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EFFECTS OF DIETARY PROTEIN UPON CONCENTRATIONS OF UREA IN PERIPHERAL PLASMA AND UTERINE FLUID IN DAIRY CATTLE

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Abstract: There have been concerns that elevated urea nitrogen concentrations in uterine fluid had significant effects on bovine spermatozoal motility and survival. However, it is still uncertain whether dietary protein has any effects upon concentration of urea nitrogen and ammonia in the peripheral plasma and uterine fluid in dairy cattle. The objectives of the study were to investigate the effects of dietary protein intake on concentrations of (i) urea nitrogen in the uterine fluid, (ii) ammonia in the blood and (iii) urea nitrogen in the peripheral plasma. Six lactating, non-pregnant and normally cycling Friesian cows were used in two randomly divided equal groups, receiving either a low or high dietary protein intake. Uterine fluids and blood samples were collected from each cow and uterine urea nitrogen; blood ammonia and plasma urea nitrogen concentrations were measured. The study showed that the cows fed the high protein (HP) diet had significantly higher uterine urea nitrogen, blood ammonia and plasma urea nitrogen concentrations than cows fed the low protein (LP) diet. The results revealed that the concentration of urea nitrogen in the uterine fluid were higher in cows fed HP diet compared with those fed a LP diet. These results suggested that high concentrations of urea in the plasma could diffuse into the uterine lumen and might create a sub-optimal environment in the uterus, which might act as an antagonist to fertility. The high level of blood ammonia and plasma urea nitrogen concentration in the HP fed cows, which could occur due to high protein intake, may affect the viability of sperm, ova and embryos and the growth and survival of the foetus by producing biochemical, endocrionological and tissue derangement. Therefore, it is likely that excessive concentration of urea nitrogen in peripheral plasma and uterine fluid and plasma ammonia of high protein fed lactating cows may be detrimental to the viability of sperm, ova, embryos or foetus.

(**Keywords:** Crude Protein, Blood & Uterine Urea-N and Ammonia in Dairy Cattle)

Introduction

There have been concerns that elevated urea nitrogen concentration in uterine fluid had significant effects on bovine spermatozoal motility and survival. The moot question is whether urea solutions have similar effects on *in vitro* bovine embryo development or survival. Solutions with urea concentrations as low as 0.006 g/100 ml have been shown to be spermicidal and higher concentrations (0.6 g/100 ml and 6.0 g/100 ml) have resulted in complete degeneration of rabbit eggs (Dasgupta *et al,* 1971). Feeding diets with high concentrations of crude protein may decrease reproductive efficiency in dairy cows (Gould, 1969; Jordan & Swanson, 1979a; Edwards *et al.*, 1980; Ferguson *et al.,* 1988 and Norton *et al.,* 1989). However, it is still uncertain what factors might be responsible for this decreased reproductive efficiency. The results of the experiments of Jordan & Swanson (1979a) showed that the highest crude protein (CP) group (19.3%) had fewer days open to observed oestrus (27 days) than the lowest CP groups (16.3 & 12.3%).

Heap (1962) pointed out that, there is no method for measuring the actual volume of the uterine fluid recovered and thus, the assays for the various constituents would be imprecise. To solve this problem, Pycock and Allen (1990) used Hank's Balanced Salt Solution (HBSS) containing phenol red to flush the uteri of mares. Knowing the concentration of phenol red in the flushing medium, and measuring the concentration in the recovered flushing, permitted calculation of any dilution of the flushing medium. In the present study HBSS containing phenol red was used to flush the cows' uteri so that the assays for uterine constituents were more precise. This study was undertaken to investigate the effects of dietary protein intake on concentration of (a) urea nitrogen in the uterine fluid, (b) ammonia in the blood and (c) urea nitrogen in the peripheral plasma.

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. Six lactating, non-pregnant and normal cycling Friesian cows were used in the experiment. Cows were on average 9.6 weeks post calving at the start of the experiment. The six cows were randomly divided into two equal groups, a control group – low protein group (LP) and a treated group–high protein group (HP). Both groups of cows

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were given the diets: 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 0.72% except that the high protein supplement cows were given an additional 3.0 kg soybean meal/head/day after each morning milking. A two-week adaptation period was used to accustom the cows to the experimental diets. Amounts of concentrate offered were near the cows' maximum consumption and refusals were non-existent. Because these cows were also grazing dry-land pastures, the percentage of protein in the diet could not be calculated. Mean (±SEM) age (years) of the cows was 2.73.(±0.251) for the LP group and 2.83 (±0.251) for the HP group. Mean (± SEM) live weight (kg) of the cows was 448.33 (±19.177) for the LP group and 434.66 (±19.177) for the HP group on the day the experiment commenced.

Oestrous cycles were synchronized using Synchro-mate B and Prostaglandin $F_{2\alpha}$ injections. Oestrus was detected by visual observation and by using KaMaR heat mount detectors. Jugular blood samples were obtained daily from each cow during the preliminary feeding period and from the day of the estrous synchronization program commenced until 7 days later. Plasma was separated and stored at -20^{0}C for plasma urea nitrogen assays. A single blood sample was collected into EDTA tubes from each cow on the day of oestrus and plasma from these samples was used for ammonia assays within 2 hours of collection.

Uterine fluids were collected and processed from each cow on the day of oestrus following the standard procedure except that 30 ml Hank's Balanced Salt Solution (HBSS) containing phenol red (Cat no. 18-100-54, Flow Laboratories, Irvine, Scotland) was used as the flushing medium instead of 0.9% sterile saline. Uterine fluid samples were stored at -20^{0}C for uterine urea nitrogen measurements. Blood and uterine fluid samples were collected between 2 and 3 hours after feeding the concentrate.

Results

The cows fed the high protein diet had significantly higher ($P<0.01$) uterine urea nitrogen and blood ammonia concentrations ($P<0.05$) than cows fed the low crude protein diet on the day of oestrus (Table-1). Mean (± SEM) concentrations of plasma urea nitrogen in the cows were 8.57 (± 1.41) mg/100 ml for the HP group and 8.88 (± 1.41) mg/100 ml for LP group on day 1 of preliminary feeding. However, the plasma urea concentration in the cows fed the soybean meal was significantly elevated by the third day after commencement of feeding of the meal. It stabilized at approximately 20-mg/100 ml by the eight day after commencement of feeding. The concentration in the HP group stabilized at approximately double that in the LP group (Table-2).

Table 1. Mean (±SEM) uterine urea nitrogen (mg/100 ml) and blood ammonia concentrations (µg/100 ml) in cows fed high or low protein diets on day of oestrus (day=0).

		Treatment	SEM	Level of Probability
Factor	High Protein	Low Protein		
Urea-N	357.83[a]	138.85[b]	21.976	0.0021
Ammonia	79.53[a]	34.24[b]	10.627	0.0394

[ab]Differing superscripts within a row indicate significant difference (P at least <0.05).

Table 2. Mean (± SEM) plasma urea nitrogen concentrations (mg/100 ml) in cows fed high or low protein diets during 7 days following beginning of the oestrus synchronization (SYN) program.

Days following SYN	Treatment		SEM
	High Protein	Low Protein	
1	26.98[a]	13.60[b]	2.702
2	25.97[a]	14.35[b]	0.980
3	24.30[a]	10.96[b]	0.911
4	22.57[a]	12.62[b]	1.851
5	20.78[a]	11.88[b]	1.493
6	26.70[a]	15.41[b]	1.905
7	20.92[a]	12.75[b]	1.241

[ab] Differing superscripts within a row indicate significant difference (P at least <0.05).

Discussion

Several authors have suggested that high crude protein (CP) intake increases the concentration of urea nitrogen in the uterine fluid and that this might create an environment less than optimal for conception. Jordan *et al.* (1983) suggested that high concentrations of urea in the plasma could diffuse into the uterine

lumen and might act as an antagonist to fertility. Haour and Saxena (1974), Jordan *et al.* (1983) and Carroll *et al.* (1988) suggested that high local urea nitrogen concentrations may inhibit LH binding to its receptors on luteal cells and thereby could decrease plasma progesterone concentrations. However, Howards, Jessee and Johnson (1976) detected a permeability barrier to urea that existed between the blood and seminiferous tubule fluid whereas Johanson and Woodbury (1978) found similar barriers to urea between blood and cerebrospinal fluid. Subsequently, McRae and Kennedy (1981) found a permeability barrier to urea between blood and the uterine lumen in rats when treated hormonally to mimic early pseudopregnancy. These authors suggested that further experiments were required to determine whether micromolecules such as protein, minerals (ions) or energy substrates might have any influence on preimplantation embryonic survival or growth.

In the present study, the concentrations of urea nitrogen in the uterine fluid were greater in cows fed a HP diet compared to those fed an LP diet. Similar results were reported by Ferguson *et al.* (1986). However, the concentrations in the low protein group were greater in the present study than those reported by these authors. Although the results of this experiment were different from those reported earlier, the group means (high protein/low protein treatments) were significantly different in both cases. The higher concentration found in the present study can be explained by the fact that the dilution of the flushing medium (HBSS) in the uterus was estimated from the dilution of phenol red in this solution. In the previous study, no estimate of the extent of dilution of the flushing medium was made. The urea nitrogen concentration values obtained in this study suggest that the results of the previous study underestimated urea concentrations by at least a factor of 15.

When the data in experiments were pooled, it was found that the average concentration of urea nitrogen in the uterine fluid was much higher than in the plasma both in LP (38 versus 9 mg/100 ml) and HP (96 versus 17 mg/100) groups. These results were similar to those of Ferguson *et al.* (1986) who found average concentrations of 22.3 versus 16-mg/100 ml and 119 versus 23-mg/100 ml in uterine fluid and plasma of LP and HP fed cows, respectively. The factors that could be responsible for this increased concentration of urea nitrogen in the uterine fluid compared with that in the plasma are still uncertain. However, it is possible that urea may preferentially diffuse into the uterine lumen. Jordan *et al.* (1983) also suggested that high concentrations of urea in the plasma could diffuse into the uterine lumen.

The effect of different concentrations of ammonia on *in vitro* growth of embryos and their subsequent viability was not tested in this experimental program. As per discussion by several author, for example Chalupa (1984) and Visek (1984), it was known that excess ammonia concentrations in the blood, uterine fluid and rumen, which could occur due to high protein intake, may affect the viability of sperm, ova and embryos and the growth and survival of the foetus by producing biochemical, endocrinological and tissue derangement.

As found in experiment, supplementing cows with high protein resulted in elevated plasma urea concentration. The concentration of blood ammonia in the lactating dairy cows in experiment were significantly higher in the HP-fed cows than in those fed the LP diets. This finding is also similar to those of the previous studies with non-lactating and lactating dairy cows reported by Hossain *et al. (1998)* and Jordan *et al.* (1983). In the present and the previous studies, significant relationships between concentrations of ammonia in the blood and urea nitrogen concentrations in the uterine fluid were detected.

The high level of blood ammonia and plasma urea nitrogen concentration in the HP fed cows, which could occur due to high protein intake, may affect the viability of sperm, ova & embryos and the growth and survival of the fetus by producing biochemical, endocrionological and tissue derangement. Therefore, it is likely that excessive concentration of urea nitrogen in peripheral plasma and uterine fluid and plasma ammonia in lactating cows fed high protein diet may be detrimental to the viability of sperm, ova, embryos or fetus. This would be worthy of further investigation.

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