Usefulness of Milk Urea Concentration to Monitor the Herd Reproductive Performances in Crossbred Karan-fries Cows

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ABSTRACT : The study was undertaken to investigate the validity of milk urea concentration as an index of the reproductive performances in crossbred Karan-Fries (Holstein Friesian×Tharparkar) cows under farm condition. Milk urea was analysed in noon milk samples (1200 to 1300 h) to interrelate with the interval from parturition to first service, number of insemination per conception, first service conception rate and service period. Milk progesterone (P4) was analysed in noon milk samples on the day 1, 10, 20 and 30 post insemination to study the effect of milk urea concentration on early embryonic mortality. The interval from parturition to first service was found significantly ($p<0.01$) higher (77.2±5.5 days) when milk urea concentration was ≥63.4 mg/dl. The average milk urea concentrations (mg/dl) were found 42.1±2.5, 47.9±1.5 and 50.3±3.1, respectively in cows that conceived at 1st, 2nd and 3rd insemination. However, the variation was not statistically significant. The first insemination conception rate was found significantly ($p<0.05$) higher (68.8%) when milk urea level was ≤32.4 mg/dl. The service period was found significantly ($p<0.05$) higher (125.4±8.8 days) when milk urea concentration was ≥45.1 mg/dl. The milk P4 level indicated that the cows, those were detected as non-pregnant on day 60 post insemination were initially pregnant but the pregnancy was terminated sometime during the day 30 to 60 post insemination. The study indicates that the milk urea values may be used as an index of reproductive performances in dairy herd when individual animals are not being monitored for nutritional status. The altered milk urea values may be utilised by the farmers as ready reference to rectify the protein and energy nutrition in cows to achieve the better reproductive performances in herd. ***(Asian-Aust. J. Anim. Sci. 2006. Vol 19, No. 1 : 26-30)***

Key Words : Milk Urea, Milk Progesterone, Conception Rate, Service Period, Cow

INTRODUCTION

Fertility is often associated with the nutritional status. The effect of protein nutrition on fertility appears complex. Several mechanisms of protein effects on fertility have been described (Ferguson and Chalupa, 1989). These include impairment of gonadal activities by toxic products of nitrogen metabolism from rumen, altered function of hypothalamo–pituitary-ovarian axis by nitrogen by-products or altered protein to energy ratio. Proposed effects are not mutually exclusive and may occur together in an additive or synergistic fashion. In dairy cows the excess dietary protein increases blood urea level, alters uterine fluid composition, decreases uterine pH and reduces conception rates (Jordan et al., 1983; Elrod and Butler, 1993; Elrod et al., 1993). The plasma P4 concentration has been reported to be lower in cows fed high dietary protein (Jordan and Swanson, 1979; Elrod and Butler, 1993; Elrod et al., 1993). It has been found that the heifers maintained on high protein diet exhibit extended luteal phase and inter-estrus interval, which indicates that the embryonic death occurs some after the critical period that is day 15 to 16 post breeding (Elrod and Butler, 1993).

Excess feeding of easily degradable protein results in an accumulation of ammonia in the rumen, which in turn increases the formation of urea in liver (Ropstad and Refsdal, 1987). The protein intake, particularly the ratio of dietary protein to energy affects the urea level in blood and milk (Oltner et al., 1983). Earlier report indicates the possibility of using milk protein content and urea concentration in either blood or milk to monitor the dietary energy and protein intake in dairy cows (Hwang et al., 2000). Measurement of milk urea provides a useful index for studying the association between the dietary protein metabolism and reproductive efficacy in dairy cows (Butler, 1998). However, literatures are not available on the validation of these hypotheses using milk urea level as a pointer in large-scale dairy farm, where animals are not being maintained under experimental situation.

The present investigation was conducted to interrelate the milk urea concentration with different reproductive parameters in crossbred Karan-Fries cows to validate its utility as an index of reproductive performances under farm condition.

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MATERIALS AND METHODS

Animals and management

The study was conducted on crossbred Karan-Fries (Holstein Friesian×Tharparkar) cows maintained at the dairy farm, National Dairy Research Institute, Karnal, Haryana, India. Only healthy breedable cows, free from any reproductive disorders were selected for the study. Animals were maintained in loose house system. Estrus detection was carried out in an open paddock by vasectomised bull parading twice daily and milk P4 analysis by Radio Immuno Assay (RIA) (Gupta and Prakash, 1993) and was presented for Artifical Insemination (AI). Cows were not bred before the day 40 postpartum. The frozen semen used for AI was checked periodically for quality. Cows were checked for pregnancy on the day 60 post insemination per rectal by herd veterinarian and by milk P4 analysis.

Feeding

Animals were fed farm grown green fodders *ad libitum* twice daily in morning (0900 to 0930 h) and in afternoon (1500 to 1530 h). Cows were provided a let down ration of 0.5 kg concentrate and an additional amount of 1.0 kg concentrate was fed for every 2.5 milk produced above 5.0 kg daily yield. The daily concentrate requirement was divided into three equal parts and fed during each milking in morning (0500 to 0600 h), noon (1200 to 1300 h) and evening (1800 to 1900 h). Animals had free access to water through out the day.

Collection of samples and data

Noon (1200 to 1300 h) milk samples were collected from the cows on 1st and 3rd day of each insemination to study the relationship between milk urea concentration and conception rate at different insemination. Average milk urea values on 1st and 3rd day of each insemination for individual animals were used for interpretation. For freshly calved cows noon milk samples were collected on the day of parturition followed by at 7 day interval till the first service to study the interrelationship between milk urea concentration and the interval from parturition to first insemination. The average milk urea values of individual animals were used for interpretation. The samples were collected from milk weighing bucket after complete milking and thorough mixing and were kept at 4°C until analysed for urea on the same day. For P4 analysis noon milk samples were collected on the day 1, 10, 20 and 30 post insemination. The animals that expressed estrus within 21 days post insemination were discarded form the experiment. For milk P4 estimation the samples were stored in plastic storage vial in refrigerator for a maximum period of 2 weeks after addition of 100 µl milk preservative (2.64 g potassium dichromate+0.61 g mercuric chloride per 100 ml distilled water) per 10 ml of milk. The noon milk samples were also collected separately for urea analysis on the same day. The data were collected from farm record for the date of calving, interval from parturition to first service, service period and number of insemination per conception. To study the diurnal pattern of milk urea concentration the milk samples of all the experimental animals were analysed once from morning (0500 to 0600 h), noon (1200 to 1300 h) and evening (0600 to 0700 h) milking.

Estimation of milk urea and milk progesterone

Milk samples were analysed for urea content using a colorimetric p-dimethylaminobenzaldehyde (DMAB) procedure (Dhali et al., 2005). Briefly, the milk samples were warmed at room temperature (30°C) and mixed well. Milk (10 ml) was deproteinised with 12% cold TCA solution (10 ml), allowed to stand for an hour, centrifuged at 3,000×g for 30 minutes and then filtered. Clear supernatant (2 ml) was mixed with 2 ml DMAB reagent (1.6 gm DMAB+90 ml ethanol+10 ml concentrated HCl). Spectrophotometric absorbance was 425 nm. The whole milk P4 was estimated by direct radioimmunoassay as per the method described by Gupta and Prakash (1993). The sensitivity of the assay was 12.5 pg/tube which corresponded to 1.25 ng/ml milk with 50% binding sensitivity being 102 pg/tube. The intra- and inter-assay coefficients of variations were 8.3% and 12.6%, respectively.

Statistical analysis

To study the effect of milk urea concentration on interval from parturition to first service, number of insemination per conception and service period, the data were subjected to ANOVA using general linear model procedure of SPSS (SPSS 10.0.1., 1999). To study the variations in milk urea and milk P4 concentrations on different post insemination days in pregnant and non-pregnant cows the data were subjected to ANOVA using general linear model procedure of SPSS (SPSS 10.0.1., 1999). Duncan multiple range test was used to separate the means if found significant (SPSS 10.0.1., 1999). The statistical significance of the conception rate at different inseminations was analysed with χ^2 test as described by Snedecor and Cochran (1994).

RESULTS

A positive relationship was found between milk urea concentration and the interval from parturition to first service. The interval increased with the increasing milk urea concentration and was found significantly ($p<0.01$) higher (77.2±5.5 days) when milk urea concentration was ≥63.4 mg/dl (Table 1). A positive relationship was found between

Table 1. Relationship between the milk urea concentration and interval from parturition to first service

Milk urea (mg/dl)	Interval from parturition to first service (days)	SE
≤38.4 (17)	64.1[a]	7.3
38.5-50.8 (14)	64.7[a]	3.3
50.9-63.3 (13)	66.0[a]	8.9
≥63.4 (8)	77.2[b]	5.5

[a, b] Indicates the values within column differ at p<0.01; The values within the parenthesis indicate the number of experimental animals.

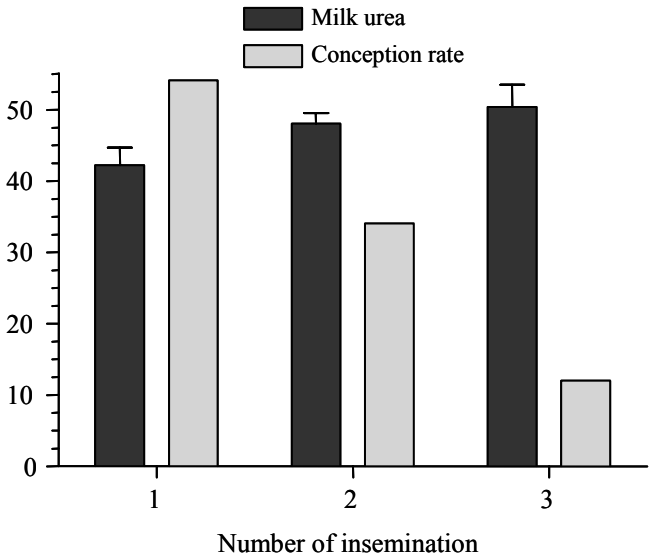

Figure 1. Milk urea concentration (mg/dl±SE) and conception rate (%) in cows by number of insemination required for conception; Number of experimental animal was 67.

the milk urea concentration and number of required insemination for conception. Milk urea concentrations were found 42.1±2.5, 47.9±1.5 and 50.3±3.1, respectively in cows that conceived at 1st, 2nd and 3rd insemination (Figure 1). However, the variation was not statistically significant. The first insemination conception rate was found significantly (p<0.05) higher (68.8%) when milk urea level was ≤32.4 mg/dl (Table 2). When milk urea level was within the range of 32.5 to 45.0 mg/dl, animals were mostly conceived at second insemination. The conception rate was found significantly (p<0.05) poor at both 1st and 2nd insemination when milk urea level was ≥45.1 mg/dl (Table 2). The service period was also found significantly (p<0.05) higher (125.4±8.8 days) when milk urea level was ≥45.1 mg/ dl (Table 3). Milk P4 level (ng/ml) on the different post

Table 3. Relationship between the milk urea concentration and service period

Milk urea (mg/dl)	Service period (days)	SE
≤32.4 (14)	89.9[a]	10.2
32.5-45.0 (19)	107.7[b]	15.6
≥45.1 (36)	125.4[c]	8.8

[a, b] Indicates the values within column differ at p<0.05; The values within the parenthesis indicate the number of experimental animals.

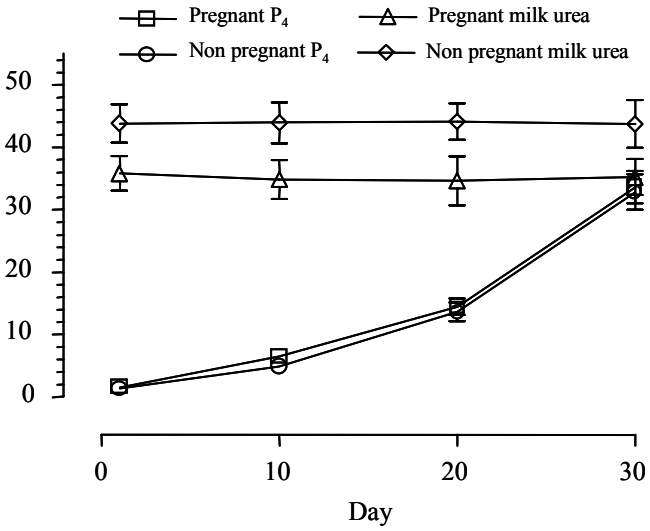

Figure 2. Milk progesterone (P4) level (ng/ml±SE) and milk urea concentration (mg/dl±SE) on different post insemination days in pregnant and non-pregnant cows; Number of experimental animal was 58.

insemination days did not vary significantly between the pregnant and non-pregnant cows (Figure 2). The P4 levels indicated that the cows, which were detected as non-pregnant on the day 60 post insemination were actually pregnant initially but the pregnancy was terminated some time during the day 30 to 60 post insemination. However, the milk urea level was found significantly (p<0.01) higher in non-pregnant cows than in the pregnant cows (Figure 2). A clear diurnal pattern of milk urea concentration (mg/dl) was observed and the concentrations were found significantly (p<0.05) higher in noon samples (34.1±4.1) than morning (27.1±2.4) or evening samples (30.4±4.3).

DISCUSSION

Postpartum milk samples were analysed for urea content to investigate whether the high milk urea concentration had

Table 2. Conception rate at different insemination in different levels of milk urea

Milk urea (mg/dl)	Conception rate at different insemination (%)			
	Insemination 1	Insemination 2	Insemination 3	Non-pregnant
≤32.4 (16)	68.8* (11)	6.2* (1)	6.2 (1)	18.8 (3)
32.5-45.0 (16)	37.5* (6)	50.0* (8)	- (0)	12.5 (2)
≥45.1 (48)	39.6* (19)	29.2* (14)	14.6 (7)	16.6 (8)

* Indicates the values within column differ at p<0.05 by χ^2 test of association; The values within the parenthesis indicate the number of experimental animals

any relationship with the onset of postpartum estrus and the subsequent first service. The result showed a positive relationship between the milk urea concentration and interval from parturition to first service. The interval was found highest (77.2 days) when milk urea concentration was ≥63.4 mg/ dl. Gustafsson and Carlsson (1993) have reported that the number of days from parturition to first service increases with increasing urea concentration in milk. The interval has been found 128 days in dairy cows with average milk urea nitrogen concentration of 20 mg/dl. Figuera et al. (1992) have found that with same CP content in diet, the interval from parturition to first service increases (34 vs. 50 days) with increasing RDP content in diet (60 vs. 65%), which also increases the blood urea concentration (20 vs. 21 mg/dl). Our results are comparable with these findings. The increased interval from parturition to first service with increasing milk urea concentration was probably due to the altered uterine environment in these animals that probably delayed the postpartum uterine involution process and subsequent onset of estrus. In earlier studies it has been found that the ionic concentration in uterine secretion (Jordon et al., 1983) and uterine pH (Elrod and Butler, 1993) differ in cows maintained on high protein diet or diet with excess degradable protein than in cows on normal diet. During the current study the milk urea levels were found comparatively higher than the earlier reports. This was probably due to the fact that the noon milk urea concentrations were used for interpretation, which were found significantly higher than the morning or evening concentrations.

To study the relationship between milk urea concentration and number of insemination required for conception, the milk urea concentrations on 1st and 3rd day of all inseminations were pooled and the average values were taken into account. The first insemination conception rate was reduced significantly when milk urea values were more than 32.4 mg/dl. Ropstad and Refsdal (1987) have suggested a significant interaction between the milk urea level and number of insemination per conception. The first service conception rate has been registered as 71% (Carroll et al., 1994) and 68% (West Wood et al., 1998) with plasma urea nitrogen concentration of approximately 23 mg/dl. In virgin heifers too the poor first service conception rate (82% vs. 61%) has been found when the peak plasma urea values have increased from 17.5 to 23.6 mg/dl (Elrod and Butler, 1993). During current study a negative relationship was found between the milk urea concentration and service period or conception rate. The result is in agreement with the earlier reports. Elrod and Butler (1993) have reported that the conception rate decreases considerably when plasma urea nitrogen concentration exceeds 16 mg/dl. They have recorded the conception rate of 87.5% and 42.8% with the plasma urea nitrogen concentration of <9 mg/dl and >16 mg/dl, respectively. Ferguson et al. (1993) have showed that the likelihood of conception rate decreases with increasing serum urea nitrogen level above 20 mg/dl. Butler et al. (1996) have found the mean pregnancy rate of 68% and 47% with the milk urea nitrogen concentrations of <19 mg/dl and >19 mg/dl, respectively. Ferguson et al. (1993) have also reported that the 21 mg/dl plasma urea nitrogen is high enough to depress the pregnancy rate. Wenninger and Distl (1994) have concluded that the reproductive traits are optimum when milk urea concentration is within the range of 15 to 25 mg/dl.

To investigate the effect of urea concentration on early embryonic mortality, the milk P4 and milk urea concentrations were estimated on day 1, 10, 20 and 30 post insemination. It was observed that the milk P4 level did not vary significantly among the pregnant and non-pregnant cows till the day 30 post insemination. However, the concentration of milk urea was found significantly higher in the non-pregnant cows. It has been reported earlier that the increased urea concentration in plasma or milk causes the impairment of fertilisation and embryo development. The uterine environment may be modified adversely and the normal process of fertilisation, embryo development and implantation of conceptus is hampered. When cows are maintained on high protein diet the urea concentration in plasma and uterine secretion increases along with low magnesium, potassium and phosphorus concentration during luteal phase (Jordon et al., 1983). Weibold (1988) has reported that the embryonic mortality is associated with the uterine environment, which is significantly different form those of cows with normal embryo and most of the embryonic death occurs before day 5 during cleavage. The concentrations of glucose, total protein, calcium, magnesium and potassium have been found significantly higher in the uterine flushing of cows with abnormal embryo. But the plasma P4 did not differ among the cows with normal and abnormal embryo. It has been proposed that the excess degradable protein acts through some undefined mechanism to decrease the uterine pH during luteal phase, which may play a crucial role in the observed fertility. Heifers on high protein diet also exhibit extended luteal phase and inter estrus interval of 26 to 36 days. This prolonged phase probably indicates the embryonic death, which occurs some time after the critical period that is the day 15 to 16 post breeding (Elrod and Butler, 1993). It has been observed earlier that the uterine pH decreases approximately by 0.1 pH unit for each 5 mg/dl increase in plasma urea nitrogen concentration (Elrod et al., 1993; Butler, 1998). Milk P4 concentration has been found to be lower (<2 ng/ml) on day 21 of estrous cycle in the cows with high milk urea content (Larson et al., 1997). In endometrial cell culture the presence of urea significantly diminishes the effect of P4 in maintaining a pH differential between apical and basal compartment (Gilbert et al., 1996).

The current investigation indicated that the embryonic death occurred some time during the day 30 to 60 post insemination. However, a similar P4 profile in both pregnant and non-pregnant animals indicated that the embryonic death probably did not occur due to sub optimal P4 level. Rather it was probably due to the altered uterine environment in animals with high milk urea concentration.

In conclusion, in present study, the increased number of insemination for conception, longer service period and embryonic death in animals with high milk urea concentration were probably due to the altered uterine environment. Perhaps it was not due to the sub optimal P4 level and associated impairment of the normal fertilisation process and embryo development. The study indicates that the milk urea values may be used as a valuable index of reproductive performances in dairy cows under farm condition, when individual animals are not being monitored for nutritional status. The altered milk urea values may be utilised by the farmers as ready reference to rectify the protein and energy nutrition in cows to achieve the better reproductive performances in herd.

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