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Effect of Feeding Quickly Degradable Nitrogen on the Development of Ovarian Follicles in Lactating Dairy Cows

Research Article

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ABSTRACT

The objective of this study was to examine the effect of an excess intake of quickly degradable nitrogen (QDN) on ovarian follicle development. Twenty lactating dairy cows were fed mixed silage and concentrates twice daily. The control diet was a typical ration for high producing dairy cows in the United Kingdom (crude protein (CP)=17.5%; metabolizable energy (ME)=11.8 MJ/kg DM). The cows were randomly divided into two groups, control diet (control; n=10) and excess QDN diet (QDN; n=10). The QDN group was fed an additional 250 g of urea per cow per day; for 17 days. Ovaries were scanned daily using B-mode ultrasonography for 17 days. The excess QDN diet resulted in significantly elevated ($P<0.05$) plasma urea concentrations four days after starting urea feeding and these were maintained until the end of the experiment. The QDN treatment did not significantly affect ($P>0.5$) the number of small (≥0.5 cm diameter), medium to large follicles (0.5-1.4 cm diameter) and pre-ovulatory (1.5-2.3 cm diameter) follicles. The results of the current study has shown that feeding excess QDN, as urea, for 17 days has no effect on ovarian follicle development in lactating dairy cows.

KEY WORDS lactating dairy cows, ovarian follicles, urea.

INTRODUCTION

Fertility of dairy cows depends on many factors including quality and quantity of nutrition, milk yield, management practices and environmental stress. Infertility occurs if animals are driven too long in a negative energy balance by one or more of these factors. Hormonal imbalance will likely occur and oocyte development and maturation are disturbed (Lamming and Darwash, 1998; Al-Katanani *et al.* 2002). Dawuda *et al.* 2002; Dawuda *et al.* 2004a; Dawuda *et al.* 2004b; Laven *et al.* 2004 and Laven *et al.* 2007 reported that various mechanisms such as hormonal imbalance, ammonia toxicity to the developing embryo and a hostile uterine environment that impair implantation are involved in steady decline of fertility in dairy cows in the United Kingdom, there is still a lot to be explained. Spring turnout of dairy cattle in the United Kingdom coincides with the first flush of pasture vegetation. The grass at that moment may contain very high levels of quickly degradable protein (Whittaker, 1999). This may negatively affect fertility since many dairy herds in the United Kingdom experience a short-term fall in pregnancy rates during this spring 'turnout' (Laven and Drew, 1999; Whittaker, 1999). The reduced fertility is associated with high intakes of high protein intake from the grass. It seems the problem of 'turnout' is worst on pastures heavily fertilized with nitrogenous fertilizers (Laven *et al.* 2002). Several earlier studies on the effect of protein nutrition or high nitrogen on reproduction in dairy cows (Ferguson and Chalupa, 1989; Laven and Drew, 1999; O'Callaghan and Boland, 1999; Butler, 1998;

Butler, 2000) have shown inconsistent results. Dawuda *et al.* (2004b) suggested that the effect of low protein intake has a negative effect on cattle fertility by (1) blocking the effect of oestradiol positive feedback, (2) eliminating progesterone withdrawal effect on the release of Gonadotropin-releasing hormone (GnRH)/gonadotrophins and (3) by enhancing the negative effect of opioid peptides on the activity of the GnRH pulse generator in the hypothalamus. Laven *et al.* (2004) conducted a study on follicular development in lactating Holstein dairy cows feeding them with diets high in quickly degradable nitrogen (QDN). They reported that there was no significant effect of QDN treatment on follicular development. Furthermore, Dawuda *et al.* (2002) reported that feeding dairy cows with diets high in quickly degradable nitrogen (QDN) has a toxic effect on developing embryos, however, cows were able to adjust within 10 days. Recently Edwards *et al.* (2014) reported that there was no significant difference in the number of antral follicles between groups of Zebus (*Bos indicus)* heifers fed high protein diet or fed low protein diet. Due to various mechanisms involved and the inconsistency of the effect of high nitrogen intake on fertility in lactating dairy cows, the current study was therefore, designed to investigate the hypothesis that intake of excess quickly degradable nitrogen (QDN) for 17 days in form of urea affects fertility in lactating dairy cows by inhibiting development of follicles from small to medium, medium to large and from large to pre-ovulatory follicle stages.

MATERIALS AND METHODS

Animals and treatment

The experiment was performed under the United Kingdom Animals (Scientific Procedures) Act 1986. Twenty lactating Holstein dairy cows aged between 4 and 8 years were included in the study. The cows were selected from the Royal Veterinary College herd using the following criteria: (i) third or subsequent lactation, (ii) calved in the previous 10 weeks and (iii) deemed suitable for re-breeding following veterinary examination. The basic diet was fed twice daily from the moment of calving and include maize and grass silage *ad libitum* and 11 kg of concentrate a day. The diet was calculated according to AFRC (1993) norms for cows in early lactation to meet the demands of metabolisable energy (ME) and metabolisable protein (MP). The detailed compositions of the diets have been published in an earlier paper (Dawuda *et al*. 2002).

The study was set up as a controlled balance design with a group receiving a standard diet after the animals had been synchronized for oestrus (control group: C) and an experimental group receiving the extra urea in the diet thereafter (QDN group).

In order to include comparable animals in each on the 2 treatment groups the animals were ranked according to calving date and their 9-18 day milk yield. Pairs of animals with comparable ranking were randomly assigned to one of each group. Means (±SD) for parity was 3.4 ± 0.2 in both groups. The 9-day to 18-day mean (±SD) daily milk yields for C and QDN groups were 38.1 ± 0.9 and 38.4 ± 1.1 kg per day, respectively.

Cows were housed in a cubicle building and were bedded daily with chopped straw with access to fresh water at all times. They were milked twice daily and milk yield recorded automatically on each milking occasion. Mixed maize and grass silage and concentrate diet were recorded. They were also weighed and assigned body condition scores once every two weeks (scale 0-5, MAFF, 1986).

Between day 55 and 65 postpartum, when all cows were cycling based on observed oestrus, a synchronized oestrus was induced. The cows were divided into two diet groups 5 days before PRID insertion (Figure 2). From that moment the controls continued to receive the basic diet while the QDN group had the basic diet supplemented with 250 g urea per cow per day (McEvoy *et al.* 1997) for 17 days. In order to avoid palatability problems, the urea was mixed with sugar beet nuts (2 kg per cow per day) and was fed to all the cows separately on top of the ration in two equal feeds after morning and afternoon milking. Control cows received 2 kg per cow per day of the sugar beef nuts only, mixed in 250 g of water. No problems of palatability or incomplete intake were seen.

Blood collection

Blood samples were collected into heparinised impregnanted tubes three times weekly commencing 2 days postpartum and continued throughout the experimental period (90 days postpartum) for determination of progesterone content by radioimmunoassay in order to monitor ovarian activity (Bulman and Lamming, 1978). Blood was collected into EDTA impregnated tubes (Greiner Labortechnik Ltd., Gloucestershire, UK) 3 h after feeding urea in the treatment group and 3 h after feeding the control diet in the control group for determination of plasma urea and ammonia. Blood samples were placed into iced water immediately after collection and centrifuged at 1800 × g at 4 ˚C, the plasma harvested and stored at -80 ˚C. This procedure was completed within 1 h of blood collection.

Oestrus synchronisation

A synchronised oestrus was induced in all cows by inserting a progesterone releasing intravaginal device (PRID) (Sanoffi Animal Health Limited, Watford, UK) for 9 days. On day 8 after PRID insertion, each cow received 500 µg of a prostaglandin-$F_2\alpha$ analogue (Cloprostenol) (Estrumate ^{R}),

Schering-Plough Animal Health Division, Welwyn Garden City, UK) intramuscularly (i.m.) to induce luteolysis. Three days after PRID removal animals were checked for standing oestrus behaviour.

Ultrasound scanning of ovaries

The genital tracts of all cows were examined using transrectal B-mode ultrasound (Aloka SSD 210, BCF Technologies, Livingston, UK), with ovaries being scanned using a 5 MHz linear array transducer. Cows were examined daily starting from the day urea feeding was first introduced in the QDN group and continued until day three after PRID withdrawal to monitor the final development of pre-ovulatory follicles. A similar procedure was adopted for the control group. The number and size (<0.5 cm to >1 cm) of all visible follicular structures were recorded.

Plasma metabolites assays

The concentrations of urea in plasma were determined in an autoanalyzer using the method of Talke and Schubert (1965). Plasma ammonia concentrations were determined using phase 2 of the same method, with decreased absorbance at 340 nm reflecting plasma ammonia concentrations (Mondzac *et al.* 1965). The respective intra- and inter-assay coefficient of variations (CVs), based on low and high quality control pools were 3.8 and 7.1%. The sensitivities of the assays were 0.1 and 4 µmol/L, respectively.

Statistical analyses

Follicle number i.e. the number of small (<0.5 cm), medium (>0.5 – 1 cm) and pre-ovulatory (>1 cm) follicles were analysed using the Chi-square (X_2) test. Milk yield were analysed using repeated measures analysis of variance (ANOVA) and using the mean of weeks -3 to -1 (acclimatization period) as a covariate. Sampling and analyses of grass silage feedstuff was carried out every week for determination of dry matter content (DM) (g/kg DM), pH, ammonia content (NH_3), content of crude protein (CP) (g/day), content of neutral detergent fibre (NDF), content of neutral cellulose gammase digestibility (NCGD), content of ash, content of metabolisable energy (ME) and organic matter digestibility (OMD) by the Near Infra-red (NIR) spectroscopy.

Live body weight data for the whole study period were analysed using repeated measures (ANOVA) and body condition score (BCS) using a Kruskal-Wallis test, with BCS at calving used as covariate. The effect of treatment group on mean ±standard error of the means (±SEM) plasma urea and ammonia concentrations were compared using a multivariate repeated measures ANOVA; the least significant difference (LSD) test was used to determine where differences occurred.

RESULTS AND DISCUSSION

Plasma progesterone concentrations

Mean (±SEM) plasma progesterone concentrations i.e. area under the curve (AUC) for the CONT and the QDN group was 21.9 ± 0.7 and 23.7 ± 0.6 ng/mL. Figure 1 shows typical plasma progesterone profiles for a cow in the CONT group (a) and a cow in the QDN treatment group (b).

Productivity data

Following introduction of the treatment diet, there was no significant difference (P>0.05) in milk yield or feed intake in the two groups (Table 1). The live body weights and body condition scores between the two groups (CONT and QDN) also remained similar (P>0.05) (Table 1).

Plasma urea and ammonia concentrations

Urea and ammonia concentrations 3 h after feeding are shown in Table 2. Ammonia and urea levels increased significantly 3 h after QDN feeding in QDN group over the CONT group. Ammonia and urea levels at this time were significantly higher than the CONT cows (P<0.001) (Table 2).

Follicle development

Mean follicle numbers (small, medium, large and pre-ovulatory follicles) are presented in Table 2. There were no significant difference (P>0.05) between the QDN group and the CONT group for all categories of follicles. Figure 2 shows the mean maximum size (2.5±0.2 cm) of follicles on day 17 after the beginning of urea treatment in the QDN group and in the CONT group. There were no significant differences (P>0.05) between the two groups.

Previous reports in lactating dairy cows have shown that 250 g urea per cow per day in the diet is adequate to achieve blood ammonia concentrations to impair follicle health (Laven *et al.* 2004; Dawuda *et al.* 2002).

The blood ammonia concentration of the current study is similar to these previous reports. The plasma urea concentrations for the cows fed QDN supplementation in the current study were also similar to those which resulted in reduced fertility in dairy cows and heifers receiving high levels of dietary nitrogen (Laven *et al.* 2007; Laven *et al.* 2004; Dawuda *et al.* 2002; Hammon *et al.* 2000; Elrod and Butler, 1993; Canfield *et al.* 1990; Howard *et al.* 1987; Jordan *et al.* 1983).

Dietary strategies for meeting the nutritional requirements of high producing dairy cows have been adjusted in response to gains in milk yield by their genetic potential for high milk production. Diets high in crude protein (17-19%) are normally fed in early lactation to stimulate and support high milk yield.

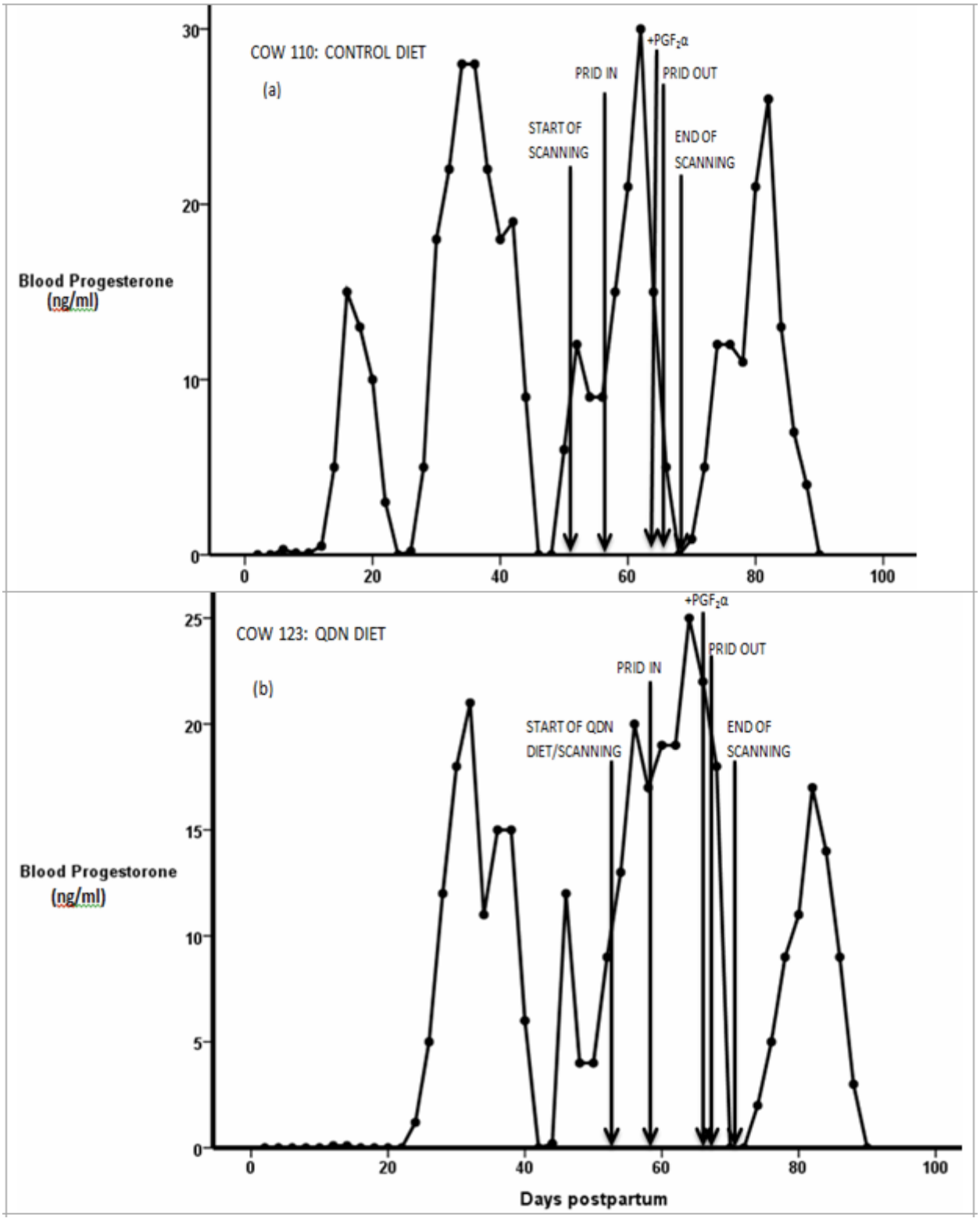

Figure 1 Blood progesterone concentrations for two cows (QDN diet for 17 days and control diet)
The day PRIDs were inserted varied between 55 and 65 days postpartum

However, feeding excess nitrogen as protein or as inorganic nitrogen to lactating dairy cows can reduce reproductive performance in some animals (Edwards *et al.* 2014; Canfield *et al.* 1990; Butler *et al.* 1996; Butler, 1998; O'Callaghan and Boland, 1999) but not in others (Caroll *et al.* 1988; O'Callaghan *et al.* 1997) despite significant increase in blood urea and ammonia.

Similarly, in sheep, there are reported effects on reproduction in ewes fed with excess QDN (McEvoy *et al.* 1997; Fahey *et al.* 1998).

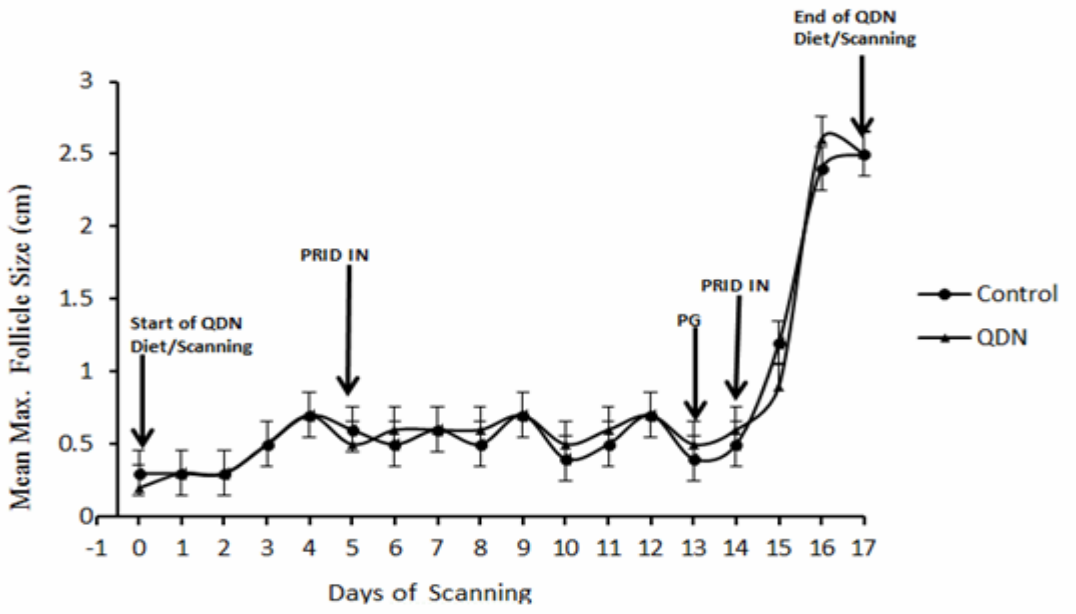

Figure 2 Mean (±SEM) maximum follicle size after treatment with QDN diet for 17 days or Control diet in lactating dairy cows
No animal ovulated after PRID OUT

Table 1 Comparison of the mean milk yield, mean dry matter intake, mean live body weight and mean body condition score following the introduction of the experimental diet till the end of the experiment

Parameter (±SEM)	Control (n=10)	QDN (n=10)
Mean milk yield (kg/day)	38.2±0.83	38.0±0.83
Mean dry matter intake (kg DM/cow/day)	23.8±0.35	23.2±0.38
Mean live body weight	640±9.0	639±9.7
Mean body condition score (scale of 5; 1=thin and 5=fat)	2.8±0.3	2.7±0.3

QDN: quickly degradable nitrogen.
SEM: standard error of the means.

Table 2 Effect of QDN feeding on mean (±SEM) blood urea and ammonia and mean (±SEM) follicular development in lactating dairy cows

Parameter	Control (n=10)	QDN (n=10)
	Mean (±SEM)	Mean (±SEM)
Plasma urea (mmol/L)	5.6±0.3[a]	8.8±0.3[b]
Plasma ammonia (NH_3) (µmol/L)	59.4±4.8[a]	98±5.1[b]
No. of small follicles (<0.5cm)	20.1±0.8	18.9± 1.1
No. of medium to large follicles (>0.5 -1 cm)	13.1±1.5	14.4±1.2
No. of pre-ovulatory follicles (>1 cm)	7.2±0.2	6.8±0.4
Mean time (hours) between removal of PRID and observed oestrus	70.2±3.7	70.5±4.1

QDN: quickly degradable nitrogen and PRID: progesterone releasing intravaginal device.
SEM: standard error of the means.

In the current study, despite the cows in the excess QDN group having significantly higher plasma urea than the control cows, there was no effect of feeding excess urea on milk yield, dry matter intake, live body weight, body condition score, the number of small follicles, the number of medium to large follicles and the number of pre-ovulatory follicles. This result suggests that there were no systemic effects associated with the feeding of 250 g of urea and that the feed intake of the QDN cows had not been adversely affected. This lack of deleterious effect of QDN intake on follicular development especially the pre-ovulatory follicle could be due to the ability of the lactating dairy cow to adjust to the toxic effect of QDN when fed beyond 10 days (Dawuda *et el*. 2002; Laven *et al*. 2004).

The results of the present study are in agreement with tose recently reported by Edwards *et al.* (2014) who has shown that feeding a high level of protein to Australian Zebus (*Bos indicus)* heifers had no effect on the number of antral follicles compared to Zebu heifers fed with low level of crude protein.

Dawuda *et al.* (2014) who demonstrated no effect of QDN feeding on follicle development in lactating dairy cows. Nevertheless, QDN feeding might have effect on the quality of developing follicles.

This is shown from the study conducted by Dawuda *et al.* (2002) who indicated that intake of urea affects reproductive performance in lactating dairy cows through impairment of embryo quality. This impairment of quality of embryos is likely to be as a result of a carry-over of deleterious effect on the quality of developing oocytes rather than on the number of follicles starting from emergence of follicular waves through to the pre-ovulatory follicle stage. It has been indicated that delayed ovulation and follicular persistency can lead to the ovulation of an aged, poor quality oocytes which is associated with low fertilization potential and high potential for early embryonic mortality (Al-Katanani *et al.* 2002). Dawuda *et al.* (2002) had earlier demonstrated that the deleterious effect of urea intake is most pronounced when it is introduced at the time of insemination. This suggests that the harmful effect of urea intake on reproductive performance in lactating dairy cows is likely to be a locally possibly epigenetic effect on the oocytes and / or embryos that compromises the normal genetic engineering and competence for survival. Another possible harmful effect of urea intake may be the interference of sperm transport within the female genitalia. The lack of significant differences in milk yield and total number of follicles between QDN and the control group further suggests that systemic effects on health are not out spoken and thus local effects are likely the explanation for the effects of QDN on fertility in dairy cows.

QDN intake might exert its negative effect is through the hypothalamo-pituitary-ovarian axis where there is reduction in the pulses and amplitude of gonadotrophin releasing hormone (GnRH), follicle stimulating hormone (FSH) and luteinizing hormone (LH) (Diskin *et al.* 2002). The reduced pulses and amplitude of LH can lead to marked reduction in oocyte quality and decreased pregnancy rates (Diskin *et al.* 2002). Similarly, poor quality of preovulatory follicles and / or of the subsequent corpus luteum may affect the quantities of oestrogen and progesterone resulting in impaired reproductive performance in the lactating dairy cow. However, Dawuda *et al.* (2014) reported no significant effect of increased intake of QDN on GH or LH release pattern or on plasma oestradiol, IGF-I and insulin. These inconsistent results suggest that other mechanisms may play a role, possibly the depletion or enhancement of receptors for GnRH, FSH, LH, oestrogen, progesterone and prostaglandin F2-alpha at target organs.

Future research may be directed to the local effect at the oocytes or embryonic or uterine level (Butler, 1998; Dawuda *et al.* 2002; Dawuda *et al.* 2004a; Laven *et al.* 2004). Another reason for these differences could be due to the timing and length of duration of treatment with urea. Studies by Dawuda *et al.* (2002); Laven *et al.* (2002) have indicated that the lactating dairy cow is capable of adjusting to the toxic effect of urea when treatment exceeds 10 days. This will however, depend on some other factors such as the timing and duration of urea treatment. It will also depend on where the effect of urea treatment is targeted along the hypothalamo-pituitary-ovario-uterine axis in lactating dairy cows.

CONCLUSION

In conclusion, the current study has indicated that intake of quickly degradable nitrogen (QDN) in the form of urea does not affect oocyte development in lactating dairy cows. Further research is necessary to clarify different aspects of this issue and to aggregate the findings in order to draw definite conclusions.

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