LIFE SCIENCES

Khulna University Studies 3(1):447-453

EFFECTS OF DIETARY PROTEIN LEVEL UPON PERIPHERAL PLASMA PROGESTERONE CONCENTRATION AND CONCEPTION RATE IN DAIRY CATTLE

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KUS-01/16-040601
Manuscript received: June 4, 2001; Accepted: August 13, 2001.

Abstract: There have been concerns that high protein intake had a significant influence on blood components and uterine constituents in non-lactating dairy cows and on spermatozoal motility. There are study that show that as dietary crude protein (CP) is increased, there is a tendency of reduced fertility in dairy cows while other studies have suggested that there is no relationship between reproductive performance and dietary CP intake. The objective of the study was to investigate the effect of dietary CP intake on blood progesterone concentrations and conception rate in dairy cattle. Fourteen lactating Friesian cows were randomly divided into two equal groups, a control group - Low Protein (LP) and a treated - High Protein (HP) group. The cows' oestrous cycles were synchronized and inseminated with frozen semen. Jugular blood samples were collected daily for at least 21 days after the first insemination starting on day of oestrus and then twice per week until they were 6-weeks of pregnant and then once per week until they were 12-weeks pregnant. The result showed that the progesterone concentrations in the control (LP) cows were significantly higher than in the treated (HP) cows during pre-conception oestrous cycles that did not result in pregnancy for 10 cows (5 in each group). Except on the last day of sampling (day 42), there were no differences in progesterone concentrations between the two groups of cows during first 6-weeks of pregnancy. The plasma progesterone concentrations were significantly lower in the HP group on days 45 and 52. However, dietary protein had no significant effect on overall group mean concentrations over this 6-week period (7 to 12 week) but the interaction, Group x time, was significant. The number of services per conception was lower for the cows fed the low CP diet. However, the median number of services per conception for the two groups was not statistically different. The results clearly indicated that cows fed the high CP rations had significantly reduced plasma progesterone concentrations when compared with those fed the low protein diet. The result also suggested that low progesterone concentrations particularly during the pre-conception oestrous cycle in the HP-fed groups were associated with a lower conception rate (32%) compared in the LP-fed group (54%).

Key words: Crude protein; Plasma progesterone; Conception rate; Bovine

Introduction

The effects of protein intake on reproductive function have not been well explained. There have been concerns that feeding diets with high concentrations of crude protein may decrease reproductive efficiency in dairy cows (Gould, 1969; Jordan and Swanson, 1979a; Edwards, Bartley and Dayton, 1980; Ferguson, Blanchard, Galligan, Hoshall and Chalupa, 1988 and Norton, Tranter, Campbell and Shepherd, 1989). It is still uncertain as to what factors might be responsible for this effect. Although all causes of embryonic mortality of dairy cows are not clear, it appears that progesterone plays an important role in embryo yield and maintenance of the conceptus at certain stages of pregnancy. Progesterone may not only determine if an early embryo survive or not, but in dairy heifers and cows it may influence whether it will progress to birth. Dasgupta *et al.* (1971) examined the aqueous solutions of urea on freshly recovered rabbit ova. The result of this experiment showed that, in the solutions containing 0.06 and 0.006% urea (low concentrations), the zona pellucida immediately become shrunken and dark, where as at concentrations of 6 and 0.6% (higher), the egg became solid, opaque and degenerated. Jordan and Swanson (1979a) fed 3 groups of 15 high-yielding dairy cows from 4 days postpartum for 91 days a diet calculated to contain 12.7, 16.3 and 19.3% crude protein (CP). The results of the experiment showed that the 19.3% CP group had fewer days open to first observed oestrus (27 days) than the 16.3 and 12.3% CP groups (41 days). However, the 12.7 and 16.3% CP groups had fewer services per conception (1.67) than the 19.3 CP groups (2.47) and the 12.7% CP group had fewer days open before conceiving than the 16.3 and 19.3% CP groups (69, 96 and 100), respectively. Linear relationships were obtained between the dietary protein intake, days before conceiving and services per conception. Similar results have been reported by Hagemeister, Lupping and Kaufmann (1980) who showed that when two levels of crude protein (16% and 19%) were fed, conception rates were 56% and 44% and services per conception were 1.79 and 2.25 respectively, although the mechanisms whereby this effect is produced are uncertain.

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DOI:https://doi.org/10.53808/KUS.2001.3.1.0116-L

The objectives of the study were to investigate the effect of dietary CP intake on blood progesterone concentrations and conception rate in dairy cattle.

Materials and Methods

All the experiments were conducted at the University of Queensland Veterinary Science Farm under the Department of Farm Animal Medicine and Production (FAMP), Brisbane, Australia. Fourteen lactating Friesian cows with an average age of 4.5 years and a live weight of 489 kg were used in the experiment. The experiment started 9 weeks after the cows had calved. The cows were randomly divided into two groups - a control group, low protein group (LP) and a treated group, high protein (HP) group. Both groups were given 4 kg premixed concentrate/head/day, 2 kg during the morning and evening milking, respectively. The composition of this concentrate was: sorghum 90.45%; meat and bone meal 4.5%; cotton seed meal 2.89%; di-calcium phosphate 1.44% and sodium chloride 0.725%. The HP group was given an additional 2.5 kg soybean meal/head/day after each morning milking. Both groups of cows were grazed together on a dry land pasture consisting of kikuyu (*Pennisetum clandestinum*), paspulum (*Paspulum dilatatum*), and couch (*Cynodon dactylon*). Both groups of cows had access to molasses (estimated consumption 3 kg/head/day). The intake of pasture was not measured as the animals grazed as part of the whole commercial herd and so it was not possible to calculate the percentage of protein in the diet. The cows remained on their experimental rations from the beginning of the experiment until they were 6-weeks pregnant. The protein supplement was withdrawn from the treatment group after 6-weeks of pregnancy and then both the groups of cows consumed the same diets. Drinking water was freely available all the times. The cows were fed their experimental diets for two weeks before any samples were collected. The cows' oestrous cycles were synchronized by intramuscular injections of two doses (25 mg each) of prostaglandin $F_{2\alpha}$, 11 days apart. Oestrus was detected by visual observation and by using KaMaR heat mount detectors. Cows were inseminated with a single dose of frozen semen at least 12 hours after being observed in standing oestrus. Cows detected in heat during the morning were inseminated in the afternoon and those detected in the afternoon were inseminated the following morning. The inseminations were performed by the same AI technician. Pregnancy was confirmed by rectal palpation at 42 days after insemination and also by monitoring progesterone values during the first 12-weeks of pregnancy. The number of services per conception of each cow was recorded. Jagular blood samples were collected daily for at least 21 days after the first insemination starting on day 0 (0=day of oestrus). Four cows (2 in each group) became pregnant after the first insemination. Additional blood samples were obtained from these four cows twice per week until they were 6-weeks pregnant and then once per week until they were 12-weeks pregnant. The ten cows (5 in each group) that did not conceive after their first insemination were re-inseminated during their subsequent natural oestrus periods until they conceived. Sampling procedures for these animals were the same as those described above. All blood samples were obtained between 2 and 3 hours after concentrate feeding. Heparinised blood was centrifuged immediately after the extracted plasma was stored at -20°C for plasma progesterone assays.

Statistical Analysis: The Statistical Analysis Systems (SAS, 1988) was used for all analyses. Data were analysed using Analysis of Variance by way of the General Linear Models (GLM) procedures. Time (day) effects were tested using Repeated Measures Analysis of Variance. Relationships between variables were assessed using the Correlation and Regression procedures. Frequency data were analysed using Kruskal-Wallis Test (SAS, 1988).

Results

Plasma Progesterone Concentrations: Mean plasma progesterone concentrations on each day of those oestrous cycles that did not result pregnancy for 10 cows (5 in each group) are given in Table 1. The length of the oestrous cycles varied from 19 to 22 days with a mean of 21 days. To allow for this variation, progesterone concentrations for the first 14 days after oestrus and the last 6 days before the next oestrus are presented in Table 1. The progesterone concentrations in the control (LP) cows were significantly ($p<0.05$) higher than in the treated (HP) cows on days 6, 7, 8, 9, 11, 12 and –6 of the oestrous cycles. These results are also presented in Figure 1 which shows that progesterone concentrations for both group of cows were lowest on days 0-2, increased to a maximum between days 12-15 and then decreased rapidly in the last 4 days of the cycle. The analysis of variance in Table 2 indicates that dietary protein significantly depressed ($p<0.01$) progesterone concentrations. The pattern of means over sampling times (days) was also significant ($p<0.0001$) for both groups while the interactions, Group x Times, was not significant.

Except on the last day of sampling (day 42), there was no differences in progesterone concentrations between the two groups of cows during the first 6-weeks of pregnancy (Table 3). Figure 2 illustrates these data and shows that by day 14 of the cycle the plasma progesterone concentrations had increased to a level close to maximum values of the luteal phase of the pre-conception oestrous cycles. The figure also indicates that progesterone concentrations remained fairly constant from day 14 onwards during the first 6-weeks of pregnancy for both group of cows. Although mean progesterone concentrations were not significantly different during the first 6-weeks of pregnancy, there was a trend suggesting that the concentrations were lower in HP-fed cows than in the LP-fed cows. Analysis of variance (Table 4) showed that there were no significant differences ($p>0.05$) between the group mean plasma progesterone concentrations during the first 6-weeks of pregnancy. However, the pattern of means over sampling times was significant ($p<0.0001$) while the interaction, Group x Time, was not significant.

Table 1. Mean plasma progesterone concentrations (ng/ml) in dairy cows (5 in each group) fed low or high protein diets during the pre-conception oestrous cycle.

Day of cycle	*Treatment*		*SEM*
	Low protein	High protein	
0	0.30	0.37	0.074
1	0.53	0.42	0.132
2	0.72	0.47	0.160
3	1.27	0.76	0.257
4	1.89	1.13	0.336
5	2.50	1.81	0.262
6	3.92^{a}	2.45^{b}	0.436
7	4.06^{a}	2.92^{b}	0.285
8	4.93^{a}	3.59^{b}	0.255
9	5.49^{a}	3.95^{b}	0.325
10	5.81	4.75	0.535
11	6.06^{a}	4.75^{b}	0.352
12	6.45^{a}	5.09^{b}	0.328
13	6.43	5.61	0.260
14	6.85	5.33	0.511
-6*	7.06^{a}	5.21^{b}	0.564
-5*	5.21	5.07	0.768
-4*	3.71	4.49	0.684
-3*	2.90	2.35	0.864
-2*	0.53	0.96	0.328
-1*	0.35	0.43	0.066

^{ab}Differing superscripts within rows indicate significant differences (p at least <0.05)
*Due to variation in the length of individual oestrous cycles, mean values for the last 6 days of the cycle have been related to subsequent days of oestrus

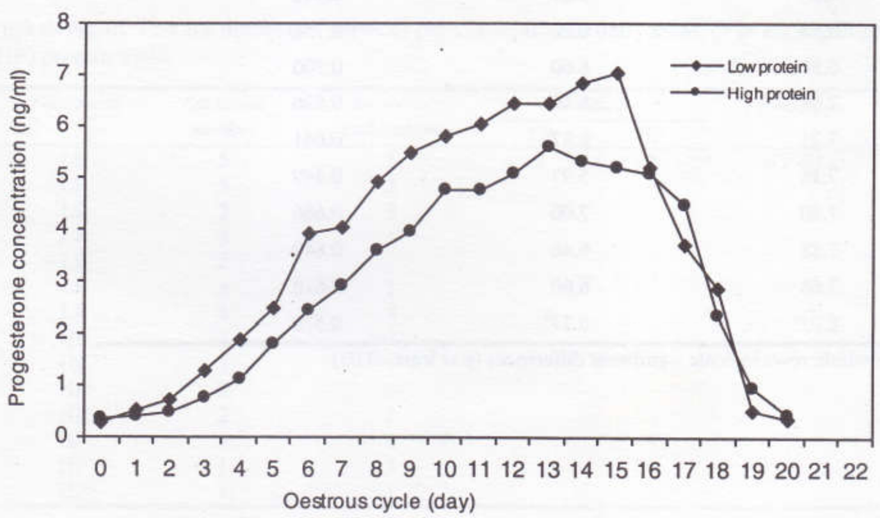

Fig. 1. Mean plasma progesterone concentrations in cows fed low or high crude protein diets during the oestrous cycle.

Table 2. Repeated Measures Analyses of Variance of plasma progesterone concentrations in dairy cows fed low or high protein diets during the pre-conception oestrous cycle.

Source	*DF*	*MS*	*F*	*Probability*
Group	1	27.03	12.12	0.0083
Error (group)	8	2.23		
Times	20	46.99	55.69	0.0001
Group x Times	20	1.30		
Error (times)	160	0.84	1.55	0.1936

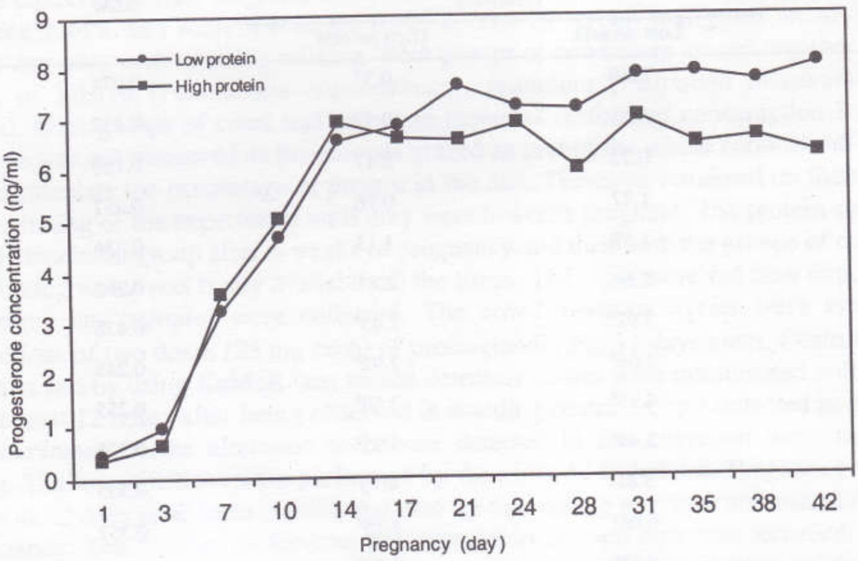

Fig. 2. Mean plasma progesterone concentrations in cows fed low or high crude protein diets during 6-weeks of pregnancy following successful insemination.

Table 3. Mean (± SEM) plasma progesterone concentrations (ng/ml) in dairy cows (7 in each group) fed low or high protein diets during the first 6-weeks of pregnancy following successful artificial insemination (AI).

Days following AI	*Treatment*		*SEM*
	Low Protein	High Protein	
1	0.46	0.36	0.050
3	0.97	0.67	0.221
7	3.27	3.57	0.418
10	4.69	5.03	0.441
14	6.55	6.92	0.758
17	6.86	6.60	0.700
21	7.61	6.56	0.536
24	7.21	6.87	0.641
28	7.16	5.97	0.449
31	7.80	7.00	0.666
35	7.88	6.46	0.840
38	7.68	6.60	0.616
42	8.02^{a}	6.27^{b}	0.576

[ab] Differing superscripts within rows indicate significant differences (p at least <0.05)

Table 4. Repeated measures analysis of variance of plasma progesterone concentrations in dairy cows fed low or high protein diets during the first 6-weeks of pregnancy following successful artificial insemination (AI).

Source	*DF*	*MS*	*F*	*Probability*
Group	1	14.25	0.91	0.3592
Error (group)	12	15.67		
Times	12	85.57	72.92	0.0001
Group x Times	12	1.74		
Error (times)	144	1.17	1.48	0.1552

Table 5. Mean (± SEM) plasma progesterone concentrations (ng/ml) in dairy cows (7 in each group) fed low or high protein diets from 7th to 12-weeks of pregnancy following successful insemination (AI).

Days following AI	*Treatment*		*SEM*
	Low Protein	High Protein	
45	8.30[a]	6.78[b]	0.499
52	8.78[a]	6.52[b]	0.614
59	8.00	7.00	0.737
66	8.73	6.80	0.861
73	8.60	7.63	0.738
80	7.51	8.44	0.449

[ab]Differing superscripts within rows indicate significant differences (p at lest <0.05).

The plasma progesterone concentrations from the 7th to the 12th week of pregnancy are shown in Table 5. The concentrations were significantly lower in the high protein fed group on days 45 and 52 ($p<0.05$). However, the analysis of variance (Table 6) shows that dietary protein had no significant effect on overall group mean concentrations during this period. The pattern of means in concentrations over this 6-weeks period (7-12 week) was not significant but the interaction, Group x Time, was significant ($p<0.05$).

Table 6. Repeated Measures Analysis of Variance of plasma progesterone concentrations in dairy cows fed low or high protein diets from the 7th to 12th weeks of pregnancy following successful artificial insemination (AI).

Source	*DF*	*MS*	*F*	*p*
Group	1	26.51	2.46	0.1430
Error (group)	12	10.79		
Times	5	0.85	0.54	0.7435
Group x Times	5	4.45	2.85	0.0224
Error (times)	60	1.56		

Conception Rate: The number of services per conception was lower for the cows fed the low CP diet than for those fed the high CP diet. However, the median number of services per conception for the two groups was not statistically significant (Table 7).

Table 7. Kruskal-Wallis Test for number of services per conception in dairy cows (7 in each group) fed low (LP) or high (HP) protein diets.

Cow No.	*Treatment*	*Lactation number*	*Services/ conception*	*Median* LP	*Median* HP	*Probability*
184	LP	5	3	2	3	0.2097
188	LP	5	2			
204	LP	3	3			
231	LP	2	1			
241	LP	2	3			
243	LP	3	1			
921	LP	1	2			
202	HP	4	7			
213	HP	3	4			
222	HP	3	2			
235	HP	2	2			
628	HP	4	4			
648	HP	1	3			
838	HP	1	1			

Discussion

Cows fed the high CP rations had significantly reduced plasma progesterone concentrations when compared with those fed the low protein diet ($p<0.01$). This finding is similar to that of the previous study which showed that in non-lactating cows progesterone concentrations differed significantly between HP and LP treatment groups during the first and the second oestrous cycle ($p<0.05$), particularly on days 4 to 14 of the oestrous cycle. Depressed progesterone concentrations in dairy cows fed high protein diets have also been reported by Jordan and Swanson (1979b) on day 14 of the oestrous cycle and by Sonderman and Larson (1989) on day 12 of a synchronized oestrous cycle and during the mid luteal phase of the subsequent oestrous cycle. Similar depressions have also been reported in ewes on day 12 after mating (Parr *et al*, 1992). However, these results are in contrast with those of Jordan *et al* (1983), Blauwiekel *et al* (1986) and Sonderman *et al* (1987) who could not detect any depression in progesterone concentrations when diets with a high CP concentration (>20%) were fed.

It was previously suggested that reduced plasma progesterone concentrations, particularly during the pre-conception cycle, may be associated with depressed conception rates. In the present study low plasma progesterone concentrations during the pre conception oestrous cycle in the HP fed group were associated with a lower conception rate (32%) than in the LP fed group (54%). Due to the small number of animals in the experiment this difference was not significant. Rosenberge *et al* (1977) also considered that conception rate was related to progesterone concentrations in the cycle preceding mating or insemination. On the basis of the present results it is suggested that elevated progesterone concentrations (>4 ng/ml) during the luteal phase of the oestrous cycle before insemination is positively associated with conception.

In this experiment, increased protein intake by cows was associated with depression of plasma progesterone concentrations during the early stage of pregnancy. Had feeding the protein supplement to the cows continued beyond the 6th week of pregnancy, the depressions may have been sustained for a loger period. Few other studies have examined progesterone concentrations during pregnancy but Joprdan *et al* (1979b) reported a depression on day 14 of the conception cycle in cows fed high protein diets. The significance of the depressed progesterone concentrations during the early stages of pregnancy is not clear but it is possible that it could lead to embryonic death. Low progesterone concentrations cause asynchrony of endometrial changes essential for uterine survival of fertilized eggs (Echternkamp and Hansel, 1973).

Lactating dairy cows fed high levels of crude protein during the oestrous cycle had lower conception rates than those fed the low protein diet. In the HP fed group, 7 pregnancies resulted from 22 inseminations (conception rate, 32%) whereas in LP fed group, 7 pregnancies resulted from 13 inseminations (conception rate, 54%). Kaim *et al* (1983) also showed an overall conception rate of 57% and 43% for LP and HP fed cows. The results of their experiment also indicated that the fertility of older cows (4th and later lactation) was more adversely affected by HP diets than was that of younger animals (1st to 3rd lactation). Similar trends were observed in the result reported here. Cows in their 3rd and later lactation fed HP diets required 4.25 services per conception whereas 1.67 services per conception were required for the younger cows (1st to 2nd lactation) when fed the same HP diets. Cows fed LP diets required 2 services per conception, whereas 1.75 services per conception were required for younger cows (1st to 2nd lactation) fed the same LP diets. Although conception rates were not significantly different between treatment groups (low number of animals may be the reason), the trend suggested that the conception rate was lower in the HP fed group than in the LP fed group. This finding is similar to those reported by Kaim *et al* (1983) who found that in three experiments, the conception rate was lower in HP fed groups, but the difference was only statistically significant when the data from three experiments were combined. Carrol *et al* (1988) reported that while the conception rate of cows fed diets containing 20% CP were not statistically different from those fed diets containing 13% CP, there was a trend towards lower conception rates in cows fed the HP diets (1.8 versus 1.5 services/conception).

Jordan *et al* (1979a) observed that the number of services per conception were 2.47,1.87 and 1.47 for cows fed diets containing 19.3%, 16.3% and 12.7% CP diets respectively. This could be compared with the present study where the number of services per conception was 3.14 and 1.86 in the HP and LP fed groups, respectively. Folman *et al* (1981) found that conception rates were 69, 56, and 44% when cows were fed 16% CPSP (crude protein containing protected soybean meal), 16% CP and 20% CP, respectively. In this study soybean meal was not protected but when data from 16% and 20% CP fed cows were compared, the results were very similar to the findings in present experiment where conception rates were 53.85 and 31.82% in the low and high CP groups, respectively. Therefore, the fertility of the cow may be adversely influenced by excess protein in the diet.

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