THE IMPACT OF FEEDING PROPYLENE GLYCOL, BYPASS FAT AND BYPASS PROTEIN ON PROGESTERONE CONCENTRATION IN POSTPARTUM DAIRY CATTLE


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Received: June 25, 2015; Accepted: July 12, 2015

Abstract: High yielding dairy cows cannot consume adequate nutrients in early lactation to support the level of milk yield. The peak milk production, at about 5-8 weeks postpartum occurs earlier than maximum feed consumption causing cows to be in negative energy balance and to mobilize fat from adipose tissue. Negative energy balance affects gonadotropin secretion, ovarian physiology and ovarian hormone production which in turn adversely affects the reproductive performance of cattle during postpartum period. The present study was undertaken to evaluate the influence of propylene glycol, bypass fat and bypass protein on postpartum luteal activity as the interest was being grown among dairy farmers, veterinarians and advisers. A total of twenty eight postpartum dairy cows were randomly allotted to four groups with seven animals in each group. The Group-I animals served as control without being fed any feed additive. Group-II animals received propylene glycol once daily as a drench at the rate of 300 ml per head while Group-III and Group-IV animals were fed 100 and 200 gms of commercially available bypass fat and bypass protein respectively. In all the groups, the mean serum progesterone concentration was at basal level on day 7 and 14 postpartum and subsequently its level increased with clear higher level from day 21 onwards. The difference in mean progesterone levels at different stages of the study was significant (Pd”0.05) and the increase seen was almost linear with highest levels on day 63 postpartum. Although, the stage variation between groups was significant, the higher or lower values recorded at different stages was not consistent with a given group.

Key words: Propylene glycol, Bypass protein, Bypass fat, Progesterone, Dairy cattle

INTRODUCTION

The early postpartum period is most critical for a dairy animal in achieving optimum number of calvings during its lifetime. The main factor identified to influence the reproductive function during the postpartum period is nutritional status [1,2]. Earlier studies have shown that high yielding dairy cows cannot consume adequate nutrients in early lactation to support the level of milk yield. The peak milk production, at about 5-8 weeks postpartum occurs earlier than maximum feed consumption causing cows to be in negative energy balance and to mobilize fat from adipose tissue in early lactation [3]. It has been put forth that negative energy balance affects gonadotropin secretion, ovarian physiology and
ovarian hormone production which in turn adversely affects the reproductive performance of cattle during postpartum period [2].

The timing and magnitude of negative energy balance alters hypothalamic secretion of GnRH and its effect on gonadotropin dependent secretion of progesterone, which affects expression of estrus and support of the uterus during early pregnancy [4]. Britt [5] suggested that severe negative energy balance on ovarian function could result in abnormal follicular and /or luteal development in the third, fourth and fifth estrous cycles which comprise the most desirable period for dairy cows to conceive. In recent days, the recommendation of feeding bypass protein and bypass fat for dairy cattle and buffaloes has become a debatable issue. Further, the interest in using propylene glycol as a feed additive has grown among dairy farmers, Veterinarians and Advisers. Feeding bypass fat to high producing lactating cows can enhance energy density of ration and energy intake in early lactation without compromising rumen cellulolytic bacterial activity [6]. Thus, the deleterious effect of acute negative energy balance on lactation can be avoided. Unlike Rumen degradable protein, feeding bypass protein to dairy cows was found to have beneficial effects on reproduction [7]. In India, most studies have been conducted to investigate the effect of feeding bypass protein on growth and milk production. Unlike the effect on growth and milk production, the studies pertaining to reproductive performance are relatively less [8]. Infact, there is scarcity of literature available regarding the effect of rumen undegradable protein on reproductive performance in early lactating cows.

Propylene glycol (PG) is a glycogenic substrate that has beneficial effects on carbohydrate and fat metabolism during early lactation in cows [9]. The first ovulation occurred significantly earlier and the first luteal phase was significantly longer indicating better quality of the follicles and the improved ovarian function in propylene glycol fed than control cows [3]. Gamarra et al. [10] observed that PG tended to increase the amount of P4 during the luteal phase in heifers. On the contrary, Rizos et al. [11] found that treatment with PG had no effect on follicular dynamics. The detectable luteal function in PG-treated heifers was more compared to control animals [12].

Cholesterol serves as a precursor for the synthesis of progesterone by ovarian luteal cells and high density lipoproteins (HDL) and low density lipoproteins (LDL) demonstrated a similar ability to synthesize progesterone. Progesterone not only prepares the uterus for implantation of the embryo, but also helps to maintain pregnancy by providing nourishment to the conceptus. Between 25 and 55 per cent of mammalian embryos die in early gestation and many of these losses are due to inadequate function of luteal cells [13]. The greatest concentration of serum cholesterol did coincide with the greatest concentration of serum P4 [14]. Tyagi et al. [15] observed the plasma progesterone concentration to be higher in treatment groups fed with 2.5% Ca salts of fatty acids (Dry matter intake).

Waterman et al. [16] reported the pregnancy rate was not affected by the amount of rumen undegradable protein (RUP) consumed in beef cows. Arias et al. [17] reported that the interval from calving to first estrus and the period to first luteal activity were not different between 45% rumen undegradable protein (RUP) treated group compared to 30% RUP treated group. The interval from parturition to first normal luteal activity (P=0.15) and the percentage of animals with luteal activity (P=0.14) during the experimental period tended to be improved in the 45% RUP treated group compared to the 30% RUP treated group. Aboozar et al. [18] reported increased plasma cholesterol and progesterone in cows fed high levels of rumen undegradable protein.

In India, no literature was available with regard to impact of feeding these three nutrients on luteal activity viz. propylene glycol, bypass fat and bypass protein in a single study. Hence, the present work was designed to study the impact of feeding propylene glycol, bypass fat and bypass protein on postpartum circulating levels of progesterone.

**MATERIALS AND METHODS**

The present investigation was carried out utilizing Holstein Friesian crossbred dairy cows of different parities, varying from two to seven were maintained at Military Dairy Farm, Bangalore. The pregnant animals were dried at the completion of 7th month of pregnancy and were shifted to individual calving pens at least 15 days prior to the expected date of calving. The periparturient animals were closely monitored for the onset of calving. During the experimental period, in addition to treatment diet, all the cows were fed a basal standard diet (concentrates) based on
the quantity of milk produced.

For the present study a total of twenty eight healthy animals which had no difficulty and no disease diagnosed at the time of parturition or seven days postpartum and considered normal [19] were randomly allotted to four groups with seven animals in each group. The Group-I animals which served as control were managed and received basal herd ration routinely practiced in the farm. The Group-II animals received propylene glycol once daily as a drench at the rate of 300 ml [20] per head in addition to the standard feeding and management received by the Group-I animals. The propylene glycol was drenched to these animals 90 minutes after concentrate feeding in the morning hours. The Group- III animals in addition to standard management and feeding routinely practiced, also received once daily 100 gms of commercially available bypass fat (NUTRI JOULE, M/s Vetcare Ltd., Bangalore) with routinely fed concentrates at the time of morning milking. The Group-IV cows in addition to routine management and feeding also received once daily 200 gms of commercially available bypass protein (NUTRI PRO, M/s Vetcare Ltd., Bangalore) with routinely fed concentrates at the time of morning milking. The Group-II animals received propylene glycol for a period of 35 days during day 7 and 42 postpartum whereas, Group III and IV animals received bypass fat and bypass protein respectively for a period of 60 days starting from day 7 postpartum. About 10 ml blood was collected aseptically from jugular vein at an interval of one week starting from day 7 till 63 postpartum for the estimation of serum progesterone. The blood samples were drawn on specified day about 6 hour post feeding and allowed to clot. The serum was harvested by centrifuging the clotted blood at 2500 rpm for 10 minutes and the serum samples so obtained were stored at -20 °C till analyzed. The serum progesterone concentration was estimated using serum Autopack Elisa kit supplied by Anand Brothers (India) Pvt. Ltd.

Statistical analysis: The collected data was analyzed [21]. Data were transformed by using Arc sin^{-1} x transformation. The matrix of data was tested by different normality test. Due to small sample size (df<12), 1.5 times of standard deviation was added to the original values. Multiple linear models were employed to draw the significant inference (LSD). The statistical methods like descriptive statistics, multivariate linear model and Least significant difference were used to test the hypothesis.

RESULTS

The blood serum progesterone was estimated at weekly interval from day 7 postpartum to day 63 postpartum to assess the effect of supplementation of propylene glycol, bypass fat and bypass protein (Table 1). In Group-I (control) cows under routine management the mean serum progesterone was very low on day 7 postpartum. Subsequently its level increased with clear higher level from day 21 onwards. Its level recorded on day 28 was significantly (P<0.05) higher than its level recorded at immediate, preceding and succeeding stages. The difference in mean serum progesterone levels at different stages of the study was significant (P<0.05) and the increase seen was almost linear with highest levels reaching on day 63 postpartum (Table 1; Fig.1). In Group-II animals which received propylene glycol supplementation, the mean serum progesterone was

<table>
<thead>
<tr>
<th>Days after calving</th>
<th>Mean serum progesterone concentration in ng/ml (Least square mean ± SE)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Control (Group - I)</td>
</tr>
<tr>
<td>7</td>
<td>0.16±0.08^*</td>
</tr>
<tr>
<td>14</td>
<td>0.63±0.70^*</td>
</tr>
<tr>
<td>21</td>
<td>1.23±0.79^*</td>
</tr>
<tr>
<td>28</td>
<td>1.99±0.67^*</td>
</tr>
<tr>
<td>35</td>
<td>1.74±0.97^*</td>
</tr>
<tr>
<td>42</td>
<td>1.88±0.76^*</td>
</tr>
<tr>
<td>49</td>
<td>1.91±0.42^*</td>
</tr>
<tr>
<td>56</td>
<td>1.87±0.07^*</td>
</tr>
<tr>
<td>63</td>
<td>2.02±0.86^*</td>
</tr>
<tr>
<td>P-value</td>
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at basal level on day 7 and 14 postpartum. The stage variation of mean serum progesterone level was significant (P≤0.05). Its level was clearly high from day 21 onwards reaching highest level on day 56 and 63 postpartum. There was also significant dip in mean serum progesterone concentration on day 35 and 49 with significant difference from preceding and succeeding stages (Table 1; Fig 1).

In Group-III animals which received bypass fat supplementation the mean serum progesterone was at basal level on day 7 and 14 postpartum. The stage variation in mean serum progesterone concentration was significant (P≤0.05) with higher values from 21 days onwards. The mean serum progesterone concentration was continued to be high from day 21 postpartum with significant dip recorded on day 42 and 56 postpartum (Table 1; Fig 1).

Similar to other groups, the mean serum progesterone was at low level on day 7 and 14 postpartum in Group-IV cows. The stagewise difference recorded was significant (P≤0.05) with higher levels on day 21 and 56 postpartum. The difference in mean serum progesterone recorded at other stages was non-significant.

The stage variation in mean serum progesterone concentration between groups showed non-significant variation on day 7 and 14 postpartum. The variation between treatment groups recorded at all other stages (from day 21 to 63 postpartum) was significant (P≤0.05). The stage variation between groups though significant, the higher or lower values recorded at different stages was not consistent with a given group (Table 1; Fig 1).

**DISCUSSION**

In the current study, in all the groups, the mean serum progesterone concentration was at basal level on day 7 and 14 postpartum and subsequently its level increased with clear higher level from day 21 onwards. Usually, the first ovulation after calving in dairy cattle occurs 10 to 14 days after the nadir of negative energy balance [22]. The difference in mean progesterone levels at different stages of the study was significant and the increase seen was almost linear with highest levels on day 63 postpartum (Table 1; Fig 1).

In cows which received supplementation of propylene glycol (Group II), the mean serum progesterone was at basal level on day 7 and 14 postpartum. The stage variation in mean serum progesterone concentration was significant with higher values from day 21 postpartum onwards. The mean serum progesterone concentration was continued to be high from day 21 onwards reaching highest level on day 56 and 63 postpartum. There was a significant dip recorded on day 35 and 49 postpartum with significant difference from preceding and succeeding stages. Miyoshi et

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**Fig.1:** Effect of supplementation of propylene glycol, bypass fat and bypass protein on serum progesterone

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![Graph showing the effect of supplementation on serum progesterone levels](image)
al. [3] observed longer first luteal phase in the propylene glycol (PG) treated cows than in the control cows. Similarly, Hoedemaker et al. [12] reported detectable luteal function in PG treated and control animals. It was reported by Hidalgo et al. [23] that PG drenching had resulted in improved quality of the CL and increased IGF-1 and P₄ levels in serum when heifers in the treatment group orally received PG for 20 days prior to embryo transfer. Further, the administration of propylene glycol (PG) to cyclic heifers increased the amount of P₄ during the luteal phase [10].

In Group-III animals which received bypass fat supplementation, the mean serum progesterone was at basal level on day 7 and 14 postpartum. The stage variation of mean serum progesterone was significant. Its level was clearly high from day 21 onwards. There was also significant rise in mean serum progesterone concentration on day 35 and 49 compared to its level recorded at preceding and succeeding stages. According to Hawkins et al. [14] increase in plasma progesterone in cows fed fat supplemented diets may not be due to increased synthesis but rather due to reduced clearance of progesterone from circulation. Moreover, the fatty acids profile of the dietary fat may influence the propensity of animals to increase plasma progesterone [24]. Interestingly, although higher plasma progesterone concentration in bypass fat supplemented groups compared to control was observed, the difference between the two groups were statistically non-significant [15].

Similar to other groups, in cows supplemented with bypass protein (Group-IV), the mean serum progesterone was at low level on day 7 and 14 postpartum. The stage variation of mean serum progesterone level was significant. Its level was clearly high on day 21 and 56 postpartum. This study reveals difference in mean serum progesterone at other stages was non-significant as evident from table 1; figure 1. Arias et al. [17] found that the interval from calving to first luteal activity were not different between 45% rumen undegradable protein (RUP) treated group and 30% RUP treated group. While, the interval from parturition to first normal luteal activity and the percentage of animals with luteal activity during the experimental period tended to be improved in the 45% RUP treated group as compared to the 30% RUP treated group.

The stage variation in mean serum progesterone concentration between groups showed non-significant variation on day 7 and 14 postpartum. The variation between groups recorded at all other stages (day 21-63 postpartum) was significant. However, the stage variation recorded between groups was devoid of any particular trend. The blood serum progesterone recorded in different groups does not indicate relationship with occurrence of first estrus postpartum. The interval from calving to first heat postpartum was significantly less in all the treatment groups compared to the interval recorded in Group-I (control). The interval recorded in treatment groups did not vary between themselves. Similar was the findings with respect to interval from calving to first service which was significantly (P<0.05) less in treatment groups compared to that of control (Group-I) animals. The failure to find any definite relationship between serum progesterone levels recorded and occurrence of first estrus postpartum may be due to long interval followed for serum sampling. However, the study has indicated better luteal activity postpartum as evidenced by serum progesterone levels recorded after two weeks postpartum which might play a pivotal role in occurrence of postpartum estruses. Mean progesterone concentration increase throughout the postpartum period [25], probably as a result of a greater frequency of ovulation and luteal development as the interval from parturition progresses [26,27]. An elevated progesterone concentration during the luteal phase is one of the criteria of normal cycling activity where cycling activity accompanied by behavioural estrus began only during the 2nd month after parturition [28]. Erb et al. [29] found no evidence that progesterone levels were different in postpartum abnormal and normal cows, however the first postpartum estrus was a better indicator of postpartum ovarian activity than changes in progesterone profiles [30]. The use of continuous serum or milk P₄ profiles in postpartum reproductive management is still not widely practiced [31].

ACKNOWLEDGEMENTS

The authors acknowledge M/s Vetcare Ltd., Bangalore for the supply of bypass fat (NUTRI JOULE) and bypass protein (NUTRI PRO) to conduct this research work. The authors wish to thank the Director, National Institute of Animal Nutrition and Physiology, ICAR, Adugodi, Bangalore, India for the facility provided to estimate the hormone...
progesterone. The authors also have due regards to Dr. Basavaraj, Assistant Professor, Department of Statistics, Veterinary College, Bangalore for the valuable help rendered towards the statistical analysis of the data.

REFERENCES