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# Influence of different levels of dietary protein on serum biochemical parameters of dairy cows during the transition period

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**Abstract**

*A study was carried out to find the influence of different dietary protein levels on serum biochemical parameters of dairy cows during the transition period. Twenty cross bred cows, three weeks prior to their expected date of calving were selected from University Livestock Farm and Fodder Research and Development Scheme (ULF&FRDS), College of Veterinary and Animal Sciences, Mannuthy as experimental animals. The cows were randomly allotted to two dietary treatments – T1 (12 per cent CP (crude protein) TMR (Total Mixed Ration)) and T2 (14 per cent CP TMR). After calving, half of the animals in T1 were allotted to T3 (16 per cent CP TMR) and remaining half to T4 (18 per cent CP TMR). Similarly half of the animals in T2 were allotted to T3 and remaining half to T4. All these rations were iso-caloric. The feeding trial was carried out for a period of 3 weeks prepartum and 45 days postpartum. The serum biochemical parameters such as serum glucose levels were significantly higher ($p<0.05$) for groups receiving treatments T2 & T4 (84.87±2.64 mg/dL) and total protein levels were significantly lower for groups receiving treatments T1 and T3and the levels of all other biochemical parameters under observation remained unaffected in all treatment combinations, but the values of NEFA (non-esterified fatty acids) were below the normal range. Furthermore, there was no occurrence of metabolic diseases in any of the treatment groups, indicating that dietary treatments were sufficient to meet the requirement of the animals. Dietary protein levels did not have any significant effect on serum metabolites studied in the present work.*

**Key words:** *Total mixed ration, Transition period, Dietary protein*

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The transition period in dairy cows is defined as the time period from three weeks prior to parturition to three weeks after parturition. Animals in the transition period require nutrients for maintenance, foetal growth and production. Judicious feeding during the prepartum period will ensure sufficient body reserves to meet the high nutrient demands of early lactation (Goff and Horst, 1997).

Protein and energy are the two nutrients that are critical during the transition period. Even though much work has been done to address the negative energy balance during the transition period, research regarding negative protein balance are scanty. During the transition period and early lactation, dry matter intake will not be sufficient enough to meet the requirements, leading to onset of metabolic disorders. Serum biochemical parameters during this period could be used as indicators for the detection of metabolic disorders (Roche, 2013).In such a scenario, the metabolic responses to different levels of dietary protein in transition cows and influence of dietary protein levels in pre and postpartum crossbred cows were evaluated in the present study.

## Materials and methods

This study was conducted at the University Livestock Farm and Fodder Research Station (ULF & FRDS), College of Veterinary and Animal Sciences, Mannuthy, Kerala, India.

### *Preparation of total mixed rations*

Four types of total mixed rations were formulated using commonly used ingredients along with paddy straw as a source roughage.

The four experimental rations were:T1 - 12 per cent CP TMR, T2 - 14 per cent CP TMR, T3 - 16 per cent CP TMR and T4 - 18 per cent CP TMR. The chemical composition of each of the four rations used are presented in the Table.1

### Feeding trial

Twenty dairy cows, around 325-340 kg body weight with 11-12 L of average milk production in previous lactation, three weeks prior to their expected date of calving were selected and randomly assigned to two groups in a completely randomised block design and were then randomly allotted to dietary treatments,T1 and T2. After calving, five animals from each group were allotted to T3 and the remaining five to T4 making four experimental groups. The feeding trial was carried out for a period of 3 weeks before the expected date of calving and 45 days after calving. All the experimental animals were fed as per standards (ICAR, 2013)

**Table 1. Ingredient composition of paddy straw based TMR offered to experimental animals maintained on four dietary treatments**

| Ingredients | T1(kg) | T2(kg) | T3(kg) | T4(kg) |
|---|---|---|---|---|
| Maize | 27 | 20 | 20 | 14 |
| Rice Polish | 9 | 10 | 9 | 10 |
| Tapioca Starch waste | 4 | 4 | 4 | 2 |
| De-oiled rice bran | 7 | 10 | 8 | 12 |
| CGF | 10 | 13 | 9 | 15 |
| Coconut Cake | 5 | 5 | 9 | 9 |
| Alfalfa | 5.5 | 8.5 | 14 | 15 |
| Straw | 31 | 28 | 24 | 20 |
| Calcite | 0 | 0 | 1.5 | 1.5 |
| Salt | 0.5 | 0.5 | 0.5 | 0.5 |
| Mineral Mixture | 1 | 1 | 1 | 1 |
| Total | 100* | 100* | 100* | 100* |

*To every 100 kg of complete feed, 10g of Vitamin AD3E supplement (containing 10,00,000 I.U of Vitamin A, 2,00,000I.U of Vitamin D3 and 1,00,000 I.U of Vitamin E) were added

**Biochemical Analysis**

Blood samples were collected from all the experimental animals on the 14thday before the expected date of calving, on the day of calving andon the 21st day after calving. Serum samples were analysed for total protein (Jong and Vegeter, 1950), albumin (Bromocresol green method), glucose (GODPAP methodology), Non esterified fatty acids (NEFA) and beta hydroxy butyrate (BHB) (Hosaka *et al*. 1981) using standard kits.

**Results and discussion**

***Prepartum biochemical profile***

Biochemical parameters of the experimental animals recorded two weeks before the expected date of calving and on the day of calving are listed in Table 2

Serum glucose levels of the animals fed with T1 and T2 were 75.18±3.62 & 84.89±2.11 mg/dL and 63.81±3.06 & 67.92±2.19 mg/dL, respectively at two weeks prior to the expected date of calving and on the day of calving. There was a decrease in serum glucose levels in both groups on the day of calving. This could be due to reduced feed intake at the time of calving. A decreased serum glucose level (46.33 mg/dL) at calving was also reported by Abdel *et al. (*2016). Valiente (2018) observed elevated serum glucose levels 20 days prior to calving.

Serum total protein levels for the groups receiving treatments T1 and T2 before calving were 7.40±0.14 and 7.62±0.24 mg/dL respectively and those on the day of calving were 8.61±0.39 and 8.90±0.27 mg/dL respectively. Farahani *et al.* (2017) also reported that the serum total protein levels of 7.06 mg/dL in animals fed conventionally.

**Table 2. Biochemical parameters of experimental cows maintained on two experimental diets two weeks before and at calving**

| Parameter[1] | 2 weeks before calving | | | At calving | | |
|---|---|---|---|---|---|---|
| | T1 | T2 | P value | T1 | T2 | P value |
| Serum glucose (mg/dL) | 75.18 ±3.62 | 84.89 ±2.11 | 0.08 | 63.81 ±3.06 | 67.92 ±2.19 | 0.02 |
| Total protein (g/dL) | 7.40 ±0.14 | 7.62 ±0.24 | 0.04 | 8.61 ±0.39 | 8.90 ±0.27 | 0.18 |
| Albumin (g/dL) | 4.28 ±0.07 | 4.29 ±0.15 | 0.97 | 3.71 ±0.13 | 3.68 ±0.15 | 0.88 |
| NEFA(ng/dL) | 0.09 ±0.02 | 0.13 ±0.01 | 0.01 | 0.18 ±0.01 | 0.17 ±0.01 | 0.97 |
| BHBA(ng/dL) | 0.28 ±0.01 | 0.30 ±0.01 | 0.81 | 0.31 ±0.01 | 0.32 ±0.01 | 0.82 |

[1]Mean values are based on ten replicates with SE

**Table 3. Biochemical parameters of experimental cows maintained on two experimental diets after 21 days of calving**

| Parameter[1] | Treatment combination | | | | P value |
|---|---|---|---|---|---|
| | T1T3 | T1T4 | T2T3 | T2T4 | |
| Serum glucose (mg/dL) | 79.05±1.25$^{ab}$ | 74.37±2.16$^{b}$ | 78.03±1.98$^{b}$ | 84.87±2.64$^{a}$ | 0.019 |
| Total protein (g/dL) | 7.30±0.17$^{b}$ | 8.88±0.36$^{a}$ | 8.61±0.39$^{a}$ | 8.90±0.27$^{a}$ | 0.006 |
| Albumin (g/dL) | 4.28±0.15 | 4.14±0.09 | 3.82±0.24 | 4.17±0.30 | 0.886 |
| NEFA(ng/dL) | 0.12±0.01 | 0.13±0.01 | 0.12±0.01 | 0.13±0.01 | 0.860 |
| BHBA(µg/dL) | 0.31±0.01 | 0.28±0.01 | 0.32±0.01 | 0.29±0.01 | 0.832 |

[1]Mean values are based on five replicates with SE

Mean ± SE of different treatment having different alphabets (a-b) as superscripts differs significantly with in a row at p<0.05

Albumin levels for the groups receiving treatments T1 and T2 before calving were 4.28±0.07 and 4.29±0.15 g/dL respectively and those on the day of calving were 3.71±0.13 and 3.68±0.15 g/dL respectively. A decrease in albumin could be associated with an increase in globulin. Globulin is known as a positive acute phase protein and its liver synthesis is commonly increased during the inflammatory condition (Bertoni *et al.*, 2008). Higher globulin may be associated with some degree of inflammation during calving.These values are in accordance with those reported by Seifi *et al*. (2005).

The NEFA levels in cows receiving 12 per cent CP prepartum increased from 0.09±0.02 two weeks before calving to 0.18±0.01 ng/dL at the day of calving while in those animals receiving 14 per cent CP, it increased from 0.13±0.01 to 00.17±0.01 ng/dL and these changes were similar between the groups. The increased NEFA levels on the day of calving could be due to fat mobilization, but the increase was not up to the level for causing a negative energy balance. This could be because, the feed given was sufficient to meet energy requirements. These values were in agreement with those of Ospina *et al.* (2010) and who observed pre partum NEFA concentrations of 0.27 mEq/L. Similar increase in NEFA levels (0.17 to 0.29 mmol/L ) on the day of calving was also observed by Huzzey *et al.* (2011).In contrast, Perumbilly *et al.* (2019) observed a higher value for NEFA (0.702 ± 0.18 mmol/L) during two weeks before calving in cross bred dairy cows .

BHB levels were similar during the two weeks before and on the day of calving for all treatment groups. In contrast to values observed in this study, BHBA levels of 5.73 and 6.42 mg/dL for primigravid and multiparous cows fed during the prepartum period with moderate protein diet (12.7% CP, 36% rumenundegradable protein) was reported by Santos *et al.*(2001)

A perusal of serum biochemical parameters before and on the day of calving indicated that dietary protein levels had no significant effect on serum metabolites either two weeks prior to calving or on the day of calving.

***Post partum biochemical profile***

Biochemical parameters of experimental animals recorded after 21 days of calving are listed in Table 3

Serum glucose levels for the groups receiving treatment combinations T1T3, T1T4, T2T3 and T2T4 were 79.05±1.25, 74.37±2.16, 78.03±1.98 and 84.87±2.64 mg/dL respectively. The animals receiving T2 and T4 had higher serum glucose levels. Glucose levels of 3.03 and 3.23 mmol/L after 11 and 21 days of calving (Reynolds *et al.,* 2003) and 55 to 70 mg/dL after 1 week of calving (Zaworski *et al.,* 2014) were reported for dairy cows.

Total protein levels for the groups receiving treatment combinations T1T3, T1T4, T2T3 and T2T4 were 7.30±0.17, 8.88±0.36, 8.61±0.39 and 8.90±0.27 respectively. Albumin levels for the groups receiving treatment combinations T1T3, T1T4, T2T3 and T2T4 were 4.28±0.15, 4.14±0.09, 3.82±0.24 and 4.17±0.30 g/dL. Total protein levels were significantly lower for groups receiving treatments T1 and T3. In contrast, higher serum total protein levels (7.07 mg/dL) were reported by Farahani *et al.* (2017).

NEFA levels for the groups receiving treatment combinations T1T3, T1T4, T2T3 and T2T4 were 0.12±0.01, 0.13±0.01, 0.12±0.01 and 0.13±0.01 ng/dL. Low NEFA levels indicated an absence of negative energy balance after calving. In contrast to this observation, higher levels of NEFA concentration(were 0.59 mmol/L) in dairy cows 25 days after calving was reported by De Souza *et al*. (2019).

BHBA levels for the groups receiving treatment combinations T1T3, T1T4, T2T3 and T2T4 were 0.31±0.01, 0.28±0.01, 0.32±0.01 and 0.29±0.01 µg/dL respectively which were lower than the values reported by Fiore *et al.* (2017) and Perumbilly*et al.* (2019). This clearly indicates that the energy requirement for the animals was met from the feed given.

Levels of all biochemical parameters, except total protein (lowest in T1 and T3) and serum glucose (highest in T2 and T4), under observation remained unaffected in all treatment combinations, but the values of NEFA (Non esterified fatty acids) were below the normal range.There was no interaction between pre- and postpartum CP levels on the serum concentrations of total protein (P = 0.68), albumin (P = 0.80), globulin (P = 0.82), or albumin:globulin ratio (P = 0.67) between the treatment groups.

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