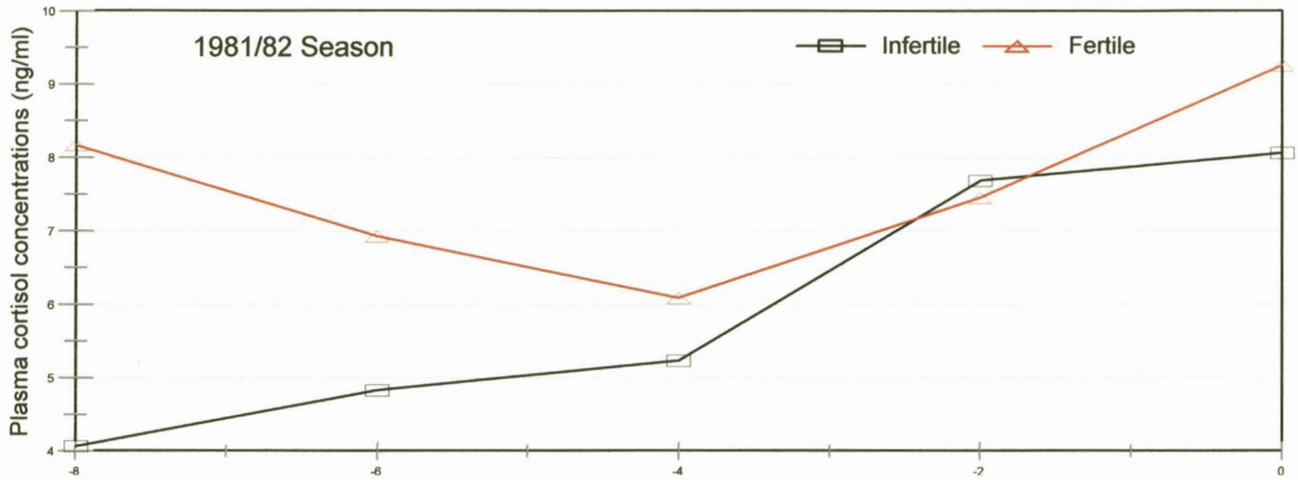
Body weight trends revealed interesting information. During 1981/82, there was a significant ($P < 0.001$) difference in body weight between females that conceived and those that did not conceive (Table 3.7). During the following season (1982/83), there was virtually no difference between the 2 groups. However, body weights of females that conceived, showed an increase during the period before being mated (Figure 3.32).

Table 3.7 Mean (± SE) plasma cortisol and thyroxine concentrations, and body weight differences in fertile and infertile cows during the 2 mating seasons

Constituent	1981/82 Season		1982/83 Season	
	Fertile	Infertile	Fertile	Infertile
Plasma cortisol (ng/ml)	$7.6^{a} \pm 0.42$	$6.0^{b} \pm 0.54$	7.3 ± 0.47	6.2 ± 0.59
Plasma thyroxine (ng/ml)	$43.1^{c} \pm 1.45$	$37.7^{d} \pm 1.85$	40.7 ± 1.36	37.9 ± 1.72
Body weight (kg)	426 ± 5.74	370 ± 7.35	445 ± 5.97	448 ± 7.57

b < a, 0.05

d < c, 0.05



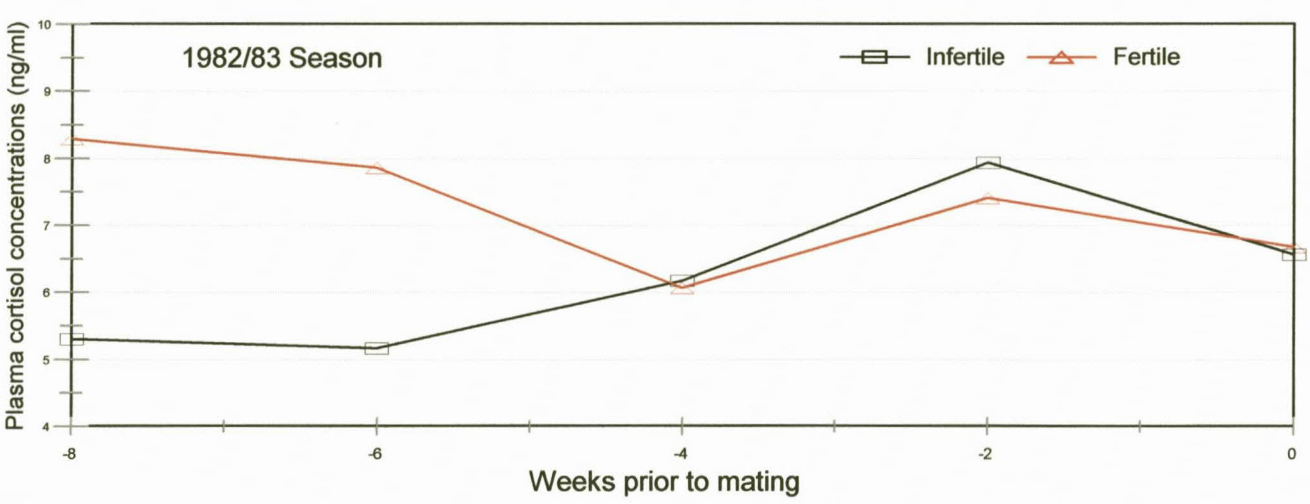


Figure 3.30 Mean plasma cortisol concentrations (ng/ml) of fertile and infertile cows from 8 weeks prior to the 1981/82 and 1982/83 mating seasons

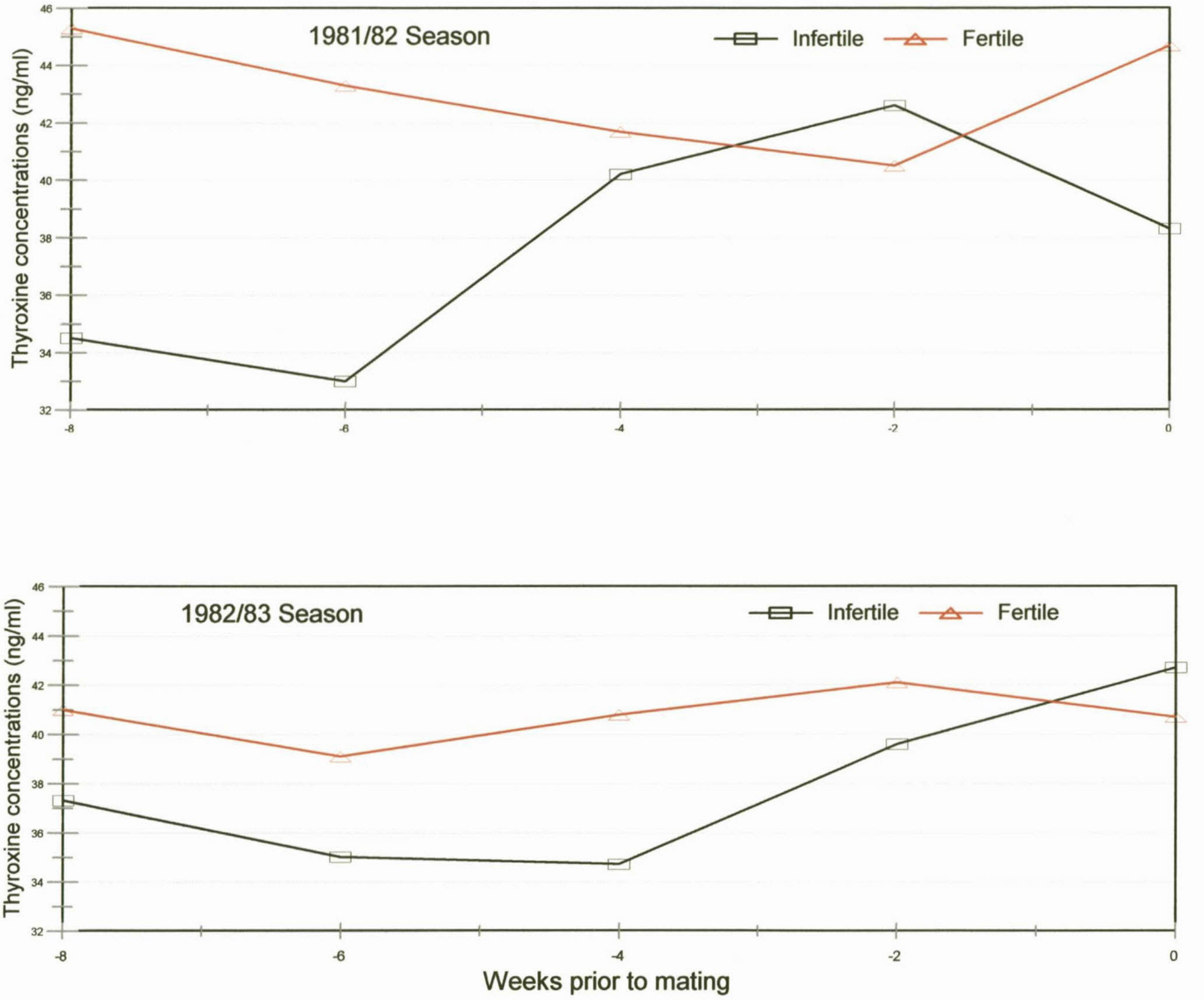


Figure 3.31 Mean plasma thyroxine concentrations (ng/ml) of fertile and infertile cows from 8 weeks prior to the 1981/82 and 1982/83 mating seasons

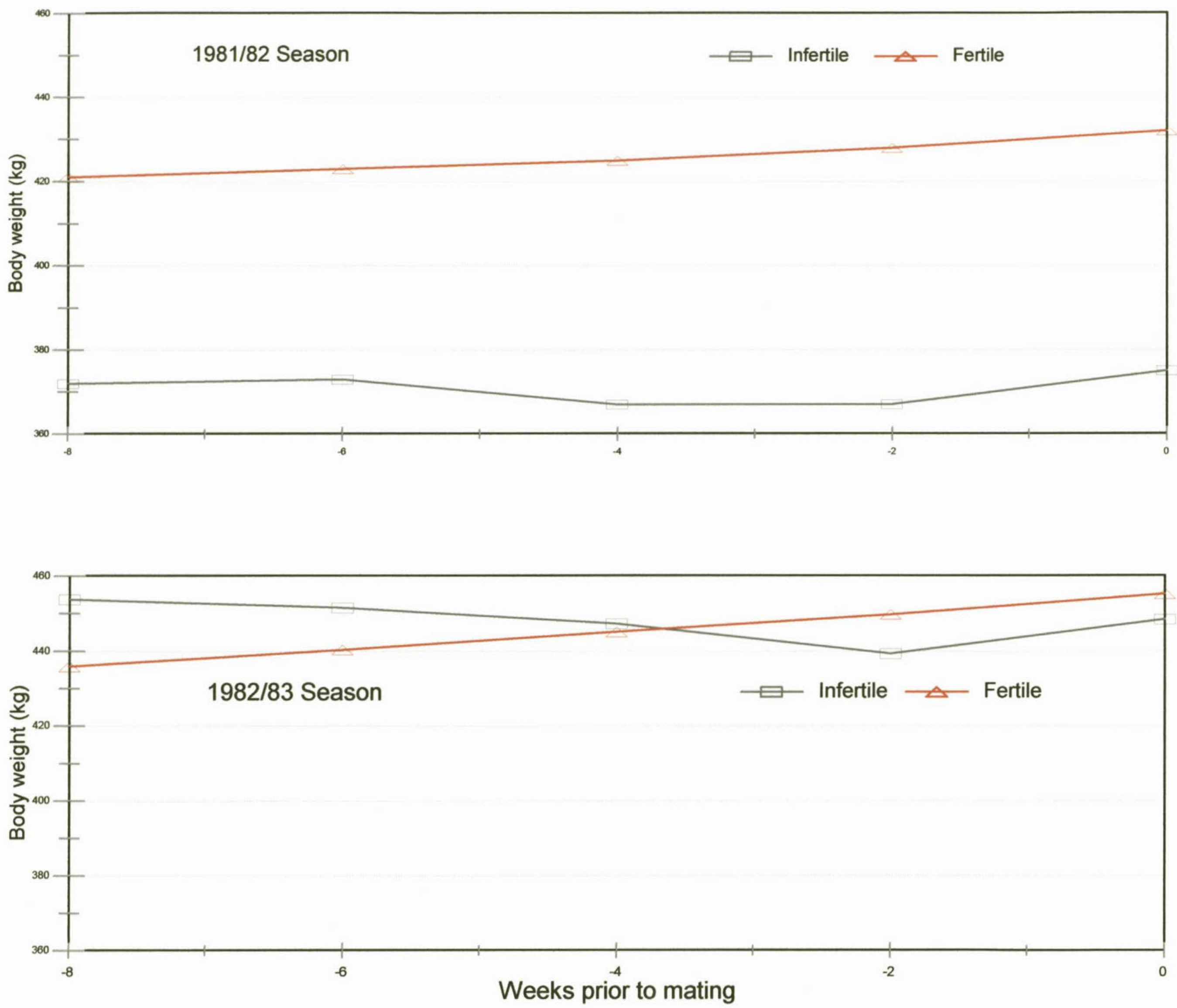


Figure 3.32 Mean body weight trends (kg) of fertile and infertile cows from 8 weeks prior to the 1981/82 and 1982/83 mating seasons

3.4 DISCUSSION

3.4.1 Influence of pregnancy on energy metabolites

Results obtained indicate that there was a tendency for blood and plasma glucose concentrations to be suppressed during pregnancy. These results are in agreement with those reported in sheep (Fukui *et al.*, 1986). In a similar vein, Hanks *et al.* (1992) observed that total volatile fatty acid concentration was lower for pregnant crossbred Angus cows compared to open cows.

It would appear as if energy metabolite levels either increased, or decreased during pregnancy and nearer to calving. Plasma glucose concentrations were slightly higher during pregnancy in crossbred Holstein cattle (Chaiyabutr *et al.*, 1998), and Vihan and Rai (1983) reported that in sheep, blood levels of glucose increased at parturition. In Suffolk sheep, no significant change in mean plasma glucose concentrations was recorded during pregnancy, although levels tended to decrease (Patterson *et al.*, 1964). These authors noted significantly raised mean plasma glucose levels at the time of lambing. In Holstein cows, the concentration of plasma-free fatty acids (FFA) and blood sugars were highest at calving. This was probably due to the hormonal and stress conditions of parturition (Radloff *et al.*, 1966). Graham (1964) reported plasma glucose levels of all Merino sheep to be fairly stable over the last few weeks of gestation, but that an increase in concentration occurred towards the end of pregnancy in those sheep which had an energy deficiency. A decrease in blood glucose of Friesian cows was observed at calving (Rowlands *et al.*, 1980). Studies with Alpine does revealed that plasma glucose decreased 7, 6 and 3% between week 13 and 21 of gestation for low, medium and high protein diets, respectively (Sahlu *et al.*, 1992).

From the literature it is obvious that, although results tend to vary, most workers report an increase in glucose levels at or around the time of parturition. Present results indicate that both increased and decreased blood glucose concentrations occur during pregnancy and parturition. Large differences between the 3 groups were prominent. e.g. in the cows on the sourveld supplemented with NPN, the suppressed blood glucose levels of pregnant animals were easily identifiable, with levels in both pregnant and non-pregnant animals increasing over time (Figure 3.1). In females on the sourveld - N treatment, blood glucose levels decreased sharply in both groups, and increased again at the onset of spring. Although there was a tendency for glucose concentrations in blood, plasma and RBC to be lower during pregnancy, it appears that nutritional status has an overriding effect on energy metabolites, since in most cases, pregnant and non-pregnant profiles responded in a similar manner to nutritional status.

An interesting observation, is that the relatively low blood glucose levels in pregnant females were recorded mostly on the sourveld. Glucose requirements for the nutrition of the foetus and for lactose production during lactation, are considerably greater than in non-pregnant and non-lactating animals (Wilson *et al.*, 1985). It is especially during the winter months, when the nutritive value of the sourveld decreases rapidly, that the foetal demands for glucose increase. It can be postulated that since foetal requirements for glucose are high, females on a low feeding regime cannot synthesize sufficient glucose from insufficient dietary nutrients to provide for the nutritional requirements of the foetus.

3.4.2 Influence of pregnancy on protein metabolites

During the first season (1981/82), pregnancy had little or no influence on the total plasma protein profiles. During the subsequent season, plasma protein levels of first-calf cows on the sourveld + N increased, whilst those on sourveld - N decreased during pregnancy (Figure 3.8). There was also a tendency for plasma protein profiles in animals to decrease on mixed sweetveld. In sheep, a decrease in total plasma protein concentrations during pregnancy occurred in control, high and low protein groups (Sykes & Field, 1973). On the other hand, it was found that total protein concentrations tended to be higher in pregnant than in cyclic goats (Huang *et al.*, 1994). In sheep, plasma protein concentration decreased, but increased in goats at parturition (Vihan & Rai, 1983).

Kim (1983) observed that total protein in milk samples of dairy cows did not change significantly during pregnancy. Similar results were obtained in sheep (Fukui *et al.*, 1986).

Present results indicate that pregnancy does not have a strong influence on plasma albumin concentration, although levels tend to decrease slightly during pregnancy. Similar results were reported by Sykes and Field (1973), who recorded a gradual decrease in sheep on a low and high protein diet. Similarly, several other workers reported a decrease in serum albumin levels in dairy cows at, or close to calving (Hojovcova, 1966; Little, 1974; Manston *et al.*, 1975; Rowlands *et* *al.*, 1975; Szulc, 1975; Treacher *et al.*, 1976; Rowlands *et al.*, 1980) and in sheep (Vihan & Rai, 1983). However, albumin concentrations increased in goats at parturition (Vihan & Rai, 1983).

Most of the present results regarding the effect of pregnancy on plasma globulin concentration is contradictory to that of Sykes and Field (1973). These authors found that globulin levels in sheep decreased gradually throughout pregnancy, with levels being 30% lower by week 20 to 21 of pregnancy. Vihan and Rai (1983) also reported that globulin levels of sheep decreased over pregnancy. Similarly, other workers found that globulin concentrations decreased during late pregnancy (Dixon *et al.*, 1961; Williams & Millar, 1979; Rowlands *et al.*, 1980). However, in goats it was reported that globulin levels tended to be higher in pregnant than cyclic females (Huang *et al.*, 1994).

Regarding plasma urea concentrations, results indicate a rise in concentration during pregnancy, except in females on the sourveld - N treatment (Figure 3.13 and 3.14). This is contradictory to results in sheep, where low and high protein diets led to decreased protein concentrations during pregnancy (Sykes & Field, 1973). Plasma urea levels in control sheep, however, increased sharply. During late pregnancy, with a sudden reduction in food intake (25 to 70% of energy requirement), urinary urea nitrogen excretion and blood urea nitrogen concentrations were highest in those sheep with the greatest foetal burden (Guada *et al.*, 1976). Herriman and Heitzman (1978) found no significant change in urea concentrations in late pregnant ewes.

It is clear that a large variation exists in the literature. While definite trends regarding the effect of pregnancy and lactation on blood metabolites were observed by some, other reports indicate no effect. So, for example, total proteins, albumin and globulin concentrations were found not to differ significantly before and after parturition in dairy cows (Pestevsek *et al.*, 1980).

It is known that a considerable increase in plasma volume of up to 35% occurs during pregnancy (Barcroft *et al.*, 1939; Sykes & Field, 1973). It, therefore, stands to reason that because of the dilution effect, a reduction in plasma concentration should occur. However, present results indicate that in only a few instances, metabolites decrease as pregnancy progresses. Of all the protein profiles investigated during the study, plasma proteins and globulins of the 1982/83 season, and plasma urea concentrations of the 1981/82 season, decreased during pregnancy. It is noteworthy that in all 3 instances, these decreases occurred in animals on a low plane of nutrition (sourveld - N group).

3.4.3 Influence of pregnancy on hormonal concentrations

3.4.3.1 Plasma cortisol concentrations

It appears that the effect of pregnancy on plasma cortisol concentrations showed no distinctive trend. There was, however, a strong tendency, in most cases, for plasma cortisol levels to decrease during pregnancy and parturition. Data were obtained over a period of 4.5 years, with animals sampled every fortnight. From the literature, it would appear that a lot of research was directed to shorter sampling periods, especially towards the end of gestation, when the frequency of sampling should be higher. According to Convey (1974), estimates of serum corticoid concentrations collected throughout pregnancy in non-lactating ruminants are not readily available. However, present results are in accordance with those of Koprowski and Tucker (1973), who found pregnancy to have no significant effect on serum corticoid concentrations. It should be noted that in the latter case, results were obtained from cows that were simultaneously lactating and pregnant.

In most other studies, definite relationships were established between corticoid concentrations and pregnancy. It was reported that glucocorticoids remained low (below 5.0 ng/ml) until a day before parturition, and increased dramatically to 10.3 ng/ml approximately 12 hours before parturition (Smith *et al.*, 1973). Similar results were obtained in Jersey and Jersey/Friesian cows, where prepartum adrenal corticoid concentrations were 6 ng/ml, rising sharply to 19.2 ng/ml at calving (Hudson *et al.*, 1976). Eissa and El-Betely (1990), reported that total corticosteroids of cows did not vary during the first 3 months of pregnancy. However, there was a significant decrease in the concentration of total corticosteroids during the fourth month of pregnancy, after which it remained fairly constant until the 9 th month. Concentrations increased 6 days before calving, and again 24 hours before calving.

In buffalo cows, the corticosteroid concentration was 1.7 ng/ml throughout gestation, increasing significantly at day 12 prepartum to 5.3 ng/ml, and peaking at 16.8 ng/ml at parturition (Eissa *et al.*, 1995). In another study with buffaloes, mean plasma cortisol levels showed little fluctuation between Day 30 and Day 2 prepartum. A small increase was observed a day before parturition, followed by a sharp increase at parturition (Prakash & Madon, 1984). In sows, plasma corticoid concentrations in late pregnancy varied appreciably from day to day, and showed no consistent pattern (Ash *et al.*, 1973). An increase in serum corticoid concentration was not recorded at parturition in sheep (Bassett & Thorburn, 1969).

Results obtained regarding plasma glucocorticoid concentrations near parturition, are in line with those reported by Adams and Wagner (1970). Levels plateaued 4 days prepartum, through to Day 2 postpartum. Brush (1958) found no significant change in plasma glucocorticoid concentration during the last 5 days prepartum and parturition. Present results seem to indicate that pregnancy per se does not influence adrenal cortex activity to a large extent, but in conjunction with feeding regime, it may have a cumulative effect. From Figure 3.17 it is evident that pregnant first-calvers on the sourveld - N feeding regime, had consistently depressed cortisol concentrations when compared with non-pregnant cows. With the supplementation of an NPN lick, this effect was, however, negligible. The plasma cortisol concentration of females on the mixed sweetveld was not influenced by pregnancy. This supposition is confirmed when the corticoid trends of both pregnant and non-pregnant animals are compared. In both sourveld groups, plasma cortisol concentrations decreased during the winter period until after calving (August/September), whereafter an increase occurred. This trend was not observed in animals on the mixed sweetveld. However, the detrimental effect of pregnancy on animals on the low feeding regime (sourveld - N) is obvious from Figure 3.17. During the 1982/83 period (Figure 3.18), results may have been clouded by the combined influence of suckling from calves born during the previous year, as well as the effect of pregnancy.

3.4.3.2 Plasma thyroxine concentrations

Thyroxine levels seemed to be less influenced by gestation, compared to adrenal cortex activity. In 1981/82, non-pregnant heifers had higher plasma T4 levels in 2 out of the 3 treatments, while the opposite trend was observed during the 1982/83 season (Table 3.3). However, there appeared to be an increase in plasma thyroxine concentration as pregnancy progressed. In all the treatments, thyroxine concentrations decreased from about 6 weeks prior to parturition, and reached a basal level at or just after parturition.

Generally, reports in the literature do not always agree on the effect of pregnancy on thyroxine concentrations, although more supported the view of decreased thyroxine concentrations at or around the time of parturition. Mel'nik *et al.* (1989) found that total thyroxine concentrations reached a precalving peak of approximately 65 nmol/l, and a minimum concentration of about 15 nmol/l from 1 to 6 weeks after calving in Russian Black Pied cows. In dairy cows, it has been noted that plasma thyroxine concentrations increased during gestation (Vanjonack & Johnson, 1975). Blood concentrations of thyroxine in crossbred Zebu cows maintained at high temperatures decreased from the first to the third trimesters of gestation (Campos *et al.*, 1993). Pethes *et al.* (1985) observed that thyroxine in blood serum decreased 14 days before and at parturition, and was significantly lower when compared to cows fed a 21% higher energy diet.

No significant change in maternal thyroxine levels was recorded in pigs between week 10 and 13 of pregnancy. A significant decrease in levels occurred during the last 2 weeks of gestation (Atinmo *et al.*, 1978).

A reason for the lower concentration of thyroid hormone before parturition may be enhanced adrenocorticotropin and cortisol secretion during this period (Pethes *et al.*, 1985). However, present results indicate that plasma T4 levels start to decrease 4 weeks before parturition. During this time, plasma cortisol concentrations were not elevated in all the groups. It has been reported that close to the time of calving, considerable transport of thyroxine occurs into the mammary gland (Roti *et al.*, 1981).

3.4.4 Influence of lactation on energy profiles

During the 1981/82 season, blood glucose concentrations increased in all 3 groups of first-calf cows after parturition, followed by a decrease during week 14 to 16 of lactation (Figure 3.1). In dairy cows, a high digestible diet led to the maintenance of high blood glucose concentration, whereas diets containing less protein and carbohydrate, led to decreased concentrations which rose 6 to 10 weeks after parturition (McClure, 1977a). Similarly, glucose concentrations in Friesian cows increased during the first week of lactation (Wittwer *et al.*, 1983).

Blood glucose levels were highest in Hereford cows receiving a balanced protein and energy diet, compared to those receiving a high protein and low energy, low protein and high energy, or low protein and low energy diets 60 to 70 days after calving (McClure, 1977b). Present results support these findings. Lactating females on the mixed sweetveld had significantly ($P < 0.01$) higher blood glucose concentrations (2.29 mmol/l), compared to cows on sourveld receiving NPN supplementation (2.14 mmol/l), or those on sourveld without NPN supplementation (2.13 mmol/l). In goats, it was found that glucose concentrations were lower in pregnant than in lactating animals, and levels increased during lactation (Mbassa & Poulsen, 1991). Arterial plasma glucose concentrations were reported to be slightly higher during gestation, but were significantly higher during lactation in crossbred Holstein cattle (Chaiyabutr *et al.*, 1998).

Oxenreider and Wagner (1971) recorded the lowest glucose concentrations in lactating Holstein cows receiving a low energy diet and suckled or milked. The highest values were recorded in non-lactating cows receiving a high energy diet. These results are in accordance with present findings, as depicted in Figure 3.1. However, the lactating cows in the study of Oxenreider and Wagner (1971) had a greater TDN deficiency on the low energy diet, compared to non-lactating cows. This was reflected in the blood glucose concentrations. Bines and Hart (1978) found that the glucose levels in Friesian heifers were almost 20% higher during the dry period, than in early lactation. Hereford-cross heifers, on the other hand, showed no marked difference in glucose levels between lactation and the dry period. Blood glucose concentrations were usually lower in early lactation than at other times (Rowlands *et al.*, 1975). Denbow *et al.* (1986) also reported that although glucose concentrations were not significantly different from early to late lactation in Holstein cattle, there was a tendency for glucose concentrations to increase as lactation progressed. In pregnant and lactating Friesian x Ayrshire cows, blood constituents were maintained within narrow limits during long-term nutritional deprivation (Roberts *et al.*, 1978).

The fact that lactation led to a decrease in blood and plasma glucose concentrations in beef heifers, indicates the importance of this process in the ruminant body. The pronounced influence also occurred in animals on the mixed sweetveld, indicating the strong and overriding influence of lactation over nutrition. However, the value of better quality natural pasture is of prime importance, as animals on the mixed sweetveld were able to maintain a blood glucose level comparable to that recorded by Payne *et al.* (1970) (45.5 mg/100ml), Hunter (1976) (44.9 mg/100ml) and McClure (1977b) (43.9 mg/100ml).

It would appear that, although foetal requirements for glucose increases during pregnancy, the process of lactation adds a greater burden on gluconeogenesis for production of milk. This is evident from the experimental animals on the mixed sweetveld (Figure 3.3), sourveld + N and sourveld - N (Figure 3.4).

3.4.5 <u>Influence of lactation on protein profiles</u>

Lactation did not appear to have a major impact on the total plasma protein concentration in the present study. However, it was reported that total serum proteins in Danish Landrace goats increased during lactation, directly proportional to parity (Mbassa & Poulsen, 1991). Smith and Kesler (1969) found that serum albumin levels in Holstein cows remained stable throughout lactation. Present results of the 1981/82 season are in accordance with those of Wittwer *et al.* (1983), who found albumin levels to decrease a week before, to 8 weeks after calving in Friesian cows. Rowlands *et al.* (1980) reported that albumin concentrations decreased at calving in some, but not all cows, and was low for up to 2 weeks.

Little (1974) noted average daily rates of increase during the first 100 days of lactation in 5 dairy herds. This observation is substantiated by Kitchenham *et al.*, (1975) and Rowlands (1978). When a low protein diet was fed to dairy cows, however, albumin concentrations failed to rise after calving (Manston *et al.*, 1975). According to Rowlands *et al.* (1975), this failure may be indicative of a low protein status in the preceding months. Present results partially support the results.

Plasma albumin concentrations continued to decrease in first-calf cows in the 2 sourveld groups after calving during the 1981/82 season, but increased in animals on the mixed sweetveld (Figure 3.9). During the 1982/83 season, this influence of lactation was not so evident.

Smith and Kesler (1969) found that α-globulin levels were lowest prepartum (1.01g), and rose to 1.4 g at week 12 postpartum. ß-globulin followed the same trend, and was highest at week 30 postpartum. In Friesian cows, globulin concentrations increased during the 3 weeks after calving (Rowlands *et al.*, 1980). Results during 1981/82 are thus in agreement with previous data. Rowlands *et al.* (1975) reported that albumin and urea concentrations were lowest during the first month of lactation, but that globulin concentrations showed the reverse trend.

In some ways, the slight decrease in urea levels in some groups of animals in the present study, corresponds to results obtained by Treacher (1978). In cows receiving a high protein diet, urea concentrations decreased just before calving, but increased immediately postpartum. In cows receiving a low protein diet, urea levels decreased from 7 months before, to 7 months after calving. Hess *et al.* (1998) reported that milk urea N concentration of *Bos taurus* cows increased significantly ($P < 0.05$) as the lactation period progressed. In Danish Landrace goats, urea concentrations decreased at early and mid-lactation, directly proportional to parity (Mbassa & Poulsen, 1991). It is in agreement with Szulc (1975), namely that the blood level of urea is influenced by type of diet rather than by stage of lactation.

Present results indicate that the energy metabolites were especially influenced by lactation, the largest effect being recorded 10 to 14 weeks postpartum. Rowlands *et al.* (1975) observed that the most significant changes in concentrations were confined to the periods up to 3 months either side of calving. It appears that species differences may add to the variation in results. For example, in sheep it was found that the levels of glucose increased at parturition, but that total protein, albumin and globulin levels decreased. In goats, the mentioned protein parameters increased at parturition. In most cases, dairy cows are managed in set practices, whilst free-grazing stock on natural pastures are more subjected to changes in feeding regimes and environmental factors such as climate.

3.4.6 **Influence of lactation on hormonal secretions**

3.4.6.1 Plasma cortisol concentrations

The increase in plasma cortisol concentrations in first-calf cows on both sourveld groups after parturition (1981/82), is in line with results of Dunlap (1981), who found cortisol levels to increase after suckling, in beef cows. Hall *et al.* (1991) also observed that cortisol release in cows was significantly above baseline levels within 15 minutes of suckling in 77% of the observed suckling events.

From Table 3.4 and 3.5 it is evident that lactation depressed adrenal cortex activity. This is particularly well illustrated in Figure 3.18. However, in their study, Smith *et al.*, (1972) came to the conclusion that the milking stimulus per se, and exteroceptive stimuli can cause increased serum corticoid levels in dairy cows. Cortisol concentrations in buffaloes decreased within 6 hours after parturition, and varied narrowly between 1.74 and 2.01 ng/ml for up to 50 days postpartum (Prakash & Madan, 1984). Plasma corticoid concentrations did not differ significantly in cows (Edgerton & Hafs, 1973) and heifers (Smith *et al.*, 1973), during the prepartum period and early lactation.

Koprowski and Tucker (1973) found that total corticoid concentrations changed little as lactation progressed, whilst lactating sheep had slightly higher basal levels of cortisol than their non-lactating counterparts (Cook, 1997). At this point, one should consider the influence of dietary nutrients on adrenal cortex activity during lactation. It appears that on a low feeding regime, such as natural pasture during winter and early spring, a definite increase in plasma cortisol concentrations occurs after parturition in first-calf cows (Figure 3.17). In the cows on the mixed sweetveld, and thus a higher feeding level, hormone concentrations fluctuated postpartum, but did not show an upswing.

Second-calf cows on the sourveld supplemented with a nitrogen lick followed a similar trend, but cows not having access to this supplementation showed decreasing plasma cortisol concentrations, except for a period of 6 weeks, 6 weeks after calving (Figure 3.18). The low plasma cortisol concentrations of mixed sweetveld cows during gestation and lactation may be the result of previous reproductive efficiency. More cows were pregnant and suckled for 2 consecutive years in this group than the 2 sourveld groups. These influences may drain body reserves, especially in animals kept on natural pasture throughout the year. This supposition is supported by blood glucose levels during the 2 years. In the first year, blood glucose concentrations showed a tendency to rise during lactation in all 3 groups. During the following year, this tendency initially materialised in the 2 sourveld groups, but not in the mixed sweetveld group. The rise in plasma corticoid concentrations postpartum, is attributed to the action of corticoids released at milking. These findings are substantiated by other researchers (Paapse *et al.*, 1971; Smith *et al.*, 1972), and may be required for maintenance of lactation (Convey, 1974).

3.4.6.2 Plasma thyroxine concentrations

Fortnightly samples indicate that plasma thyroxine concentrations were significantly depressed during lactation in most cases. These findings are in agreement with those of most other workers. Thyroid activity appeared to be depressed in lactating dairy cows, even when energy intake was low (Bertoni *et al.*, 1982). The researchers conclude that this may be a mechanism for conserving glucose for use by the mammary gland. Plasma thyroxine levels were suppressed postpartum, and during the first postpartum oestrous cycle, for a period of 3 weeks (Kesler *et al.*, 1981). According to Bines and Hart (1978), total thyroxine concentrations were not significantly different between Friesian and Hereford-cross heifers, and did not appear to change between peak lactation and the dry period in either breed. However, thyroxine concentrations for the 2 types of heifers were 44.8 and 52.0 ng/ml during the dry period, and 34.7 and 40.3 ng/ml during lactation, respectively. Thyroxine levels in cows at peak lactation are reported to be 54% of those in dry, non-pregnant cows (Lorscheider *et al.*, 1969). These results are in agreement with present findings that lactation appears to suppress thyroid activity.

There appeared to be a definite increase in plasma thyroxine concentration as lactation progressed (Figure 3.21 and 3.22). In dairy cows, there were significant differences at early, middle and late lactation in serum T4 levels. Concentrations increased as the stage of lactation progressed (Akasha *et al.*, 1985). Convey (1974) also expressed the possibility of an increase in thyroid stimulating hormone concentration following milking. Tucker

(1981), on the physiological control of lactation, states that among others, thyroid hormones may synergise with the low prolactin in ergot-treated animals and may be responsible for substantial secretion of milk postpartum. Current results indicate that plasma T4 concentrations increased in all females in different groups during both years, indicating that lactation has a stronger influence on thyroid than adrenal cortex activity, and that this gland is important to maintain the process of lactation.

3.4.7 <u>Metabolic and hormonal secretions in relation to fertility</u>

3.4.7.1 <u>Energy profiles</u>

Although there were initial decreases in blood and plasma glucose concentrations in the females that conceived, they showed higher levels 8 weeks prior to mating, compared to those animals that did not conceive. Similar results were obtained in Hereford cows that were sampled at weekly intervals. Cows that became pregnant had significantly higher plasma glucose levels during the first 12 weeks postpartum, compared to cows which did not conceive (Selk *et al.*, 1985). Buffaloes and cows with normally functioning ovaries recorded higher levels of glucose than those with completely inactive ovaries (Abdel-Reheim, 1982). Profiles in German Simmental cows indicated that there was a tendency for cows not conceiving to have a low plasma glucose concentration (Baur, 1984). More recently, it was observed that primiparous beef cows (over 3 years of age), having a higher body condition score at parturition, also had higher glucose concentrations during breeding (Vizcarra *et al.*, 1998). However, these authors concluded that concentrations of metabolites in blood were not predictive of luteal activity. Rowlands *et al.* (1980) found no relationship between conception rate and glucose concentration. Similar results have been reported in Italy (Gentile *et al.*, 1978).

3.3.7.2 <u>Protein profiles</u>

Present plasma protein profiles were not consistent enough during the postpartum period, to enable the establishment of a definite relationship with fertility. However, in most cases, plasma protein concentrations of cows which conceived were lower after parturition, and this was especially true of plasma urea levels. These results are contradictory to those of Rowlands *et al.* (1980), who found average concentrations of albumin to be significantly lower between 0 and 2 weeks after calving. This is reflected by the 8 cows which required 4 or more services, compared to 32 cows which conceived after fewer services. The authors maintain that cows which are better able to maintain a stable albumin concentration are likely to have a higher fertility. Low conception rates were associated with low glucose and plasma albumin concentrations of pasture-fed dairy cows at the commencement of mating (Wilson *et al.*, 1985).

Rowlands *et al.* (1977) found that the concentration of albumin was inversely related, and the concentration of globulin was directly related to the number of services required for conception. On the other hand, higher levels of total serum protein were related to fertility (Abdel-Reheim, 1982). The issue is contradicted by results of Cheng and Su (1980), who found that cattle with a reproduction disorder had higher concentrations of serum protein. Gentile *et al.* (1978) and Parker and Blowey (1976) observed no relationship between blood total proteins and albumin levels with fertility.

Present plasma urea results are also not in accordance with previous work. In dairy cows that failed to conceive after 3 to 5 inseminations, values for urea were low (Meli *et al.*, 1984). However, in Holstein cows in early lactation, plasma urea nitrogen was not found to be different between oestrus and day 7 of the oestrous cycle (Elrod *et al.*, 1993). Thus, results from the literature and in the present study, seem to indicate that a more consistent relationship exists between glucose concentrations and fertility, than protein concentrations and fertility.

3.4.7.3 Hormonal profiles

As was the case with blood glucose concentrations, plasma cortisol and thyroxine concentrations differed substantially between fertile and infertile cows, just after calving. This difference was nullified as time elapsed postpartum (Figure 3.30 and 3.31), until small differences were recorded at mating. Abeygunawardena (1988) came to the conclusion that the frequency of LH peaks was negatively related to the mean corticosteroid concentrations in cows, suggesting that suckling does not extend the postpartum anestrous period, primarily by a mechanism involving adrenocorticoids. This may also imply that elevated adrenocortical levels lead to depressed fertility, which is in contrast to the findings of the present study. However, it should be stressed that no significant relationship between adrenal cortex activity and fertility was recorded in this study. A study was conducted with ewes to evaluate the time-course of thyroid hormone involvement in the development of anoestrus, in consequence of the thyroid gland's role in the termination of seasonal reproductive activity in a variety of birds and mammals. Thrun *et al.* (1996) came to the conclusion that thyroid hormones need not be present for much of the breeding season (mid-September through late December) for anoestrus to develop in the ewe. It was also postulated that these hormones need to be present for only a brief period of time near the end of the breeding season for the neuroendocrine changes that lead to anoestrus.

During the period calving to mating, body weight changes between cows calving and not calving were significant during 1981/82 (Figure 3.32). Cows calving during 1982, showed an increase in body weight 8 weeks prior to mating, and during the mating season of 1981/82. The open cows maintained body weight during this period. During the following year (1982/83), cows not calving showed a downward body weight trend during the mating period, whilst the cows that calved, showed a consistent increase in body weight. These results are in line with those reported in British Friesian heifers, where the highest pregnancy rate was recorded for heifers that gained weight (Baishya *et al.*, 1982). Although Boadi and Price (1996) reported significant differences in body weights and body condition scores between restricted and supplemented beef cows, the precalving conditions did not affect the calving rate. It is interesting to note that in the second year (1982/83) of the present study, the difference at mating between calvers and non-calvers was only seven kg per heifer. It is thus deduced that in order to achieve a high calving rate, cows should be gaining weight during the period after calving to the end of the mating season. This appears to be a better strategy to follow, than attaining a minimum target weight at mating.

3.5 CONCLUSIONS

Blood and plasma glucose concentrations were depressed in heifers pregnant with their first calves. These 2 parameters may thus be useful tools in diagnosing pregnancy in heifers. In addition, low blood glucose concentrations during pregnancy may assist in identifying low nutrient intake. This was illustrated in animals on the sourveld, and not supplemented with non-protein nitrogen during the winter. The sharp decrease in blood glucose concentrations (March to August) from approximately 2.79 mmol/l to 1.68 mmol/l in pregnant and non-pregnant first-calf cows, indicated a severe depletion in energy reserves. In order to curb this condition, it is recommended that the supplementation of NPN commence earlier in the year, viz. at the beginning of March instead of the beginning of May, as is usually practised. Further research is required to validate findings. Should this recommendation be insufficient to maintain homeostasis in the pregnant female, to enhance nutrient supply to the foetus and provide for lactational demands, further supplementation, in the form of silage and/or hay, should be considered for first-time pregnant beef females.

It is difficult to establish a norm for blood glucose concentrations during pregnancy, the reason being that levels tend to increase or decrease during gestation, depending on nutritive intake as seen in the results. Nutritional factors appear to have an overriding effect over pregnancy per se. However, as females on the mixed sweetveld have a high annual reproductive rate, it is suggested that a minimum blood glucose concentration of 2.23 mmol/l be used as a norm for maintaining pregnancy in cows on the high rainfall natural pastures of the Eastern Cape.

Results indicate that plasma and RBC glucose concentrations are less sensitive as indicators of nutritive deficiencies during gestation. Protein metabolites varied between treatments. Neither total plasma protein, albumin, globulin, nor urea concentrations showed any tendency towards being influenced by pregnancy.

Plasma cortisol concentrations in pregnant first-calf cows were influenced by the nutritive value of the natural pastures. The fact that lower plasma cortisol concentrations occurred during pregnancy on the sourveld, and not on the mixed sweetveld, indicate the nutritive shortfalls of the sourveld pasture during this period. As gluconeogenesis is stimulated by corticoids, the low plasma cortisol concentrations inhibited this process, causing lower blood glucose concentrations. However, plasma cortisol concentrations of the animals on the mixed sweetveld were regulated at a higher level, which thus stimulated gluconeogenesis and led to higher blood glucose concentrations.

Blood samples were taken at too long intervals to effectively study the influence of parturition on plasma cortisol concentrations. Plasma thyroxine concentrations did not appear to be drastically influenced by pregnancy. Levels tended to rise as pregnancy progressed, but declined nearer to parturition.

Results indicate that lactation influenced blood and plasma glucose levels during the first 6 to 8 weeks after parturition. Levels were depressed, presumably because of the draining of glucose into the mammary gland. The increase in blood and plasma glucose concentrations is attributed to the increased nutritive value of the natural pasture during spring. As was the case during pregnancy, plasma protein profiles were not influenced by lactation to a large extent.

Plasma cortisol concentrations increased after parturition, in both lactating and non-lactating first-calvers on sourveld during the first year. However, the increase seems to be subject to a nutritional influence - levels in females on the sourveld - N increased at a slower rate than animals on the sourveld + N. Cows lactating for the second consecutive year were under nutritional stress, even on the mixed sweetveld. It is apparent that this influence suppressed gluconeogenesis, hence the lowered blood glucose concentrations experienced during this period.

Plasma thyroid activity was also dramatically influenced, especially during second lactation. During the first lactation, plasma T4 levels were suppressed for 6 to 12 weeks postpartum, whereas during the second lactation, plasma T4 concentrations were suppressed for a period of 22 weeks after parturition. It appears that previous reproductive performance influences subsequent plasma T4 levels during lactation to a large degree. Thyroid activity was more suppressed in cows in their second lactation and on a higher feeding regime, than dry and lactating animals on a lower plane of nutrition.

Results obtained in this trial indicate that normal metabolic activity is strained in females on the sourveld without NPN supplementation in the winter, especially during pregnancy and lactation. Beef females on the sourveld, supplemented with NPN, may have normal metabolic activities during their first reproductive season, but not during the second. The lower blood and plasma glucose, cortisol and thyroxine concentrations of animals even on the mixed sweetveld, indicate the need for additional feeding, such as hay or silage in order to supplement the requirements during the first 8 weeks after parturition. This should not only provide the nutritional needs for the process of lactation, but also improve conception rates in the subsequent mating period.

Blood glucose, and to a lesser extent plasma glucose concentrations after calving, may indicate whether cows will conceive during the subsequent mating period. Cows that conceived had a blood glucose concentration of 2.46 mmol/l, compared with 1.90 mmol/l of open cows after parturition. During the second year, the blood plasma concentrations were 2.21 vs. 2.07 mmol/l, respectively. Similarly, both plasma cortisol and thyroxine concentrations were significantly elevated in fertile cows after calving. While blood glucose results substantiate previous findings, additional research is required to verify the relationship between plasma cortisol and thyroxine concentrations, and fertility in beef females.

Although body weight per se is an important parameter to be used as a guide for mating, body weight trends, such as an increase or loss of weight during the mating season, may be a better criterion for predicting successful reproductive efficiency.

3.6 SUMMARY

Pregnancy did not influence blood glucose concentrations in Bonsmara first-calf cows to any large extent, the mean concentrations being 2.06 ± 0.03 vs. 2.25 ± 0.04 ($P < 0.01$), 2.16 ± 0.04 vs. 2.13 ± 0.04 and 2.27 ± 0.04 vs. 2.28 ± 0.05 mmol/l for animals on the sourveld + N, sourveld - N and mixed sweetveld + N treatments during 1981/82, respectively. During 1982/83, all the cows on the mixed sweetveld + N treatment conceived. The mean concentrations for females on the sourveld + N and sourveld - N treatments were 1.90 ± 0.03 vs. 2.16 ± 0.04 ($P < 0.001$) and 2.21 ± 0.04 vs. 2.18 ± 0.04 mmol/l, respectively. Mean plasma glucose concentrations were 3.20 ± 0.06 vs. 3.25 ± 0.06, 3.26 ± 0.06 vs. 2.98 ± 0.06 ($P < 0.01$) and 3.50 ± 0.05 vs. 3.21 ± 0.07 mmol/l for animals on the 3 treatments respectively during 1981/82, and 3.09 ± 0.05 vs. 3.26 ± 0.05 (P < 0.05) and 3.37 ± 0.06 vs. 3.25 ± 0.05 mmol/l respectively during 1982/83. Mean red blood cell glucose levels were generally elevated in pregnant first-calf cows during 1981/82, viz. 1.13 ± 0.04 vs. 1.0 ±0.04 (P < 0.05), 1.1 ± 0.04 vs. 0.85 ± 0.04 (P < 0.01) and 1.23 ± 0.03 vs. 0.93 ± 0.05 (P < 0.001) mmol/l respectively. During the following year, the influence of pregnancy on RBC glucose was less; namely 1.20 ± 0.04 vs. 1.10 ± 0.04 and 1.16 ± 0.04 vs. 1.08 ± 0.04 mmol/l respectively.

Pregnancy did not have a significant effect on total plasma protein concentration. Pregnant and open first-calvers on the sourveld + N, sourveld - N and mixed sweetveld + N had concentrations of 65 ± 0.92 vs. 68 ± 10, 70 ± 0.81 vs. 66 ± 0.78 (P < 0.05) and 71 ± 0.55 vs. 72 ± 0.75 g/l respectively during 1981/82, and 73 ± 1.20 vs. 74 ± 1.30 and 76 ± 1.40 vs. 75 ± 0.1.20 (P < 0.05) g/l respectively during the 1982/83 season. Albumin concentrations were not significantly different between pregnant and open first-calf cows during the 1981/82 season, viz. 32 ± 0.44 vs. 32 ± 0.48, 33 ± 0.49 vs. 31 ± 0.47 (P < 0.05) and 34 ± 0.44 vs. 34 ± 0.06 g/l respectively. However, during the 1982/83 season, levels were significantly elevated in pregnant cows on the 2 sourveld treatments, viz. 31 ± 0.40 vs. 29 ± 0.50 (P < 0.05) and 32 ± 0.40 vs. 30 ± 0.40 (P < 0.05) g/l respectively. Globulin concentrations were unaffected by pregnancy status (35 ± 0.75 vs. 35 ± 0.81, 37 ± 0.91 vs. 36 ± 0.87 and 38 ± 0.62 vs. 38 ± 0.85 g/l respectively for the females on the 3 treatments during 1981/82, and 42 ± 1.20 vs. 44 ± 1.30 and 44 ± 1.30 vs. 45 ± 1.20 g/l respectively for second-calf cows on the sourveld + N and sourveld - N respectively during 1982/83. Plasma urea levels were generally elevated in pregnant females, namely 1.80 ± 0.18 vs 1.70 ± 0.20, 1.47 ± 0.12 vs. 1.25 ± 0.11 and 3.80 ± 0.17 vs. 3.76 ± 0.23 mmol/l during 1981/82, and 1.83 ± 0.09 vs. 1.73 ± 0.10 and 1.57 ± 0.11 vs. 1.71 ± 0.09 mmol/l respectively during the 1982/83 season.

Pregnancy resulted in significant (P < 0.05) higher plasma cortisol concentrations during the 1979 season, with values being 11.6 ± 0.41 vs. 9.6 ± 0.71 ng/ml for animals on the sourveld + N. However, during 1981, a reverse situation occurred, with pregnant females on the sourveld + N having concentrations of 9.4 ± 0.58 vs. 18.2 ± 0.92 ng/ml (P < 0.001) for open females, whilst those on the mixed sweetveld + N had concentrations of 11.8 ± 0.0.89 vs. 14.6 ± 1.11 ng/ml respectively. During the 1981/82 season, plasma cortisol concentrations were 9.8 ± 0.60 vs. 7.7 ± 0.70 ($P < 0.05$), 7.1 ± 0.50 vs. 7.6 ± 0.50 and 10.9 ± 0.45 vs. 8.5 ± 0.66 ($P < 0.01$) ng/ml for the 3 treatments respectively. During the subsequent year, virtually no difference was noted between pregnant and open cows; 6.7 ± 0.30 vs. 6.8 ± 0.35 and 6.9 ± 0.36 vs. 6.8 ± 0.31 ng/ml for cows in the sourveld + N and sourveld - N groups respectively.

Pregnancy also influenced plasma thyroxine concentrations to varying degrees. During 1979, plasma T4 levels were similar, viz. 50.1 ± 1.04 vs. 50.1 ± 1.84 ng/ml respectively. During 1980, values recorded on the sourveld + N treatment were 52.8 ± 1.13 vs. 57.6 ± 1.77 ($P < 0.05$), and on the mixed sweetveld + N treatment 53.3 ± 1.52 vs. 52.0 ± 1.91 ng/ml respectively. Inconsistent results were also obtained during the 1981/82 season (40.0 ± 0.98 vs. 43.8 ± 1.14 [$P < 0.05$], 37.0 ± 1.56 vs. 44.7 ± 1.56 [$P < 0.01$] and 49.8 ± 1.05 vs. 44.2 ± 1.52 [$P < 0.001$] ng/ml for the 3 treatments respectively), and the 1982/83 season (39.3 ± 0.96 vs. 39.4 ± 1.13 and 43.4 ± 0.99 vs. 38.5 ± 0.85 for the sourveld + N and sourveld - N treatments respectively). Al the cows in the mixed sweetveld + N group were pregnant during the 1982/83 season.

Lactation suppressed blood and plasma glucose concentrations for 8 to 10 weeks after parturition. RBC glucose concentrations were not consistently influenced, although in this case, too, levels were lower during lactation. The influence of lactation on total plasma protein, albumin and globulin concentrations were not significant, and variable. Plasma urea levels were considerably suppressed in lactating first-calf cows on the sourveld + N, sourveld - N and mixed sweetveld + N treatments during the 1981/82 season, viz. 1.28.± 0.23 vs.1.96 ± 0.17 ($P < 0.05$), 1.02 ± 0.15 vs. 1.53 ± 0.10 ($P < 0.01$) and 3.05 ± 0.33 vs. 3.98 ± 0.16 ($P < 0.05$) respectively. Little differences were noted during the subsequent year.

The adrenal cortex and thyroid activity were suppressed by the effect on lactation in first-calf cows on the sourveld - N and mixed sweetveld + N groups during the 1981/82 season.

Cows having a mean blood glucose concentration of 2.49 mmol/l after first parturition (8 weeks prior to mating) conceived at next mating, whilst infertile cows had mean concentrations of 1.93 mmol/l. During the following year, fertile and infertile cows had glucose concentrations of 2.20

and 2.07 mmol/l, respectively. Similar results were obtained for plasma glucose (3.57 vs. 2.75 mmol/l during 1981/82, and 3.36 vs. 3.27 mmol/l during 1982/83), cortisol (8.2 vs. 4.0 ng/ml, and 8.3 vs. 5.3 ng/ml for both seasons) and thyroxine (45.2 vs. 34.5 ng/ml, and 41.0 vs. 37.2 ng/ml for both seasons). Fertile cows were in a weight gaining phase from parturition to mating.

It is concluded that blood and plasma glucose concentrations may be useful tools in diagnosing pregnancy in heifers pregnant with their first calves. The sharp decline in blood glucose concentrations from March to August, from 2.79 mmol/l to 1.68 mmol/l in both pregnant and open first-calf cows, indicate that this parameter may assist in identifying low nutrient intake. The present practice of providing an NPN supplementation from May onwards, is insufficient to curb the severe depletion of energy reserves of Bonsmara females on the Dohne Sourveld. Further supplementation in the form of hay and/or silage should be provided to enhance nutrient supply to the foetus and provide for lactational demands.

Blood glucose, and to a lesser extent plasma glucose concentrations after calving, may indicate whether cows will conceive during the subsequent mating period. Similarly, plasma cortisol and thyroxine concentrations were significantly elevated in fertile cows after calving. These parameters, measured just after calving, may thus also prove to be useful in predicting subsequent fertility in cows.

CHAPTER 4

INFLUENCE OF SEASON ON BLOOD METABOLITES, AND PLASMA CORTISOL AND THYROXINE CONCENTRATIONS IN BONSMARA FEMALES

4.1 INTRODUCTION

Blood chemistry is known to be affected by a variety of factors and stimuli. These have been discussed in the preceding chapters. Thus far, the influences of nutrition, gestation and lactation have mainly been discussed, and their influences on blood metabolic profiles outlined.

Although the Compton Metabolic Profile Test (Payne, 1972; Payne *et al*., 1970) was designed to monitor dairy herds for abnormal blood chemistry, it soon became apparent that season of the year influenced the blood parameters. Evidence was gained to demonstrate the influence of season of the year on blood profiles (Payne *et al*., 1973; Payne *et al*., 1974; Rowlands *et al*., 1974). Johnson *et al*. (1995) reported a seasonal variation in immunoglobulin levels in goats, regardless of diet provided. Levels were highest during the dry season, which is from June to December, and lowest during the rainy season, which is from January to May. Indeed, most abnormalities are related to time of year (Payne, 1978). It is known that only small quantities of blood glucose are absorbed from the alimentary tract in ruminants. Yet, the effects of high ambient temperatures on blood glucose metabolism may be of specific importance to livestock, with their reliance upon gluconeogenesis (Sano *et al*., 1983). Shell *et al*. (1995) reported that, although unaffected by nutrition, shading significantly influenced plasma urea-nitrogen change in beef cattle. According to McDowell (1972), the tropical areas of the world are becoming more important in contributing food supply to the world. However, these regions are known for the high environmental temperatures during the summer season, and the consequent environmental stresses on reproduction have been reviewed. Moberg (1976), stated that among environmental stresses, temperature stress is the most radical. Whilst it is agreed that low temperatures have a minimal effect on reproduction, elevated environmental temperature can produce a heat stress sufficient to reduce fertility.

The maintenance of homeostasis is an important factor in animal production. According to Christison and Johnson (1972), one of the most general responses of an animal to a stressor is the activation of the hypothalamic-pituitary-adrenal axis. Endocrine glands of mammals living in hot climates are undoubtedly involved in the adaptive process (Rhynes & Ewing, 1973).

Both the adrenal and thyroid glands are important in regulating many processes in the animal body. One of the factors affecting the release of hormones from these glands, is environmental stimuli. It has been reported that high ambient temperatures influence the adrenal cortex (Bergman & Johnson, 1963; Stott & Robinson, 1970; Rhynes & Ewing, 1973; Lee *et al.*, 1976). According to Turner and Bagnara (1971), adrenalectomized animals are unable to tolerate stresses of, among others, cold and heat. On the other hand, cortical hormones can fully restore the resistance of such animals to temperature changes. Similarly, researchers have reported adrenocortical responses to environmental stress (Reid, 1962; Reid & Mills, 1962; Thompson *et al.*, 1963). The reproductive process is altered in animals subjected to physiological stress (Stott & Robinson, 1970). Miller and Alliston (1974) reported the adverse environmental effects on reproduction to be greatest at or near the time of mating. It has also been reported that prolonged exposure to heat depressed plasma cortisol during 2 oestrous cycles in Guernsey heifers (Abilay *et al.*, 1975).

Normal reproduction in sheep (Thwaites, 1968) and cattle (Plasse *et al.*, 1970) was adversely affected by high ambient temperature. According to Stott (1972), physiological processes affected are short oestrous periods, abnormal oestrous cycles, increased proportion of abnormal ova shed, decreased fertilization rate, and increased embryonic and foetal mortality during gestation. In a review on the effect of season, climate and temperature on reproduction and lactation, Thatcher (1974) suggested that reduced adrenal responsiveness may exist in animals exposed to mean ambient temperatures of above 21.1°C, and that such conditions may contribute to depressions in heat production during thermal stress.

It is recognized that environmental temperature also influences the thyroxine secretion rate (Premachandra *et al.*, 1958). The importance of the thyroid gland was pointed out, when shown that thyroidectomized animals survived for only relatively short periods at low environmental temperatures (Turner & Bagnara, 1971). Thyroid function being related to season has been reported in sheep (Henneman *et al.*, 1952) and dairy cattle (Lodge *et al.*, 1957; Premachandra *et al.*, 1958).

In view of the effect which temperature, and thus season, has on blood metabolites, the adrenal cortex and thyroid activity, this study aimed to determine to what degree seasonal changes influence these parameters in heifers and cows under free-grazing conditions, and the possible effects on reproduction performance.

4.2 EXPERIMENTAL PROCEDURES

Five Bonsmara first-calf cows were used in each of the following 3 experimental groups: Group 1, females were run on the Dohne Sourveld, supplemented with a bone meal and salt lick in the summer season, and an NPN lick during the winter season (sourveld + N); Group 2, females were run on the Dohne Sourveld, supplemented with bone meal and salt lick throughout the year (sourveld - N); Group 3, females were run on the mixed sweetveld, supplemented with a bone meal and salt lick in the summer, and an NPN lick in the winter (mixed sweetveld + N).

Blood samples were obtained via the jugular vein at fortnightly intervals (09:00) in heparin tubes, packed on ice, and immediately analysed for blood and plasma glucose, total plasma protein, plasma albumin and plasma urea concentrations. Aliquots of plasma samples from each experimental animal were stored at -20°C for the later determinations of cortisol and thyroxine concentrations. The methods used for the analyses of the mentioned blood and plasma metabolites and hormone concentrations, were previously described (Experimental Procedures, paragraph 8 Methods and Data Collection, pp 11 to 12).

4.3 RESULTS

4.3.1 Influence of season on energy profiles

The influence of season on blood, plasma and red blood cell glucose concentrations during the 1981/82 season is presented in Table 4.1. In all 3 treatments, glucose concentrations were higher in the summer, compared to the winter seasons.

Table 4.1 Mean (± SE) concentrations of blood and plasma metabolites in beef females on different sourveld and mixed sweetveld treatments during 1981/82

Constituent	Sourveld + N		Sourveld - N		Mixed sweetveld + N	
	Summer	Winter	Summer	Winter	Summer	Winter
Blood glucose (mmol/l)	2.20 ± 0.04	2.11 ± 0.04	2.27[i] ± 0.04	2.02[j] ± 0.04	2.43[w] ± 0.04	2.12[x] ± 0.05
Plasma glucose (mmol/l)	3.32[a] ± 0.06	3.11[b] ± 0.04	3.33[k] ± 0.06	2.90[l] ± 0.06	3.46[y] ± 0.05	3.25[z] ± 0.06
RBC glucose (mmol/l)	1.12 ± 0.04	1.01 ± 0.04	1.06[m] ± 0.04	0.89[n] ± 0.04	1.03 ± 0.04	1.13 ± 0.04
Total protein (g/l)	67.0 ± 0.90	66.0 ± 0.10	70.0[o] ± 0.80	66.0[p] ± 0.80	72.0 ± 0.06	71.0 ± 0.07
Plasma albumin (g/l)	31.0[c] ± 0.45	33.0[d] ± 0.45	30.0[q] ± 0.05	33.0[r] ± 0.05	33.0[aa] ± 0.05	35.0[bb] ± 0.06
Plasma globulin (g/l)	38.0[e] ± 0.80	32.0[f] ± 0.80	40.0[s] ± 0.90	33.0[t] ± 0.90	40.0[cc] ± 0.70	36.0[dd] ± 0.80
Plasma urea (g/l)	1.25[g] ± 0.18	2.25[h] ± 0.19	1.13[u] ± 0.11	1.58[v] ± 0.11	3.73 ± 0.18	3.78 ± 0.22

b < a, 0.05
c < d, 0.01
f < e, 0.01
g < h, 0.01
j < i, 0.01
l < k, 0.001

n < m, 0.05
p < o, 0.05
q < r, 0.01
t < s, 0.001
u < v, 0.05
x < w, 0.001

z < y, 0.05
aa < bb, 0.01
dd < cc, 0.01

Blood glucose profiles during the year are depicted in Figure 1.5. Concentrations decreased from approximately 2.90 mmol/l in mid-March (early autumn) to 1.79 mmol/l towards the end of July (winter). This represents a decrease of 38.5%. An upswing in levels occurred early in the season (at the beginning of August) and increased significantly to higher levels by the beginning of March 1982. During this year, blood glucose concentrations were highest in the animals on the mixed sweetveld, especially during the summer season (Table 4.1).

Plasma glucose concentrations originally followed the same pattern as the blood glucose concentrations, decreasing sharply from March to May (autumn) (Figure 1.6). This was followed by a slight increase, and another decrease during January and beginning of February. For this parameter, concentrations of first-calf cows on the mixed sweetveld, were highest and concentrations of animals on the sourveld without nitrogen supplementation, the lowest. Red blood cell glucose concentrations only partially reflected seasonal trends (Figure 1.7). The sharp increase during July (winter), and decrease during February (summer) did not reflect blood and plasma glucose concentrations. Furthermore, RBC glucose levels were higher in winter than in summer in females on the mixed sweetveld.

During the 1982/83 season, opposite results were recorded, with energy profiles consistently lower in summer than in winter (Table 4.2).

Blood glucose concentrations showed a tendency to decrease towards the beginning of August (Figure 1.8). A sharp increase in levels occurred during mid-September (spring); thereafter, the concentrations decreased as the season progressed. It is apparent that cows on the sourveld - N treatment, had the highest blood glucose concentrations during both seasons. Plasma glucose concentrations also showed no relationship with time of the year (Figure 1.9). In fact, the increased levels experienced during May 1982 and 1983 are in sharp contrast with results reported during 1981/82. However, the increase in August is in line with results from the previous year. As was the case during the first year, RBC glucose concentrations did not show a strong relationship with season of the year (Figure 1.10).

Table 4.2 Mean (± SE) concentration of blood and plasma metabolites in beef females on different sourveld and mixed sweetveld treatments during 1982/83

Constituent	Sourveld + N		Sourveld - N		Mixed sweetveld + N	
	Summer	Winter	Summer	Winter	Summer	Winter
Blood glucose (mmol/l)	1.98[a] ± 0.04	2.08[b] ± 0.04	2.13[m] ± 0.04	2.27[n] ± 0.04	2.01[s] ± 0.04	2.13[t] ± 0.05
Plasma glucose (mmol/l)	3.07[c] ± 0.06	3.30[d] ± 0.06	3.25 ± 0.06	3.38 ± 0.06	3.07[u] ± 0.05	3.45[v] ± 0.06
RBC glucose (mmol/l)	1.08[e] ± 0.04	1.21[f] ± 0.04	1.11 ± 0.04	1.13 ± 0.04	1.06[w] ± 0.04	1.30[x] ± 0.04
Total protein (g/l)	70.0[g] ± 1.30	76.0[h] ± 1.20	73.0[o] ± 1.30	78.0[p] ± 1.30	72.0[y] ± 1.50	78.0[z] ± 1.70
Plasma albumin (g/l)	30.0 ± 0.50	30.0 ± 0.50	31.0 ± 0.40	31.0 ± 0.40	32.0 ± 0.40	31.0 ± 0.40
Plasma globulin (g/l)	40.0[i] ± 1.30	46.0[j] ± 1.20	42.0[q] ± 1.30	47.0[r] ± 1.20	40.0[aa] ± 1.50	47.0[bb] ± 1.70
Plasma urea (g/l)	1.52[k] ± 0.10	2.05[l] ± 0.10	1.60 ± 0.10	1.67 ± 0.10	2.91 ± 0.15	3.10 ± 0.17

a < b, 0.05
c < d, 0.01
e < f, 0.05
g < h, 0.01
i < j, 0.01
k < l, 0.01

m < n, 0.05
o < p, 0.05
q < r, 0.05
s < t, 0.05
u < v, 0.001
w < x, 0.01

y < z, 0.05
aa < bb, 0.05

4.3.2 Influence of season on protein profiles

During 1981/82, season did not have the same influence on the 4 protein constituents (Table 4.1). Total plasma protein concentrations were not significantly affected by season of the year. However, Figure 1.11 indicates a definite downward trend of total plasma protein concentrations towards the end of June, followed by a gradual increase as the season progressed. The higher protein profiles of first-calf cows on the mixed sweetveld was evident.

According to Table 4.1, plasma albumin levels were higher during winter compared to summer. This could be explained by the fact that albumin concentrations were already at a high level during April (when the winter season commenced) and increased until the end of July (Figure 1.12). Levels were slow to recover during the subsequent season.

It appears that plasma globulin levels were influenced most by season of the year (Table 4.1). These profiles followed those of total protein, decreasing during the winter, and indicating an early upswing during July (Figure 1.13). However, in this parameter, the increase continued consistently throughout the summer season until February, 1982.

Plasma urea concentrations yielded interesting results. In both sourveld groups, levels were significantly higher in winter compared to summer, with no difference being recorded between seasons in the mixed sweetveld group (Table 4.2). From Figure 1.14 it is evident that season had practically no influence on plasma urea profiles; there was rather a major influence of nutrition on this constituent. As was the case with energy metabolites, protein concentrations were higher during the winter than in the summer months, during 1982/83.

In 1982/83, total plasma protein concentrations were significantly elevated in all 3 treatment groups during the winter season (Table 4.2). Concentrations increased during the winter period towards the end of June (winter). After a sharp decrease during October, levels decreased as the summer season progressed (Figure 1.15). Plasma albumin levels were not in any way influenced by season of the year (Figure 1.16).

Plasma globulin concentrations were significantly higher in winter than in summer for all 3 treatment groups (Table 4.2). Similar to 1981/82, plasma globulin concentrations showed a striking resemblance to those of total plasma protein concentrations during both seasons (Figure 1.17). Although plasma urea concentrations were also higher in winter than in summer, the effect was very slight (Table 4.2). These concentrations showed no relation to season of the year (Figure 1.18).

4.3.3 Influence of season on adrenal cortex and thyroid activity

Season of the year did not appear to have any effect on pooled plasma cortisol concentrations of heifers and cows during 1979 (Table 4.3).

Plasma cortisol levels of both heifers and cows were influenced by time of the year. In the statistical analysis, the onset of the winter season was assumed to be April, and that of the summer season, October. Figure 2.1 reveals that adrenal cortex activity declined during late winter, and showed a slow upward trend from October onwards.

During 1980, plasma cortisol concentrations were depressed in both the sourveld + N and mixed sweetveld group of heifers during the winter season. This influence is illustrated in Figure 2.2 and Figure 2.3. Plasma cortisol concentrations decreased as the summer season progressed. However, levels of especially heifers and cows on the sourveld, fell rapidly towards the end of June, and increased during spring and summer.

The influence of season was less dramatic during 1981/82, and only first-calf cows on the sourveld + N were significantly affected. From Figure 2.4, it is clear that both groups of females on the sourveld were influenced by season, with cortisol levels declining during autumn and winter, and increasing again as spring approached. It is to be noted that plasma cortisol concentrations in animals on the mixed sweetveld were not acutely influenced by season of the year.

There was a tendency for plasma cortisol concentrations to decrease from March to July (autumn to winter), followed by an upswing during late winter. The elevated levels were maintained

Table 4.3 Mean (± SE) plasma cortisol and thyroxine concentrations of heifers and cows during the summer and winter seasons on different nutritional regimes

Hormone concentrations	Sourveld + N		Sourveld - N		Mixed sweetveld + N	
	Summer	Winter	Summer	Winter	Summer	Winter
Cortisol (ng/ml)						
1979	10.6 ± 0.64	10.40 ± 0.50	-	-	-	-
1980	14.53 ± 0.72	13.11 ± 0.74	-	-	14.62[q] ± 0.99	11.77[r] ± 0.99
1981/82	10.01[a] ± 0.63	7.47[b] ± 0.67	6.83[i] ± 0.50	7.81[j] ± 0.05	9.54 ± 0.49	9.80 ± 0.61
1982/83	8.07[c] ± 0.33	5.46[d] ± 0.32	7.32[k] ± 0.34	6.37[l] ± 0.33	6.64 ± 0.33	6.33 ± 0.34
Thyroxine (ng/ml)						
1979	46.3[e] ± 1.66	53.5[f] ± 1.30	-	-	-	-
1980	55.1 ± 1.40	55.3 ± 1.43	-	-	55.5[s] ± 1.69	49.8[t] ± 1.70
1981/82	41.4 ± 1.02	42.5 ± 1.10	35.6[m] ± 1.56	46.1[n] ± 1.56	46.0 ± 1.13	48.0 ± 1.41
1982/83	37.7[g] ± 1.06	41.1[h] ± 1.03	39.0[o] ± 0.93	42.0[p] ± 0.91	46.7 ± 1.06	46.2 ± 1.10

b < a, 0.05   e < f, 0.001   i < j, 0.05   m < n, 0.001   r < q, 0.05

d < c, 0.001   g < h, 0.05   l < k, 0.05   o < p, 0.05   t < s, 0.05

during the subsequent summer season, and started to decline again from March to May. During the 1982/83 season, plasma cortisol concentrations of cows in the 2 sourveld groups fluctuated more than that of cows in the mixed sweetveld group. Cortisol concentrations showed more variation during the 1982/83 season than during the previous season.

Plasma thyroxine concentrations were significantly ($P < 0.001$) influenced by season of the year during 1979 (Table 4.3). Levels were inconsistent, but tended to be higher in winter compared to summer (Figure 2.6). During the 1980 season, mean plasma T4 levels were significantly ($P < 0.05$) higher in heifers in summer than during winter on the mixed sweetveld, but not on the sourveld (Table 4.3). However, it is difficult to assess the influence of season on thyroid activity in heifers (Figure 2.7) and cows (Figure 2.8), as plasma T4 levels increased and decreased during both seasons.

Plasma thyroxine concentrations showed a more consistent trend during 1981/82. In all 3 treatments, plasma T4 levels were higher in winter than in the summer (Table 4.3). Although the levels of this hormone were not influenced to such a large extent as those of the plasma cortisol levels, there was a tendency for an increase during winter of 1981/82 (Figure 2.9). During the following year, plasma T4 concentrations of cows in both sourveld groups tended to be suppressed during the summer months (Table 4.3). This slight effect is illustrated in Figure 2.10.

4.3.4 Relationship between climatic factors, and metabolic and hormonal parameters

Climatic data from the Dohne Research Station were used to determine a regression on principal-component analysis (BMDP/4R). The climatic parameters served as independent variables, and the metabolites as dependent variables.

In the first analysis relating to the 1981/82 data, the mean climatic data, one week prior to blood sampling were determined to establish a relationship with metabolites. However, rainfall was always taken as the total rainfall for the period. In the second analysis, using the same metabolic data, climatic data the day before blood sampling, were used. The same method of analyses was repeated for the 1982/83 data. Climatic data recorded were maximum, minimum and mean temperatures, rainfall, windspeed, total wind, sunshine, barometer, barograph, evaporation, maximum and minimum humidity. Only the climate variables that had a more pronounced influence on the data, are listed.

Climatic data on the day of blood sampling were omitted because of the very slight influence on blood parameters. Because of the absence of a weather station at the mixed sweetveld site, no climatic data for this location were available, and were therefore omitted in these analyses. The results of the principal-component analysis of mean weekly data are shown in Table 4.4.

The first principal-component analysis of the climate variables indicated that the first principal component accounted for 36.2% of the total variation in climate. In the PC1 analysis, positive coefficients were obtained for temperature and humidity, and negative coefficients for atmospheric pressure. In a sense, this represents a warm humid condition, and were thus termed "warm".

The second principal-component (PC2) analysis of climatic variables showed that the second component accounted for 29.3% of the total variation. Positive coefficients were obtained for rainfall, maximum and minimum humidity, and negative coefficients for sunshine and evaporation (Table 4.4). These conditions were termed "rainy".

The third principal component (PC3) was responsible for 12.8% of the total variation. It had positive eigen vectors for rainfall, windspeed and total wind, and negative values for barograph and maximum humidity. Hence, these conditions were described as "windy".

Table 4.5 indicates correlation coefficients between metabolites and hormones, and principal components. Although blood glucose concentrations were significantly correlated to PC1 and PC3, these correlations were not strong. The correlation coefficients between various protein metabolites with principal components were stronger and highly significant ($P < 0.01$). This indicates that "warm" climatic conditions influenced the various protein parameters in both a positive and negative manner. Both plasma cortisol and thyroxine concentrations were significantly influenced by "warm" climatic conditions. "Rainy" and "windy" conditions had much less effect on the parameters.

Table 4.4 **Mean climatic data 1 week prior to blood sampling, reduced by principle component analysis to orthogonal predicting variables (Eigen vectors): 1981/82**

	Principle components (PC)				
	1	2	3	4	5
Eigen values	4.34	3.52	1.54	0.86	0.66
Cumulative proportion of total variance (%)	36.2	65.5	78.3	85.5	91.0
Eigen vectors:					
Minimum temperature	0.43				
Mean temperature	0.42			0.27	
Rainfall		0.36	0.34	0.26	-0.38
Windspeed			0.63		
Total wind			0.58		-0.34
Sunshine		-0.40		-0.27	-0.51
Barometer	-0.40			0.48	
Barograph	-0.35		-0.17	0.61	
Evaporation		-0.40			-0.32
Maximum humidity	0.27	0.32	-0.19		-0.40
Minimum humidity		0.48			

Table 4.5 Summary of regression analysis of metabolites on climate principle components

Dependent variables (metabolites)	Correlations between principal components and dependent variables			Multiple correlation coefficient (r)	F value for regression
	1 "Warm"	2 "Rainy"	3 "Windy"		
Blood glucose	0.29[c]		-0.12[b]	(1) 0.09	24.25
				(3) 0.33	13.74
Plasma glucose	0.17[c]		-0.13[b]	(1) 0.25	17.16
				(3) 0.30	15.13
Total plasma protein	0.22[c]			(1) 0.05	12.68
				(1) 0.13	37.10
Plasma albumin	-0.35[c]	-0.21[c]		(2) 0.23	25.29
				(1) 0.19	61.38
Plasma globulin	0.44[c]	0.18[c]		(2) 0.26	30.31
				(1) 0.06	15.73
Plasma urea	-0.24[c]		-0.11[a]	(3) 0.10	9.14
				(1) 0.07	18.32
Plasma cortisol	0.26[c]	0.11[a]		(2) 0.17	10.36
Plasma thyroxine	-0.13[b]			(1) 0.05	4.82

[a] Not significant [c] Significant ($P < 0.01$) [b] Significant ($P < 0.05$)

The results of the principal-component analysis of weather data taken one day prior to blood sampling are set out in Table 4.6.

The first principal component (PC1) was responsible for 34.8% of the total variation, the second for 21.4% and the third, for 17.8% of the total variation. These conditions were termed "warm", "cool" and "dry", respectively.

In contrast to the first analysis, it appears that "cool" climatic conditions exerted a larger influence on metabolites than "warm" conditions (Table 4.7). In PC1, both glucose and protein metabolites had limited positive correlation to principal components. Metabolites indicated a stronger relationship with PC2. As in the previous case, plasma albumin was negatively correlated, and plasma globulin concentrations positively correlated to principal components. Plasma cortisol was also more significantly related to weather conditions than plasma thyroxine levels.

In the third analysis of 1981/82 data, results of the principal-component analysis indicated that climatic data on the day (08:00) of blood sampling did not influence metabolites, and are therefore not presented.

Using the LSML76 computer program, correlation coefficients between the various weather and blood parameters were determined. Only those parameters that were significantly influenced, are presented in Table 4.12.

Metabolic and hormonal profiles were not consistently affected by windspeed, barometer, barograph or evaporation recordings. From Table 4.12 it is clear that metabolites were largely influenced by the mean ambient temperature. Correlation coefficients were generally low, with energy metabolites having positive relationships with weather data. Plasma albumin and urea concentrations were consistently negatively correlated with weather data 1 week and 1 day prior to blood sampling, during the 1981/82 season.

Table 4.6 Climatic data on the day before blood sampling reduced by principal component analysis to orthogonal predicting variables (Eigen vectors): 1981/82

	Principle components (PC)				
	1	2	3	4	5
Eigen values	4.18	2.57	2.13	1.29	0.72
Cumulative proportion of total variance (%)	34.8	56.3	74.0	84.8	91.0
Eigen vectors:					
Maximum temperature	0.42				
Minimum temperature		0.43			0.28
Mean temperature	0.39	0.32			-0.38
Rainfall				0.72	0.26
Windspeed		-0.42	-0.44	0.34	
Total wind speed		-0.42		0.35	
Sunshine			0.38		
Barometer	-0.34		0.43		
Barograph	-0.34		0.45		
Evaporation	0.38		0.25		-0.38
Maximum humidity				0.36	-0.81
Minimum humidity		0.36			

Table 4.7 Summary of regression analysis of blood metabolites on the climate principle components

Dependent variables (metabolites)	Correlations between principal components and dependent variables			Multiple correlation coefficient (r)	F value for regression
	1 "Warm"	2 "Cool"	3 "Dry"		
Blood glucose	0.20[c]	0.29[c]	0.20[c]	(1) 0.20 (2) 0.08 (3) 0.17	16.43 23.55 17.01
Plasma glucose	0.19[c]	0.13[b]	0.32[c]	(1) 0.14 (2) 0.24 (3) 0.10	20.77 13.28 29.06
RBC glucose	0.09[a]	-0.10[a]	0.26[c]	(1) 0.10 (2) 0.09 (3) 0.07	7.01 8.61 19.16
Total plasma protein	0.14[b]	0.14[b]		(1) 0.04 (2) 0.02	5.37 5.39
Plasma albumin		-0.28[c]		(2) 0.16	14.93
Plasma globulin	0.12[b]	0.31[c]		(1) 0.20 (2) 0.10	12.97 28.13
Plasma urea		-0.23[c]		(2) 0.17	26.17
Plasma cortisol	0.10[a]	0.31[c]		(1) 0.17 (2) 0.17	10.30 7.44
Plasma thyroxine	-0.14[b]	-0.13[b]		(1) 0.08 (2) 0.13	7.78 6.48

[a] Not significant [c] Significant ($P < 0.01$)

[b] Significant ($P < 0.05$)

During the 1982/83 season, the above-mentioned procedures were repeated. The results of climatic conditions on metabolites are presented in Table 4.8 to 4.11.

Results from this period differed from the previous year, in that correlations between weather and metabolite values were less prominent, and energy metabolites showed a negative relationship with climate. Correlation coefficients differed from year to year (Table 4.13). Plasma globulin concentrations also showed a negative correlation with climatic conditions, as opposed to the positive relationships recorded during the 1981/82 season. Plasma urea was the only metabolite to have consistent negative relationships with weather parameters during both years.

4.4 DISCUSSION

4.4.1 Influence of season on energy metabolites

Present results indicate that plasma glucose concentrations were elevated in first-calf cows during the summer of 1981/82. Inverse results were obtained during the following season. Literature reveals that season of the year has a varied influence on glucose concentrations. Rowlands *et al.* (1974) reported that no seasonal trend in glucose concentrations was recorded. However, blood glucose concentrations of non-lactating cows were 2.41 vs. 2.56 mmol/l, and those of lactating cows 2.35 vs. 2.47 mmol/l during the winter and summer seasons respectively.

The plasma glucose concentration of shorn sheep, exposed to a hot environment of 30°C and 70% relative humidity, changed little before and during the exposure period. However, the turnover rate of blood glucose decreased significantly, but feed intake was not affected (Sano *et al.*, 1983).

In a study with sheep, Sykes and Field (1974) reported little seasonal variation in glucose concentrations. Similarly, serum glucose concentrations in steers were not affected by the sampling date (McCracken *et al.*, 1993). Mature pregnant Hereford cows were subjected to heat stress of 36°C in a controlled environment. The concentrations of glucose were similar in the umbilical venous blood of heat-stressed and control cows (Reynolds *et al.*, 1985). Blood glucose metabolism and heat production of shorn sheep decreased, but the contribution of blood glucose to heat production was unchanged during exposure to a temperature of 30°C (Sano *et al.*, 1983).

Table 4.8 **Mean climatic data a week prior to blood sampling, reduced by principle component analysis to orthogonal predicting variables (Eigen vectors): 1982/83**

	Principle components (PC)				
	1	2	3	4	5
Eigen values	4.38	2.44	1.83	1.19	0.69
Cumulative proportion of total variance (%)	36.5	56.8	72.0	81.9	87.7
Eigen vectors:					
Mean temperature	0.42				
Rainfall		0.38		-0.51	-0.38
Windspeed			-0.51		
Total wind			-0.58	-0.32	
Sunshine	-0.23	-0.40			
Barometer	-0.37		0.30	-0.35	0.27
Barograph	-0.34		0.32	-0.39	0.36
Evaporation	0.23	-0.41	-0.29		
Maximum humidity		0.31		0.38	0.63
Minimum humidity		0.51			0.33

Table 4.9 Summary of regression analysis of blood metabolites on climate principle components for the1982/83 season

Dependent variables (metabolites)	Correlations between principal components and dependent variables			Multiple correlation coefficient (r)	F value for regression
	1 "Warm"	2 "Cool"	3 "Dry"		
Blood glucose	-0.16[a]			(1) 0.14	22.19
Plasma glucose	-0.16[a]		-0.17[a]	(1) 0.19	23.64
				(3) 0.16	29.70
RBC glucose			-0.19[a]	(3) 0.07	11.80
Total plasma protein	-0.34[a]	-0.24[a]		(1) 0.27	55.48
				(2) 0.32	48.28
Plasma globulin	-0.33[a]	-0.22[a]		(1) 0.26	53.28
				(2) 0.38	46.21
Plasma urea	-0.27[a]		-0.23[a]	(1) 0.07	23.28
				(3) 0.12	21.21
Plasma cortisol	0.15[a]			(1) 0.21	19.68
Plasma thyroxine	-0.13[b]			(1) 0.05	8.86

[a] Significant ($P < 0.01$)

[b] Significant ($P < 0.05$)

Table 4.10 Climatic data 1 day prior to blood sampling, reduced by principal-component analysis to orthogonal predicting variables (Eigen vectors): 1982/83

	Principle components (PC)				
	1	2	3	4	5
Eigen values	4.83	2.29	1.90	1.31	0.56
Cumulative proportion of total variance (%)	40.2	59.3	75.2	86.1	90.8
Eigen vectors:					
Minimum temperature		0.54			
Mean temperature	0.35	0.42			
Rainfall		0.37	-0.43		
Windspeed		-0.27	-0.40	0.28	0.27
Total wind			-0.42	0.45	0.27
Sunshine		-0.36	0.32		
Barometer	-0.34			0.30	0.40
Barograph	-0.33			0.32	0.41
Evaporation	0.41				
Maximum humidity				-0.67	0.60
Minimum humidity	-0.35		-0.37		

Table 4.11 Summary of regression analysis of blood metabolites on climate principle components for the 1982/83 season

Dependent variables (metabolites)	Correlations between principal components and dependent variables			Multiple correlation coefficient (r)	F value for regression
	1 "Warm"	2 "Cool"	3 "Dry"		
Blood glucose	-0.16[a]			(1) 0.16	28.51
Plasma glucose		-0.16[a]		(2) 0.07	10.68
Total protein		-0.36[a]		(2) 0.29	61.89
Globulin		-0.39[a]		(2) 0.15	54.28
Urea		-0.19[a]		(2) 0.04	11.90
Cortisol			-0.19[a]	(3) 0.09	15.61
Thyroxine	-0.21	-0.15[a]		(1) 0.05 (2) 0.07	14.58 11.17

[a] Significant ($P < 0.01$)

Table 4.12 Correlation coefficients between weather parameters and blood metabolites during the 1981/82 season

Blood Metabolite	Mean temperature		Total rain	
	A	B	A	B
Blood glucose	0.2537[a]	0.3879[a]		
Plasma glucose	0.1797[a]	0.3279[a]		
Plasma protein	0.1876[a]	0.2011[a]		
Plasma albumin	-0.2189[a]	-0.1887[a]	-0.2122[a]	-0.2252[a]
Plasma globulin	0.3145[a]	0.3131[a]	0.2450[a]	
Plasma urea	-0.2204[a]			
Plasma cortisol	0.2099[a]	0.2662[a]		
	Maximum humidity		Minimum humidity	
	A	B	A	B
Blood glucose	0.3715[a]		0.2454[a]	
Plasma glucose	0.2659[a]		0.2071[a]	
Plasma albumin	-0.4054[a]	-0.2520[a]	-0.3223[a]	-0.3027[a]
Plasma globulin	0.3727[a]		0.3246[a]	0.2240[a]
Plasma urea	-0.2150[a]	-0.3561[a]	-0.1939[a]	-0.2061[a]

[a] Significant $P < 0.01$

A: Mean weather data 1 week prior to blood sampling

B: Weather data 1 day prior to blood sampling

Table 4.13 Correlation coefficients between weather parameters and blood metabolites during the 1982/83 season

Blood Metabolite	Mean temperature		Total rain	
	A	B	A	B
Blood glucose	-0.1688[a]	-0.2164[a]		
Plasma glucose	-0.1712[a]	-0.1599[a]		
Plasma protein	-0.2936[a]	-0.2225[a]		
Plasma globulin	-0.3055[a]	-0.2416[a]	-0.2122[a]	-0.2252[a]
Plasma urea	-0.2862[a]			
	Maximum humidity		Minimum humidity	
	A	B	A	B
Plasma glucose	-0.2363[a]	-0.1561[a]		
Plasma protein				
Plasma globulin				-0.3080[a]
Plasma urea			-0.1746[a]	-0.3064[a]
Plasma cortisol	-0.2096[a]		-0.1939[a]	-0.2264[a]

[a] Significant $P < 0.01$

A: Mean weather data 1 week prior to blood sampling

B: Weather data 1 day prior to blood sampling

Plasma glucose and free fatty acid concentrations did not change when shorn sheep were kept at 30°C (Sano *et al.*, 1979). Oltner (1983), also found no consistent seasonal trends for glucose levels in Swedish dairy cattle.

Polwarth sheep were observed under temperatures varying from 10.5 to 46.5°C. In the shorn animals, blood glucose concentrations increased under high temperatures, from 56.3 to 60.5 mg/100ml, and in unshorn animals, the change was 54.7 to 57.5 mg/100 ml. (Silva *et al.*, 1992). The increased trend of glucose concentration in the present study during the first year, is therefore more in line with the results reported by the latter authors. On the other hand, a decrease in plasma glucose concentration of Holstein heifers was reported during heat exposure, and a significant ($P < 0.05$) increase during cold exposure (Itoh *et al.*, 1997). However, cooling did not affect plasma glucose concentrations in dairy cows (Flamenbaum *et al.*, 1995).

4.4.2 Influence of season on protein metabolites

Present results indicate that total plasma protein decreased in all 3 treatment groups during autumn, and increased during the spring and summer of 1981/82 (Figure 1.11). Sykes and Field (1974) found that total protein, albumin and globulin concentrations followed similar trends, decreasing from November to May (winter) and increasing again from May to November (summer). In contrast, the total protein concentrations in 24 dairy herds were virtually constant during the year (Rowlands *et al.*, 1974).

In hill sheep, plasma albumin concentrations showed seasonal variation, with higher values being recorded during the summer than during winter (January 1973 to July 1974). However, there was a trend for a gradual increase in concentrations with succeeding years (Sykes & Russel, 1979). The influence of season seemed to be less pronounced during this latter period.

Present results, indicating high levels of plasma albumin during the autumn and winter, and decreased levels during spring and summer (1981/82), are also in contrast with those of Rowlands *et al.* (1974), who found that albumin concentrations rose slightly but significantly during the summer months. Michel (1980) reported that total protein and albumin levels were relatively stable, with the percentage below normal decreasing in winter, and the percentage with above normal values, increasing in summer.

Plasma globulin concentrations determined during 1981/82 are in accordance with those of Sykes and Field (1974), who found that globulin concentrations tended to be higher in summer than in winter. In hill sheep, plasma globulin levels were generally also higher during summer months (Sykes and Russel,1979). In a 5.5 year study of blood metabolites in cattle, Michel (1980) reported that globulin concentrations were significantly lower during the winter periods. Sykes and Russel (1979) observed that albumin concentrations were generally below normal in summer, and were associated with hyper-globulinaemia. Moxoto goats in northeast Brazil showed a seasonal variation in immunoglobulin levels, concentrations being highest during June to December which is the dry season, and lowest during January to May, being the rainy season (Johnson *et al.*, 1995).

Plasma urea N concentrations showed large and significant annual cycles in hill ewes, with levels being higher in summer than during the winter months (Sykes and Russel, 1979). Similarly, Sykes and Field (1974) observed extremely low values during mid- and late winter. Clear seasonal patterns were also observed in urea N in dairy herds, with levels being higher during summer (Rowlands *et al.*, 1974). In Holstein steers in Illinois, USA, serum urea N concentrations were higher during May and November, than during June and September (McCracken *et al.*, 1993). These latter studies are in contrast with the present results obtained, where levels were only slightly influenced by season.

Concentration of urea N was similar in umbilical venous blood of heat-stressed (36°C) and control Hereford cows (Reynolds *et al.*, 1985). Michel (1980) suggested that the decreases in, among others, glucose and urea concentrations, were due to lack of a proper feed supplementation.

The interpretation of blood and plasma metabolites are complicated by prandial variations in concentrations. So, for instance, Lindsay (1970) stated that glucose metabolism is influenced largely by intake. This was also seen in the present study (Chapter 1). It is thus agreed with Sykes and Russel (1979), that seasonal variations follow a pattern of nutrient availability.

It has been shown that non-nutritional factors such as pregnancy and lactation influence metabolite concentrations (Sykes & Field, 1974) These are discussed in more detail in Chapter 3. As stated earlier, the plasma fraction of blood increases during pregnancy, and because glucose is confined almost entirely to the plasma fraction, it follows that glucose concentrations would be affected during pregnancy, and especially during the latter stages of June to August. These factors may be responsible for the inconsistency of present results obtained during the 2 seasons.

From the onset of the experiment, the vast differences in blood metabolite levels between the 1981/82 and 1982/83 seasons were obvious. During the first season, energy and protein metabolites were significantly elevated in first-calf cows on the mixed sweetveld. Repeatability of these results were low during the following year. Similarly, the various blood and plasma metabolites were not influenced to the same extent by processes such as pregnancy and lactation during the various seasons. These results emphasize the importance of interaction between various factors to exert different influences on the metabolism and physiology of the animal.

It should be pointed out that the early upswing in blood and plasma glucose levels during August cannot be due to the improved nutritional status of the natural pasture. In a previous study at the same location, it was observed that the crude protein content of oesophageal fistula samples from the sourveld and mixed sweetveld declined to a low level of 5.6 and 5.8%, respectively during August 1982. Thereafter, rapid increases occurred, to reach maximum levels of 8.7 and 11.3% respectively. Total non-structural carbohydrates (TNC) decreased to a low of 2.8 and 3.1% during September, followed by the maximum levels of 5.8 and 6.8% on the 2 types of pastures 1 month later (Erasmus, 1988). It is concluded that adjustments of homeostasis occurred, in response to lengthening of daylength, or due to the advent of spring in anticipation of higher nutrient intake. It has been previously shown that the magnitude of differences between summer and winter metabolic profiles vary from year to year (Payne *et al.*, 1973). Results of the present study supports the findings of Rowlands *et al.* (1974), who states plasma urea to appear to be most susceptible to seasonal fluctuations. As urea concentrations reflected the amount of nitrogen ingested, this parameter probably reflected the higher nutritive content of the natural pasture during the summer season.

4.4.3 Influence of season on hormonal profiles

During the 1979 to 1981/82 seasons, it can be seen that plasma cortisol concentrations were suppressed during the winter season, in heifers and cows, on the different feeding regimes. During the 1982/83 season, no clear effect of season on plasma cortisol profiles were observed in experimental animals.

These results appear to contradict most of what was reported earlier. Plasma cortisol concentrations in Brown Swiss, Holstein-Friesian and White Fulani heifers were generally low in a hot, humid tropical climate (Adeyemo *et al.*, 1981). Non-shaded dairy cows in Hawaii had higher rectal temperatures, but tended to have lower plasma corticoid levels than shaded cows. In some sows and gilts exposed to a temperature of 28°C, mean glucocorticoid concentrations were lower than those of control animals (Kattesh *et al.*, 1980). Similarly, other investigators also found an inhibiting effect of high temperature on the adrenal cortex activity in cattle (Bergman & Johnson, 1963; Rhynes & Ewing, 1973; Abilay *et al.*, 1975; Lee *et al.*, 1976). Stott and Wiersma (1971) observed decreased cortisol levels in cattle exposed to a hot summer.

Some workers reported acute thermal stress to result in large, rapid increases of plasma cortisol levels for a short period, whereas constant thermal stress suppresses levels (Stott & Robinson, 1970; Christison & Johnson, 1972). Miller and Alliston (1974) found that exposure of 14-month old Angus heifers to high temperatures resulted in higher corticoid levels for the first week, but lower levels for the remainder of the oestrous cycle, when compared with those heifers kept under a controlled temperature environment. In a previous study on the Dohne Sourveld, plasma cortisol concentrations of Bonsmara, Shorthorn and Afrikaner heifers declined significantly ($P < 0.01$) from winter to summer, and it was concluded that the high levels during winter indicate a stressful situation (Erasmus, 1978). The results of Collins and Weiner (1968) imply that stress imposed by sudden exposure to a high ambient temperature may increase ACTH secretion. Glucocorticoids also increased significantly in acutely heat-stressed steers (Christison *et al.*, 1970). On the other hand, chronic exposure of shorn wethers to 8 to 9°C increased plasma cortisol concentrations (Graham *et al.*, 1981). In a study with pregnant goats, Olsson *et al.* (1995) found that heat stress did not increase the plasma cortisol concentration. Interestingly, cortisol concentrations were significantly higher in Zebu cattle of Mexico during the rainy, compared to the dry season

(Lamothe-Zavaleta *et al.*, 1991).

Present results appear to be more in line with those of Welsh *et al.*, (1981), who found that concentrations of corticoids were increased in June (summer), when compared with January secretions. No variation in cortisol trends due to season was found in pinealectomised and control rams (Kennaway *et al.*, 1981). Most of the results cited, indicated a decrease in adrenal cortex activity during the warm season, or high temperatures. It has been suggested that these animals are able to adjust physiologically to elevated heat loads by decreasing adrenal corticoid output (Lee *et al.*, 1976).

A number of factors should be considered when interpreting the results. Firstly, many of the reported studies were conducted under artificial conditions, viz. controlled chambers and barns. Results obtained from these studies may not reflect those obtained from free-grazing animals on natural pasture, such as was the case in the present study.

A second and vital factor, may be the temperature range at which studies were carried out. In their trial, Lee *et al.* (1976) studied adrenal cortical function of lactating Holstein cows in Louisiana with its wide temperature variation. The Dohne Research Station has a moderate climate, with mean summer temperatures of between 18 and 20°C, and winter temperatures of between 13 and 14°C. It is, therefore, clear that if temperature per se influences adrenal cortical activity, cortisol concentrations would not be affected by seasonal variation to any large degree. It is thus concluded that the temperature variation between summer and winter was not so acute as to warrant significant homeostatic adjustments in order to maintain thermal balance.

A third and important factor may be the influence of feeding regime on the adrenal cortex. In many studies reviewed, dairy cows were used as experimental animals. These animals are usually maintained under intensive management conditions, with nutrient intake not being a limiting factor. Rhynes and Ewing (1973) exposed Hereford bulls to high ambient temperatures, and these animals received a nutritionally balanced diet at the rate of 2 kg of feed per 100 kg of body weight per day. This is a far cry from beef animals ranging on sour grassveld with a protein content of between 3 and 4% in the winter. In the latter instance, because of lack of sufficient nutrients, the metabolism of animals would be reduced, and the decrease in plasma cortisol concentrations experienced during the winter, would serve as an energy-saving mechanism (Figure 2.1 to 2.4).

Thus, present results in these trials are in disagreement with those in the literature, and it can be concluded that the absence of extreme seasonal variation, and the influence of insufficient nutrient intake under free-ranging conditions may have altered adrenal corticoid function.

Present results regarding the influence of season on thyroid activity were inconsistent. In many instances, plasma T4 concentrations seemed to increase during the winter season. Over the past 2 seasons, this increase seemed to last until September (Figure 2.10). This tendency of elevated plasma T4 levels during the winter months is in agreement with Annison and Lewis (1959). Total T4 concentrations in ram plasma also tended to be higher during the winter period. Ewe plasma indicated increased levels over the lactation period (Heaf *et al.*, 1983). Using 3 Holstein-Friesians in early lactation, Magdub (1982) found lower plasma and milk T4 concentrations at 30°C than at 20°C. Similarly, it was shown that plasma T4 concentrations increased in low, and decreased at high temperatures in male rats (Yousef & Johnson, 1968), the horse (Irvine, 1967) and cattle (Yousef *et al.*, 1967). In crossbred young bulls, T4 concentrations were reported to be lower during the hot-humid season, than during other periods (Singh & Goel, 1986). Shorn sheep exposed to a temperature of 30°C had decreased thyroxine concentrations with no reduction in feed intake (Sano *et al.*, 1983). Other studies also demonstrated a negative association between thyroid activity or T4 concentrations and ambient temperature (Lundgren & Johnson, 1964; Yousef *et al.*, 1967; Hurley *et al.*, 1980; Schillo *et al.*, 1982; Sano *et al.*, 1983).

Interesting results were obtained by Christopherson *et al.*, (1979), in which the authors observed highest plasma T4 concentrations in bullocks kept out of doors on a maintenance level of feed intake, and a temperature of -15°C. Under these circumstances, T4 levels were reduced when the feed intake was increased to twice that of maintenance. In ewe lambs, serum T4 levels were higher from July through to September in lambs fed a high energy diet, compared to lambs fed a low energy diet. However, when the low energy group of lambs were switched to the high energy diet, the difference in serum T4 levels was eliminated (Fritzgerald *et al.*, 1982). Thus, these studies underline the importance of nutritional influences on thyroid activity, and its interaction with environmental temperature.

In contrast, plasma thyroxine concentrations were found to be highest in spring, and lowest in autumn (Wallace, 1979). The changes in hormone levels could not be related to changes in ambient temperature or day-length. During this study, the wethers were kept indoors and penned, and sampled at intervals of 3 months during the year. Ingraham *et al.* (1979), found that average plasma thyroid hormone values not to be different between shaded or unshaded cows. Erasmus (1978) observed significantly ($P < 0.01$) lower T4 concentrations in Bonsmara, Shorthorn and Afrikaner heifers during winter, compared to those during the summer on Dohne Sourveld.

Neither long artificial photoperiod (16 hours), nor short artificial photoperiod (8 hours) altered the T4 concentrations in ewe lambs (Fritzgerald *et al.*, 1982). In one experiment, thyroxine concentrations in the serum of lambs kept under long day-length were consistently higher throughout, compared to the serum of short day-length lambs. In a second experiment, the results were reversed (Forbes *et al.*, 1979). However, the authors attributed the apparent effect of day-length entirely to pre-experimental differences. These results are then in accordance with those of Hoersch *et al.* (1961) and Henderson (1958).

Results indicate that the animals in the present trial were not under stress, as indicated by plasma cortisol concentrations. According to Moberg (1976), whether or not an event will constitute a stressful situation is undoubtedly modified by the physiological and nutritional state of the animal. It was, therefore, surprising that the experimental animals that did not receive nitrogen supplementation during the winter season, did not respond with increased adrenal cortex activity due to undernutrition, that was further compounded by the fact that they were either pregnant or lactating.

Moberg (1976) stated it is unlikely that thyroid stimulating hormone is involved in the effect of stress (ambient temperature) on reproduction. It also appears that seasonal influences on thyroxine are insignificant. Indeed, it was found that plasma T4 concentrations were higher in animals on a low intake, but that temperature had no effect (Macari *et al.*, 1983). These authors concluded that the metabolism of thyroid hormone is influenced independently by both ambient temperature and energy intake. Thus, in spite of being in a moderate climate, present results indicate that there was a tendency for thyroxine concentrations to increase during the winter season. It is in accordance with Premachandra *et al.* (1958) that an increasingly warm environment appears to be a greater stimulus for a reduction in thyroid secretion rate than an increasingly colder environment.

Unlike metabolic profiles, plasma cortisol and T4 concentrations did not show definite opposite trends during the various seasons. Plasma cortisol levels showed consistent signs of increasing during the summer, and declining during the winter period. Thyroxine concentrations were less influenced by seasonal effects. However, it is believed that the activity of both the thyroid and adrenal glands were more influenced by nutritive status of the pasture than by the seasonal effect.

In a previous study involving Shorthorn, Afrikaner and Bonsmara heifers on the Dohne Sourveld, negative and more meaningful relationships between ambient temperature and plasma cortisol concentrations were indicated (Erasmus, 1978). Stronger correlations were recorded when temperatures 1 week prior to blood sampling were taken, compared to those at 08h00 on the day of blood sampling. In this regard, Lee *et al.* (1976) also observed a negative correlation between plasma corticoids and environmental maximum and minimum temperatures at 3, 7 and 14 days prior to blood sampling.

In the case of plasma thyroxine concentrations, present correlations between this hormone and ambient temperature were very low. This is in sharp contrast with results obtained in a previous study (Erasmus, 1978). In this instance, both positive and negative correlations were found during the winter seasons. However, mean correlations between plasma T4 concentrations and ambient temperatures 1 day preceding day of blood sampling were 0.83, 0.77 and 0.66 for Shorthorn, Afrikaner and Bonsmara heifers, respectively.

Literature indicates an inverse relationship to exist between the rate of thyroxine secretion and temperature (Henneman *et al.*, 1952; Flamboe & Reineke, 1959; Johnson & Yousef, 1966). Although results of the present study indicated a weak relationship between plasma thyroxine levels and climatic conditions, concentrations were suppressed during the summer, and are thus in accordance with the above-mentioned study. Other workers failed to record a difference in thyroxine secretion rate (TSR) between the winter and summer seasons (Pipes *et al.*, 1963). It is obvious that there is a vast difference in the seasonal influence on blood and hormonal parameters during the 2 seasons. Results obtained during the present study seem to indicate that the seasonal effect is inconsistent, as was stated by Hewett (1974).

4.5 CONCLUSIONS

Present results indicate that, with the exception of plasma urea concentrations, season of the year did not have a consistent influence on blood and plasma metabolites. In the preceding chapters, it was shown that nutritive values of the natural pasture, pregnancy and lactation influenced the metabolic profiles. Those factors, together with the fact that the concentrations of blood metabolites vary from year to year, may be the prime factors influencing these parameters. According to Rowlands *et al.* (1974), another factor which may influence levels is the amount of exercise which cows have between summer and winter. Being under free-ranging conditions, and under similar management practices from the beginning of 1981 to mid-1983, there is no reason to believe that the experimental animals may have had more exercise during one season compared to the other.

The decrease in blood and plasma glucose concentrations towards the end of the summer season may have been due to the decreased nutritive value of the natural pastures. However, the early upswing in levels is most likely caused by increased photoperiod.

Literature is explicit on the long-term influence of temperature and/or season on adrenal cortex activity. Many of these studies were carried out with dairy and other animals receiving adequate nutrition. The present results indicate that plasma cortisol concentrations were higher in summer than in winter, indicating reduced gluconeogenesis during the latter season. This lower glucocorticoid level may signal an energy-saving mechanism in order to help the animal overwinter on low nutritive pasture. These results were obtained over a 4.5 year period, and were substantiated from year to year. However, the fact that season had a larger influence on cortisol profiles of animals on the sourveld than those on the mixed sweetveld, indicates that the nutritional status of the animal may determine to what extent the adrenal gland is influenced by season. Present results may well thus indicate that the decreased plasma cortisol concentrations experienced during the successive winters, may be caused by insufficient nutrient intake rather than a seasonal influence.

In contrast, present plasma thyroxine levels tended to be more in line with those in the literature. Even though high and low energy diets appeared to influence plasma T4 concentrations more than season did, Fritzgerald *et al.* (1982) are of the opinion that results from Falconer and Draper (1967) indicate that the decline in ambient temperature or the onset of puberty cannot be ruled out as factors leading to the stimulation of serum thyroxine concentrations.

The problem of studying different aspects of the free-grazing animal, is that environmental factors will always interact with nutritional influences to bring about a response. In this regard it should be kept in mind that the apparent influence of climate may be less prominent to nutritional changes. In order to illustrate this aspect, Payne (1978) stated that blood urea is low in winter and high in summer. From a nutritional and seasonal point of view, this is acceptable. However, this is in direct contrast with present results, with plasma urea levels being elevated during the winter months for the 2 successive years. The reason for this trend could be the results of supplementation of non-protein nitrogen during this period, which is thus reflected in the blood plasma.

It is not easy to differentiate between the influence of feeding on the one hand, and seasonal influence on the other. Therefore, it is of utmost importance to always include a statistical analysis of the responses to climatic conditions in free-grazing animals. It should be kept in mind that seasonal variations are not as extreme in tropical areas, compared to colder areas. This may partially explain the discrepancies found in literature. The inconsistent effect of season on present metabolic and hormonal parameters are, therefore, ascribed to, among others, lack of extreme climatic conditions during the summer and winter periods. The direct opposite metabolite trends during the 2 seasons may be due largely to nutritional effects. It is not clear to what extent the different groups (herds) may be a source of variance in the blood metabolites.

4.6 SUMMARY

Energy and some of the protein blood and plasma metabolites were significantly higher during the summer of 1981/82 than during the winter. Summer and winter concentrations recorded for the experimental animals on the sourveld + N, sourveld - N and mixed sweetveld + N for blood glucose were 2.20 vs. 2.11, 2.27 vs. 2.02 ($P < 0.01$) and 2.43 vs. 2.12 ($P < 0.001$) mmol/l for the 3 groups respectively. Values for plasma glucose were 3.32 vs. 3.11 ($P < 0.05$), 3.33 vs. 2.90 ($P < 0.001$) and 3.46 vs. 3.25 ($P < 0.05$) mmol/l respectively. Those for RBC glucose were 1.12 vs. 1.01, 1.06 vs. 0.89 ($P < 0.05$) and 1.03 vs. 1.13 mmol/l for the groups respectively. Plasma protein concentrations recorded were 67 vs. 66, 70 vs. 66 ($P < 0.05$) and 72 vs. 71 g/l respectively, and those of plasma globulin 38 vs. 32 ($P < 0.01$), 40 vs. 33 ($P < 0.001$) and 40 vs. 36 g/l for the 3 groups respectively. Plasma albumin and plasma urea concentrations, on the other hand, were significantly higher in females in the winter period compared to the summer period. Albumin levels were 31 vs. 33 ($P < 0.01$), 30 vs. 33 ($P < 0.01$) and 33 vs. 35 ($P < 0.01$) g/l respectively, and those for urea 1.25 vs. 2.25 ($P < 0.01$), 1.13 vs. 1.58 ($P < 0.05$) and 3.73 vs. 3.78 mmol/l for the 3 groups respectively. During the following year, results were generally reversed, with plasma albumin and urea levels being higher during the winter than summer seasons.

Plasma cortisol concentrations indicated a more consistent trend over the years. Levels were generally elevated during the summer season, compared to the winter season, viz. 10.01 vs. 7.47 ($P < 0.05$), 6.83 vs. 7.81 ($P < 0.05$) and 9.54 vs. 9.80 ng/ml for the summer and winter seasons on the 3 treatments during 1981/82, respectively. The year thereafter, concentrations were 8.07 vs. 5.46 ($P < 0.001$), 7.32 vs. 6.37 ($P < 0.05$) and 6.64 vs. 6.33 ng/ml respectively. Plasma thyroxine levels, on the other hand, were depressed during the summer season,viz. 41.1 vs. 42.4, 35.6 vs. 46.1 ($P < 0.001$) and 46.0 vs. 48 ng/ml on the 3 treatments respectively during the summer and winter of 1981/82, and 37.7 vs. 41.1 ($P < 0.05$), 39 vs. 42 ($P < 0.05$) and 46.7 vs. 46.2 ng/ml respectively during the summer and winter of 1982/83.

It is concluded that season of the year generally did not have a significant and consistent influence on blood and plasma metabolites in this study.

CHAPTER 5

GENERAL DISCUSSIONS AND RECOMMENDATIONS

There is no doubt that beef production on especially the sourveld areas in South Africa is hampered by low calving rates. A better understanding of factors prohibiting efficient reproduction function in beef heifers and cows in these areas is essential, order to create innovating and creative ideas leading to solutions to improve lifetime productivity of these animals. Since the introduction of non-protein nitrogen (NPN) supplementation to the sourveld areas in the 1960's, little has changed by way of progress, in order to find further solutions to improve pasture utilization for the purpose of increasing low calving rates and productivity.

The soil at Dohne in the Eastern Cape is acid, the pH varying between below 5.0 to 6.5. The fertility of the soil is low, with the most important deficiencies being phosphate and nitrogen (Pienaar *et al.*, 1951). These deficiencies filter through to the natural pasture, resulting in a relatively low crude protein and energy content in the natural pasture. Crude protein content of oesophageal fistulate samples average 7.2% during the summer, and 6.58% during the winter (Erasmus, 1988). These levels are higher than reported earlier, and it is postulated that during a period of subnormal rainfall, as was experienced during the present study, grass growth was less vigorous, resulting in less fibre being deposited, resulting in a higher digestibility of the pasture. Nonetheless, from the energy and protein profiles of the present study, it appears that the Dohne Sourveld is not capable of sustaining efficient and economic animal production throughout the year with Bonsmara females. As the economic viability of any beef enterprise hinges on the all-important aspect of high reproductive performance, it is clear that the overall calving percentage of between 35 and 87% on the sourveld (Erasmus, 1988), falls far short of the ideal of weaning a calf from every cow in the herd within a 365 day period, and thus making the enterprise more profitable. Two very important factors interacting to bring about this unfortunate situation of a low calving rate, is the type (including the condition) of natural pasture, and the type of animal kept on this natural pasture.

In a previous study involving oesophageal fistula samples, it was emphasized that supplementary feeding programmes for the livestock are unavoidable to increase animal production in the sourveld areas of the Eastern Cape (Erasmus, 1988). The protein and energy content of samples collected by means of oesophageal fistulas reached maximum levels for only a short period of the year (during the active growing season of the pasture), viz. October and November. Blood glucose concentrations during the first year (1981/82) appeared to reflect the nutritional status of the natural pasture, and it was obvious from the large fluctuations in the summer and winter grazing, that the experimental animals were under nutritional stress, especially from late summer (end of February) onwards. In the previous study with the same Bonsmara females on the sourveld and mixed sweetveld, the question was posed whether higher protein or energy content of the latter pasture type led to higher FSH and LH peak concentrations (Erasmus, 1988). From the present study (Table 1.1), it is clear that both protein and energy metabolites were significantly elevated in animals on the mixed sweetveld, compared to sourveld during the 1981/82 season. Of interest in this regard, is the study of Tandle *et al.* (1997), who reported that normally cycling cows had, among others, significantly higher total serum protein levels than anoestrous cows. On the other hand, it was also found that anoestrous crossbred Jersey cows had significantly lower levels of blood glucose than normal cycling cows (Ramakrishna, 1997). However, during the 1982/83 season of the present study, only plasma urea ($P < 0.01$), and to a lesser extent, plasma albumin concentrations were significantly elevated in cows on the higher feeding regime (Table 1.2). Because cows on the mixed sweetveld annually realise a higher calving rate than those on the sourveld, it is tempting to suggest that protein was the limiting nutritional factor of the 2 on the sourveld, and therefore contributes more to increased reproduction performance than energy.

As previously stated, it is worrying that the increase in body weight of Bonsmara females during the mating period (beginning December to end of February) and the active growing season cannot be maintained from year to year. Growth is curtailed from the beginning of December to the beginning of January (summer), depending on the year (Erasmus, 1988). In the past, most of the studies focussed on limiting weight loss of livestock on sourveld. If active growth of beef females are not maintained during the summer season, it is an indication of nutritional inadequacy of the natural pasture, hence the poor production results during the mating season. In South Africa, the inconsistency of especially rainfall, results in major differences in growth and nutritional values of pastures from year to year, and this impacts on the response obtained with supplementation. Comparing body weight trends of the experimental animals prior to or during the mating season with fertility in the present study (Figure 3.32), it is clear that it is of paramount importance that meaningful body weight gains before and during the mating season are essential in order to assure high conception rates.

A positive relationship exists between conception and body weight in Sussex type heifers and cows at the onset of the mating season (Meaker *et al.*, 1980). E.g. 43% of heifers conceived when the body weight at the beginning of the breeding season was 212 kg, whilst 78% conceived when body weight increased to 337 kg. These authors suggested that target weights at mating be introduced to support maximum conception. Similarly, it was reported that a calving rate of 60% was obtained with a body weight of 250 kg and less, compared with a 100% calving rate at a weight exceeding 318 kg in Afrikaner heifers (Harwin *et al.*, 1967). Present results do not support this view of setting target weights, and it is interesting to note the comments of Baker and Morris (1982) regarding heifers: "Even if there are age-related or weight-related thresholds for puberty, they cannot be generalized to other managerial situations. We suggest that farmers should be wary of thresholds and target weights." In the United States of America, farmers are encouraged to manage their beef herds in such a way as to obtain maximum reproduction efficiency. One of the recommendations to achieve this goal is to have cows gaining weight 60 days precalving and 60 days postcalving (Dunbar *et al.*, 1981). Data obtained from the Vaalharts Research Station also pointed to the fact that females should be in a weight-gaining phase during the mating season to realise a high calving rate. During the 1989/90 mating season, Bonsmara heifers had a mean body weight of 421 kg at the beginning of the mating season, gained 37 kg over this period and recorded a calving percentage of 85.7%. During the following year, mean mating body weights were down to 408 kg, but with a mean weight gain of 80 kg over the mating season, a calving percentage of 100% was obtained. During the 1991/92 season, the mating weights were highest of the 3 seasons, viz. 514 kg. The mean weight gain was a mere 5 kg, whilst a calving rate of 75% was achieved.

It is against this background (the importance of gaining body weight during the mating season), that the recommendation is made that strategic feeding (including the feeding of the high protein and energy lick) should be investigated to be part and parcel of the livestock management strategy in the sourveld, even during the summer season, regardless how inappropriate this measure may seem. However, more studies should be undertaken to validate this view and to investigate the effect of this practice on production and reproduction performance.

Generally, blood metabolites appear not to be effective indicators of successful reproduction in the bovine. In the present study, cows that conceived had higher blood and plasma glucose concentrations just after parturition, compared to those that failed to conceive during the subsequent breeding season. Although these differences were substantial during the 1981/82 season, they were less marked during the following year. Adams *et al.* (1987) concluded that blood glucose concentrations are affected by body condition score, thus influencing reproductive performance in cattle. Vizcarra *et al.* (1998) reported that cows that have luteal activity during the breeding season had 5.7 mg/dl more glucose than cows without luteal activity. It, therefore, appears that plasma glucose concentration may influence reproductive performance, but the mechanism by which plasma glucose concentration may influence reproduction, has not been elucidated (Vizcarra *et al.*, 1998). In fact, the latter researchers are of the opinion that the mechanism by which nutrition influences reproduction in cattle, remains unclear.

It has always been normal management practice to offer a high protein and energy lick to the animals from May until the first spring rains. However, the sharp decline in blood and plasma glucose concentrations commencing from the end of February (late summer), indicates that the feeding of this lick should commence from the beginning of March each year in an effort to stem the rapid depletion of the liver glucose reservoir. However, it is doubtful whether this measure will suffice for the nutritional stress of the lactating cow. In this regard, the focus should be on another strategic feeding programme, i.e. providing additional feed during late pregnancy and the first trimester of lactation. It is imperative that further studies be launched to investigate the efficiency of these proposed practices.

The inconsistent relationship blood metabolic profiles of Bonsmara heifers and cows had with nutritional status of the natural grazing, was rather disappointing. Metabolite and hormone data of the 1981/82 season reflected the seasonal nutritional fluctuations, but during the following year, no pattern could be established. This was also frequently encountered in the literature. Bishonga *et al.* (1996) reported that plasma urea levels were significantly ($P < 0.01$) affected by diet throughout the experiment in sheep, whilst Shell *et al.* (1995) found that although plasma urea-nitrogen concentrations were not affected by nutrition, they were affected by shading ($P < 0.05$). In a previous chapter, it was pointed out that nutrition, season, pregnancy and lactation may contribute to the inconsistencies recorded in the results thus far. However, the study of Vinkler *et al.* (1996) illuminates the complexity of the problem, and it was reported that compared to the controls, cortisol concentrations in cows increased when the digestible crude protein:total dietary energy ratio of 1:4.5-5 was increased to 1:81, or decreased to 1:28. The implication of these findings is that whilst researchers busy themselves with the influence of nutrition at a macro level on metabolite or hormone concentrations, variations at micro level (e.g. the ratio of elements within the nutritional realm) may intervene to confound or alter results. When this is extrapolated to animals kept under extensive conditions such as in the present study, it is then possible that rotating females between different camps with different grass species, height of pasture, condition of the grazing etc. may have caused blood metabolite and hormone trends which could not be readily ascribed to the "global" or macro nutritional effect. In this sense, it could be deduced that results of experiments where animals are kept on natural pasture may prove more difficult to interpret that those of animals kept under controlled environments.

Of interest are the results by various workers who reported that diurnal variations in blood glucose concentrations may be greater than the variations due to energy status (Hartman & Lascelles, 1965; Radloff *et al.*, 1966; Coggins & Field, 1976). This may have been the reason why previous studies failed to reveal significant responses of blood constituents to underfeeding in cattle (Fisher *et al.*, 1975; Parker, 1977). The determination of blood glucose concentrations at fortnightly intervals in the present study may not have been appropriate to obtain the maximal response of glucose levels to changes in energy content of the natural pasture.

In the present study, it was found that pregnancy did not significantly influence either energy and protein metabolites, or hormone concentrations. However, lactation suppressed blood and plasma glucose of experimental animals on all 3 treatments for 8 to 10 weeks after parturition during the 1981/82 season. During the subsequent year, only plasma glucose concentrations of lactating second-calf cows were suppressed (Figure 3.4). These results are in agreement with those of Rowlands *et al.* (1975) who found that blood glucose concentrations were usually lower in early lactation than at other times. Because glucose is the main precursor of lactose in cows, glucose demand is generally highest during early lactation (Bickerstaffe & Annison, 1974). However, present results should in a sense be viewed parallel to that of Roberts *et al.* (1978), who studied the effects of underfeeding Friesian X Ayrshire cows for 6 months during pregnancy and lactation. These researchers also obtained similar results to those reported here, and emphasized the homeostatic mechanisms of cattle are able to regulate the concentration of blood glucose, within narrow limits under very widely different conditions of inputs and outputs. These workers noted that it is probable that the precipitating cause of the failure of homeostasis in extensive situations is a factor other than prolonged energy or protein deficiency. It was also noted that the abrupt introduction of a gross imbalance of dietary constituents may have a much greater effect on blood composition than a more prolonged deprivation. This may have a direct bearing on the present results of cows on sourveld which were born and bred under conditions of nutritional inadequacy, and thus exposed to a lifetime of undernutrition. It is postulated that these animals may have become adapted to their nutritional environment, and the body weight loss during winter and part of the summer was not sufficient to impair their sensitivity towards homeostasis, the latter maintaining blood composition in the animal body. Therefore, relatively little change was observed in plasma glucose concentrations during the stressors of pregnancy and lactation, or even level of protein and energy deficiency in the natural pasture.

The environment can affect metabolic and endocrine homeostasis by altering metabolic requirements for, for example, maximum milk yields. However, it appears that the potential effects of one component thereof, viz. seasonal extreme temperatures, are poorly understood. Studies such as the nature of the present one, involving the examination of physiological differences between beef females kept extensively on natural pasture involving 3 treatments, and influenced by different internal (pregnancy and lactation) and external (ambient temperature and other weather patterns) stimuli, appears to be a rather difficult approach, with these factors interacting with one another to apparently camouflage trends and results. The point made is that whilst studying the influence of pregnancy and lactation on blood and plasma metabolites over a 12 month period, time of the year (season) is bound to cause a further physiological impact which may alter homeostasis in the animal body. Faulkner *et al.* (1980) found that exposure of the lactating goat to cold, increased the plasma glucose concentration, but reduced extraction of glucose by the mammary gland, and this could be related to reduced milk secretion. In the present study, no relationship between plasma glucose concentrations and lactation could be found, emphasising the vast difference there may be in climatic differences between the different trials. Species differences also add to inconsistent results.

In the experiment of Sun *et al.* (1994), the objective was to determine whether the magnitude of metabolic differences between rams of control and fleeceweight-selected lines were influenced by ambient temperature to which the rams were exposed, thereby isolating one of the possible factors responsible for inconsistencies in expression of the metabolic differences. Rams of the 2 lines were studied at ambient temperatures of 6°C and 25°C, but were fed equal proportions of their daily diet every 2 hours, under conditions of equal photoperiod and at a simular age, viz. 7 months. It was found that urinary outputs and clearance rates of urea and creatinine were not influenced by ambient temperature. There were, however, marked changes throughout the 24 h sampling period in plasma concentrations of urea and T4. These results do thus not support the hypothesis that metabolic differences between the fleeceweight-selected and control lines of Romney sheep are influenced by the ambient temperatures to which the sheep were exposed.

From the foregoing, it is deduced that the Dohne Sourveld was not capable of sustaining high annual reproduction and production performance of Bonsmara cows and heifers. This pasture type with its known low nutritional status for approximately 10 months of the year is a given, and it cannot be altered. Its usefulness, however, may be increased by providing a high protein and energy lick. Supplementing this pasture with additional feedstuffs such as hay or silage may be an option, but it may prove to be a costly exercise (Erasmus & Barnard, 1985). Alternatively, the type of animal farmed with on the sourveld should also be more closely scrutinized.

Dry, pregnant, mature Bonsmara cows weighing approximately 500 kg such as in the present study, require a daily allowance of 0.44 kg of total protein, and 3.8 kg of total digestible nutrients (TDN) (National Research Council, 1970). In cows nursing calves, these concentrations increase to 0.97 and 6.0 kg respectively. Clearly, the crude protein content of 7.2 (0.72 kg) and 6.58% (0.66 kg) (summer and winter) and TDN content of 46.14 (4.6 kg) and 45% (4.5 kg) (summer and winter) of fistula Dohne Sourveld samples (Erasmus, 1988), are a far cry from basic nutrients required by the cow for reproductive purposes. It is thus concluded that the Bonsmara female is not the prime choice of beef animal suited to beef cattle production on the sourveld. It is agreed with Bonsma (1951) that originally, no large cattle have been bred on acid soil country. Therefore, it is logic to conclude that larger framed animals (such as the Bonsmara) would not be well adapted to an environment of high rainfall and acid soils. This explains why the Zebu cattle of the high Himalayan mountains, the Welsh Black cattle, most of the cattle on the higher slopes of the Drakensberg and the indigenous Mashonaland cattle, are small (Bonsma, 1951). However, it is possible that a medium-framed animal, selected for high fertility under these conditions, may undergo a reduction in size after many generations and become fully adapted with regard to high annual calving rates. But it is believed that the beef producer cannot afford to take this long and tedious road of gaining adaptation for his animals, whilst producing at a loss. A smaller framed animal with a 350 kg body weight, requires 0.79 kg of protein, and 4.9 kg of total digestible nutrients (TDN), which is closer to what the sourveld has to offer to the beef female in terms of nutritional fulfilment. Economic production is thus more feasible. Selection for maternal characteristics should be a high priority, since high fertility in a herd is an indication of adaptability. Boyd (1984) reported that cows with reduced sensitivity to loss of body condition or body weight were generally in poor condition, but continued to cycle and had higher conception rates than cows with normal sensitivity.

In studies such as the present one, where beef females are kept on natural pasture throughout the experimental period, it is difficult to distinguish between the various factors affecting metabolite and hormonal concentrations in the animal body. As seen in the preceding chapters, nutrition, pregnancy, lactation and season of the year affect the metabolic processes. The question now is, how much each factor contributed towards the observed variation in the results.

To illustrate the point, the study of pregnant goats observed under heat stress is relevant. When ambient temperature increased from 20 to 39.5°C for 5.15 hours, plasma cortisol concentrations, used as an indicator of stress, was not increased. However, catching site of feed caused an abrupt, short-lasted elevation (Olsson *et al.*, 1995). In another study with goats, it was reported that plasma cortisol concentrations increased in relation to water intake (Olsson *et al.*, 1996). Although the role of the adrenal cortex is recognized in the metabolic realm, it was interesting to note the affect of mere catching sight of feed, as well as water intake, had on cortisol concentrations, apparently overriding the effect of heat stress.

In conclusion, two other factors which may also have a pronounced influence on blood metabolite concentrations, and thus reproductive performance, is the genetic make-up of the animal and mineral content of the pastures. Concerning the first-mentioned, it was found that animals from the fleeceweight-selected line of Romney sheep exhibited lower plasma concentrations of amongst others, urea and thyroxine than sheep of the control line (McCutcheon *et al.*, 1987; Clark *et al.*, 1989; Thompson *et al.*, 1989; Sun *et al.*, 1991). Sun *et al.* (1992) also observed lower basal concentrations of urea in fleeceweight-selected than control Romney rams. However, the apparent relationship between circulating urea concentrations and genetic merit for fleeceweight was not reported in Australian Merino lines. In Merino sheep of superior genetic merit for wool production, lower levels of circulating thyroxine were reported (Hough *et al.*, 1988), but this could not be substantiated in a subsequent trial of sheep from the same lines (Williams *et al.*, 1990).

Although these results do not have a direct bearing on the present study, they do imply that selection of animals in a herd for specific traits, may influence blood metabolite and hormone concentrations for better or worse, in terms of reproductive performance. Consequently, it would be wise in a cow-calf enterprise to place much more emphasis on selection for fertility, not only culling open heifers and cows annually, but also those that calve late in the calving season. It has been reported that more cows calving late in the calving season are open at the end of the following breeding season (Burris & Priode, 1958; Reynolds *et al.*, 1966; Spitzer *et al*; 1975).

Secondly, mineral deficiency may have a profound detrimental affect on production and reproduction rates of farm stock on the sourveld. As early as 1940, mention was made of the phosphate deficiency in the soils which contribute to making it difficult for farmers to make a success of sheep farming (Du Toit *et al.*, 1949). Indeed, it was reported that Merino ewes on natural pasture (Dohne Sourveld), supplemented with a mineral lick consisting of bone meal (62.3%), salt (31.1%), sulphur (4.7%) and iron sulphate (1.9%), had a mortality rate of 25% for a 3 year period, whilst in the control ewes, it was 32.3% (Kotze, 1950). The number of lambs born from 68 ewes was 114 and 81 respectively over the experimental period, whilst the percentage of lambs dying, was 36.8 and 69.9% respectively. Similarly, much was gained by providing a mineral lick in terms of wool production (3.32 vs. 2.64 kg for controls) and live weights, the latter varying on average between 30.18 and 40.90 for the mineral, and 28.86 and 35.55 kg for the control group (Kotze, 1950).

Apart from the ingestion of phosphorus and calcium, the sufficient intake of other minerals by beef cattle play an enormous role to meet the nutritional demands set for conception, growth and rearing of a calf. In this sense, reference is made to trace minerals such as copper, of which a deficiency results in reproductive disorders (Kearl, 1982). Concentration of copper was reported to be significantly lower in dairy cows with low fertility (Vijchulata, 1995), whilst supplementation of 1% copper (50 ml) resulted in the shortening of the interval from calving to conception (Manickam & Balagopal, 1993). In the case of manganese, low fertility and frequent abortions are the main deficiency symptoms (Kearl, 1982). It has also been reported that when non-protein nitrogen (NPN) is used in place of natural protein (NPN being widely used in licks on the sourveld), sulfur (inorganic) should be supplemented. In addition, selenium deficient feeds are generally grown on acidic soils. However, it is not recommended that selenium be added to mineral supplements or diets fed to animals, as the margin between the toxic level and the dietary level required to prevent deficiency symptoms is very small (Kearl, 1982). However, supplementation of heifer calves with intraruminal Se pellets led to reduced basal plasma concentration of thyroxine, and increased body weight gain (Wichtel *et al.*, 1996). Therefore, from above-mentioned, the essence of the matter is that mineral deficiency on the sourveld may be prevalent, and that further investigation into this matter should be pursued to further determine what other inhibitory factors have a detrimental influence on the reproductive processes.

Recommendations

Emanating from the aforementioned, the following recommendations are made:

1. The sharp and consistent decline of blood and plasma glucose concentrations from the end of February (late summer) onwards, indicate that the present management practice of commencing feeding urea licks at the beginning of May, is erroneous. These licks should be offered as early as beginning of March, in an effort to help stem the drop in blood glucose concentrations. Further research would be appropriate to substantiate what affect this would have on the production performance (body weight fluctuations) from the summer to winter period. Should this measure be inadequate, the feeding of urea licks throughout the year should be considered. As a non-protein nitrogen supplement may stimulate pasture intake by as much as 40%, the stocking rate will then have to be reduced accordingly.

2. The soil of the Dohne Sourveld is acid (pH ranging between below 5 to 6.5), and of low fertility. The high rainfall which results in leaching of calcium and magnesium concentrations, imply that mineral studies with free-ranging beef females should be undertaken in this area to assess possible shortages which may contribute towards the low fertility rate of beef females. Any problem relating to the health, production or reproduction aspect in a herd which is not attributable to disease, or protein and energy deficiencies, should focus on mineral studies as causative or contributing factors towards low reproductive efficiency.

4. Although target body weight and/or body condition per se at mating may be an important guide to be used for successful breeding, present results indicate that the prerequisite for heifers and cows to conceive, is that the beef female should be in a positive energy phase (i.e. gaining body weight), during the mating season in order to predict and achieve maximum reproductive efficiency.

5. It appears as if blood and plasma glucose, as well as cortisol and thyroxine concentrations after parturition, are indicative of reproductive fitness, and an indication of whether cows will possibly conceive during the subsequent mating

period. This may be a useful tool to cull cows that are prone to be open at the end of the breeding season.

6. Blood glucose concentrations in the growing heifer may be a sensitive enough barometer to indicate the energy status in the animal body. However, it appears that a process such as lactation may influence the metabolism to such an extent that this parameter is less suitable as an indicator of the energy status. Because plasma albumin profiles were a mirror-image of body weight trends during the first year, it is concluded that this metabolite may be useful to reflect the protein status in the growing, pregnant heifer. Again, this finding could not be substantiated during the following year, presumable because of the effect of lactation. Plasma urea concentrations may also be a good indicator of protein status in heifers in order to distinguish between high and low protein concentrations in the feed and/or lick supplementation.

7. Plasma cortisol and thyroxine concentrations in the blood plasma cannot be used as effective indicators of either energy or protein content of the natural pasture, or of crude protein content in NPN supplemented licks.

8. Blood and plasma glucose concentrations appear to be good indicators in diagnosing pregnancy in heifers, a minimum blood glucose concentration of 2.23 mmol/l may be used as a norm for maintaining pregnancies of cows, on the sourveld. The suppressed blood and plasma glucose, cortisol and thyroxine concentrations of beef females even on the mixed sweetveld during their second lactation, indicate that additional feeding of hay or silage is essential during strategic stages of the reproductive cycle of the cow, such as during the first 8 weeks after parturition.

9. The fact that seasonal influences (weather patterns) on blood metabolic and hormonal profiles was not always significant and consistent, may be due to the lack of extreme climatic conditions, during the summer and winter seasons. In

further studies on the sourveld, efforts should rather centre around low nutritional status of the natural pasture on production and reproduction performance of beef cattle.

10. In conclusion, the type of animal grazing on the sourveld should be carefully selected. The Bonsmara did not perform well on this native pasture type, and results point to the possibility of grazing a smaller framed animal in these regions. The Nguni breed, with its "built-in" superior nitrogen urea and ammonia concentrations in the blood stream, may be a better choice from an economic point of view. It is postulated that this characteristic trait will facilitate the utilization of the sourveld in a more efficient manner, and also consume less of the protein and energy lick. With the belief that an indigenous, small framed and hardy beef animal will be more suited to the sourveld regions of South Africa, many of the recommendations above may have to be reconsidered. Such an animal, with lower nutritional requirements, may be efficient with regards to especially reproductive performance, in its utilization of the sourveld. In this case too, further research will clarify aspects concerning this issue.

SUMMARY OF THESIS

During the 1981/82 season, first-calf Bonsmara cows on the sourveld (lower plane of natural pasture) supplemented with an NPN lick in winter (sourveld + N), and sourveld not supplemented with an NPN lick in winter (sourveld - N), had significantly lower mean blood (2.13; 2.12 and 2.30 mmol/l; $P < 0.01$), plasma (3.20; 3.10 and 3.38 mmol/l; $P < 0.01$) and red blood cell glucose (1.06; 0.98 and 1.09 mmol/l, $P < 0.05$) concentrations, compared to the cows on mixed sweetveld (higher plane of natural pasture) supplemented with an NPN lick in the winter (sweetveld + N). Similar results were obtained with regard to total plasma protein, 66.0; 68.0 and 72.0 g/l ($P < 0.01$), plasma albumin, 32.0; 32.0 and 34.0 g/l ($P < 0.01$), and plasma globulin, 35.0; 36.0 and 38.0 g/l ($P < 0.05$) for the 3 treatments respectively. Plasma urea concentrations appeared to be a good indicator of protein status of first-calf cows on the 3 treatments, concentrations being 1.75; 1.35 and 3.75 mmol/l ($P < 0.01$) respectively.

During the following year (1982/83), differences in energy and protein constituents were much smaller, and only plasma albumin and urea concentrations were indicative of the higher nutritive value of the mixed sweetveld.

First-calf cows on the sourveld + N had significantly ($P < 0.01$) lower plasma cortisol concentrations than their counterparts on mixed sweetveld + N during the 1980 period (11.94 vs. 13.02 ng/ml respectively), and the 1981/82 period (8.78; 7.32 and 9.76 ng/ml for females in the 3 treatment groups respectively. During the subsequent year, virtually no differences in plasma cortisol levels were observed. No significant difference in plasma thyroxine concentrations was noted between experimental animals on the sourveld and mixed sweetveld during the 1980 period. During the following 2 seasons, plasma T4 levels were significantly ($P < 0.001$) elevated in the experimental animals on the mixed sweetveld (41.80; 40.80 and 48.10 ng/ml during 1981/82, and 39.10; 40.80 and 46.60 ng/ml during 1982/83 for females in the 3 treatment groups respectively.

Pregnancy influenced the mentioned metabolites and hormones Bonsmara cows to varying degrees. Lactation suppressed blood and plasma glucose concentrations for 8 to 10 weeks after parturition. The influence of lactation on total plasma protein, albumin and globulin concentrations

was not significant, and variable. Plasma urea levels were considerably suppressed in lactating first-calf cows, compared to dry cows in the 3 treatment groups, viz. 1.28 vs. 1.96 ($P < 0.05$); 1.02 vs. 1.53 ($P < 0.01$) and 3.05 vs. 3.98 ($P < 0.05$) respectively. Little differences were noted during the subsequent year. The adrenal cortex and thyroid activity were suppressed by the effect of lactation in first-calf cows in all 3 treatment groups during the 1981/82 season.

Cows having higher mean blood glucose concentrations after first parturition (8 weeks prior to mating) conceived at the next mating, compared to lower concentrations of infertile cows, viz. 2.49 vs. 1.93 mmol/l, and 2.20 vs. 2.07 mmol/l during the 1981/82 and 1982/83 seasons respectively. Similar results were obtained for plasma glucose (3.57 vs. 2.75, and 3.36 vs. 3.27 mmol/l respectively), plasma cortisol (8.2 vs. 4.0, and 8.3 vs. 5.3 ng/ml respectively) and plasma thyroxine (45.2 vs. 34.5, and 41.0 vs. 37.2 ng/ml respectively). Fertile cows were in a weight gaining phase from parturition to mating.

Season of the year generally did not have a significant and consistent influence on blood and plasma metabolites. Energy, and some of the plasma protein metabolites, were significantly higher during the summer of 1981/82, than during the winter. During the following year (1982/83), results were generally reversed, with plasma albumin and urea levels being higher during the winter than summer seasons. Plasma cortisol concentrations were generally elevated during the summer season, compared to the winter season.

Key words: Sourveld, mixed sweetveld, Bonsmara cows, glucose, plasma protein, plasma albumin, plasma globulin, plasma urea, cortisol, thyroxine

OPSOMMING VAN PROEFSKRIF

Gedurende die 1981/82 seisoen, het Bonsmara eerstekalfkoeie op die suurveld (laer peil van natuurlike weiding) met 'n NPN lekaanvulling in die winter (suurveld + N), en suurveld sonder 'n NPN lekaanvulling in die winter (suurveld - N), betekenisvolle laer gemiddelde bloed- (2.13; 2.12 en 2.30 mmol/l, P < 0.01), plasma- (3.20; 3.10 en 3.38 mmol/l, P < 0.01) en rooibloedselglukosekonsentrasies (1.06; 0.98 en 1.09 mmol/l; P < 0.05) in vergelyking met die koeie op die gemengde soetveld (hoër peil van natuurlike weiding) met 'n NPN lekaanvulling in die winter (soetveld + N) getoon. Soortgelyke resultate is verkry ten opsigte van totale plasma proteïene, 66.0; 68.0 en 72.0 g/l (P < 0.01), plasma albumien, 32.0; 32.0 en 34.0 g/l (P < 0.01) en plasma globulien, 35.0; 36.0 en 38.0 g/l (P < 0.05) vir die 3 behandelings onderskeidelik. Plasma ureumkonsentrasies blyk 'n goeie indikator van proteïenstatus van eerstekalfkoeie op die 3 behandelings te wees, nl. 1.75; 1.35 en 3.75 mmol/l (P < 0.01) respektiewelik.

Gedurende die daaropvolgende jaar (1982/83) was verskille in energie- en proteïenmetaboliete heelwat kleiner en het slegs plasma albumien- en ureumkonsentrasies die hoër voedingswaarde van die gemengde soetveld gereflekteer.

Eerstekalfkoeie op die suurveld het betekenisvolle (P < 0.01) laer plasma cortisolkonsentrasies as hul eweknieë op die gemengde soetveld gedurende die 1980 periode (11.94 vs. 13.02 ng/ml) en 1981/82 periode, gehad (8.78; 7.32 en 9.76 ng/ml vir diere in die 3 behandelingsgroepe onderskeidelik). Gedurende die daaropvolgende jaar, is feitlik geen verskil in cortisolpeile waargeneem nie. Geen betekenisvolle verskille in tiroksienkonsentrasies tussen eksperimentele diere op die suurveld en gemengde soetveld gedurende die 1980 periode waargeneem nie. Gedurende die volgende 2 seisoene, was die plasma T4 peile betekenisvol hoër (P < 0.001) in die eksperimentele diere op die gemengde soetveld gewees (41.80; 40.80 en 48.10 ng/ml gedurende 1981/82, en 39.10; 40.80 en 46.60 ng/ml gedurende 1982/83 vir die koeie in die 3 behandelingsgroepe onderskeidelik).

Dragtigheid het die genoemde metaboliete en hormone van Bonsmarakoeie tot 'n varierende mate beïnvloed. Laktasie het bloed- en plasmaglukosekonsentrasies vir 8 tot 10 weke na parturisie onderdruk. Die effek van laktasie op totale plasma proteïen-, albumien- en globulienkonsentrasies was nie betekenisvol nie, en het gevarieer. Plasma ureumpeile is aanmerklik onderdruk in lakterende eerstekalfkoeie, in vergelyking met droë koeie in die 3 behandelingsgroepe, nl. 1.28 vs.1.96 ($P < 0.05$); 1.02 vs. 1.53 ($P < 0.01$) en 3.05 vs. 3.98 ($P < 0.05$) onderskeidelik. Min verskille is gedurende die daaopvolgende jaar waargeneem. Die bynierkorteks- en tiroïedaktiwiteit is deur die effek van laktasie in eerstekalfkoeie in al 3 behandelingsgroepe gedurende die 1981/82 seisoen onderdruk.

Koeie met 'n hoër gemiddelde bloedglukosekonsentrasie na eerste kalwing (8 weke voor paartyd), het bevrug geraak tydens die daaropvolgende paarseisoen, in vergelyking met die laer konsentrasie van onvrugbare koeie, nl. 2.49 vs. 1.93 mmol/l, en 2.20 vs. 2.07 mmol/l gedurende die 1981/82 en 1982/83 seisoene onderskeidelik. Soortgelyke resultate is verkry ten opsigte van plasmaglukose (3.57 vs. 2.75 mmol/l en 3.36 vs. 3.27 mmol/l onderskeidelik), plasma cortisol (8.2 vs. 4.0 ng/ml, en 8.3 vs. 5.3 ng/ml onderskeidelik) en plasma tiroksien (45.2 vs. 34.5 ng/ml, en 41.0 vs. 37.2 ng/ml onderskeidelik). Vrugbare koeie was in 'n gewigstoenemende fase van parturisie tot paring.

Seisoen van die jaar het oor die algemeen nie 'n betekenisvolle en konsekwente invloed op bloed- en plasmametaboliete getoon nie. Energie-, en van die plasma proteïenmetaboliete, was betekenisvol hoër gedurende die somer van 1981/82 as gedurende die winter gewees. Gedurende die daaropvolgende jaar, is omgekeerde resultate oor die algemeen verkry, met plasma albumien- and ureumpeile wat hoër in die winter in vergelyking met die somer was. Plasma cortisolkonsentrasies het oor die algemeen hoër gedurende die somerseisoen in vergelyking met die winterseisoen geneig.

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