

## Original Research Article

### **EFFECT OF BYPASS FAT AND BYPASS PROTEIN SUPPLEMENTATION DURING TRANSITION PERIOD ON REPRODUCTIVE PERFORMANCE OF ASSAM HILL GOAT**

#### **Abstract**

The present study was undertaken to evaluate the response of Assam Hill Goat (AHG) in terms of their productive and reproductive performances upon bypass fat and bypass protein supplementation during the transition period. Twenty-four AHG in transition period (21day prepartum to 21day post-partum) were divided into two equal groups (n=12) viz. T-0 and T-1; where Group T-1 was supplemented with 10 g of bypass fat and 5 g of bypass protein along with their normal diet for a period of 42 days. Group T-0 acted as control and was provided with a normal diet without any supplementation. Blood samples were collected on day -21, -14, -7, 0 (day of kidding), +7, +14 and +21 of the transition period for haemato-biochemical studies. Birth weight, milk yield and time taken for expulsion of foetus and foetal membrane and incidences of peri-partum diseases were recorded. The results indicated that, the supplementation had no significant effect on haematological parameters (TLC, Neutrophil count and Hb), serum Ca, P, glucose, globulin and GGT activity. Serum NEFA levels were significantly ( $p<0.05$ ) lower in supplemented groups on the day of kidding ( $0.75\pm 0.09$  mmol/L) and day 21 of postpartum ( $0.38\pm 0.04$  mmol/L). Significantly ( $p<0.05$ ) higher mean values of total protein on day -14, -7, 0, 7, 14 and 21 ( $6.96\pm 0.21$ ,  $6.99\pm 0.25$ ,  $7.02\pm 0.26$ ,  $7.24\pm 0.28$ ,  $7.03\pm 0.12$  and  $6.95\pm 0.15$  g/dL) and serum albumin values on day -14, 7 and 21 ( $4.46\pm 0.23$ ,  $4.69\pm 0.23$  and  $4.44\pm 0.27$  g/dL) were recorded in T-1 group. Significantly ( $p<0.05$ ) lower mean values of BUN were recorded in T-1 group on day 0, 7 and 21 ( $30.25\pm 1.74$ ,  $35.62\pm 1.58$  and  $42.08\pm 1.45$  mg/dL). The supplemented (T-1) group recorded significantly higher milk yield ( $303.5\pm 21.16$ ,  $306.25\pm 19.67$  and  $310.75\pm 23.93$  ml) on day 14, 21 and 28 after kidding and there was a significant ( $p<0.05$ ) reduction in time taken for the expulsion of foetus ( $89.75\pm 11.84$  mins) and foetal membranes ( $90.50\pm 5.86$  mins). The supplementation of bypass fat (10g/day) and bypass protein (5 g/day) during the transition period reduce the effect of negative energy balance and facilitate quicker recovery from NEB along with improvement in milk yield and reduce time taken for expulsion of foetus and foetal membrane.

**Keywords:** Assam Hill Goat, bypass protein, transition period, bypass fat, negative energy balance, non-esterified fatty acids (NEFA)

## 1. INTRODUCTION

Goat also known as poor man's cow known for their excellent reproductive potential and their capability to adapt to diverse agro-climatic conditions have been a source of quick income for rural households, hence occupies a significant role in uplifting the rural economy of India. The indigenous goats of Assam are now recognized as the "Assam Hill Goat", the 31<sup>st</sup> registered Goat Breed of India. With the majority of the Indian population living in rural areas, this drastic change in meat consumption pattern has provided an opportunity for the nomads, landless, marginal, and small farmers to rear livestock animals. This breed is distributed throughout Assam and in some adjoining areas of Meghalaya. Assam Hill Goats are popular for quality meat and their prolific nature of producing twins and triplets with a very less infant mortality rate. The gap between demand and production of chevon could be fulfilled by augmenting the reproductive efficiency of the animals. Reproductive performance is a key factor in the economic viability and productivity of the farm in the case of goats.

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Transition period is a critical period for female ruminants as they undergo a physical and biological transition from a non-lactating pregnant state to post parturient lactating state leads to stress caused by the abrupt and significant changes in metabolism, anatomy, and physiology. Around the transition period there will be a substantial increase in energy requirements to support own maintenance, pregnancy, and lactation which also coincides with a period of depressed feed intake, often resulting in maternal negative energy balance (NEB) in female ruminants (Sundrum, 2015). The higher the NEB, higher is the susceptibility to metabolic and infectious diseases. The environmental (pen relocation), behavioral, and nutritional changes (ingredient and energy density alterations) made during this period might have a positive impact on the animal's health.

Nutritional management during the transition period has direct and indirect effects on postpartum productive and reproductive performance of the animal. Any impairment in the management or nutrition during the transition period may lead to disturbance in the postpartum production and subsequent reproductive cycle of the animal and also increases the risk of clinical and sub-clinical diseases, which ultimately decreases the production. The relationship between nutrition and reproduction is well established and plays an important role in the reproductive performance of animals (Smith et al., 2010). Keeping in the view of previous studies and understanding of the transition animals it reveals that providing a high-energy diet and quality protein will improve the productive and reproductive performance of the animal in subsequent cycles. Although energy-dense cereal grains and oil cakes could be included in the rations to increase the energy density, however, they depress the dry matter intake, and fiber digestion and cause ruminal acidosis. If those ingredients are included in higher amounts it may predispose the animals to off-fed conditions. Because of these drawbacks, we have adopted feed-protected nutrients which are made in such a way that they escape the ruminal hydrolysis and will be available for absorption from the intestine. It is hypothesized that bypass fat could reduce the effect of NEB and enhance the performance of the animal also supplementing bypass protein

results in enhanced absorption of amino acids from the intestine and will also enhance the immune response of the animal. The present study was designed to assess the response of Assam hill goats in terms of their productive and reproductive performance against bypass fat and bypass protein supplementation during transition period.

## 2. MATERIALS AND METHODS

The present study was conducted on a total of 24 numbers of four months pregnant does of Assam Hill Goat breed maintained at Goat Research Station, Assam Agricultural University, Burnihat. The animals were maintained under semi-intensive system of management. Goats were let loose for grazing during day time and were supplied with 200 g of concentrate mixture along with 3-4 kg chaffed fodder daily to each animal and water supply was *ad libitum*. A total of 24 numbers four month pregnant does were randomly divided into two (2) groups comprising twelve (12) animals in each group, viz., T-0, and T-1. Animals of T-1 group were fed with bypass protein (5g) and bypass fat (10g) while T-0 animals were kept as control fed with normal concentration mixture. Feeding trial was conducted as following to study the effect of bypass fat and bypass protein supplementation on productive, reproductive and blood biochemical profiles.

T-0 (Control): Does were fed the basal diet which consists of concentrate mixture.

T-1 (Treatment): Does were fed the basal diet supplemented with 10g bypass fat and 5 g bypass protein/ head/ day for a period of 42 days.

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The bypass fat and bypass protein were supplemented by mixing with the concentrate feed which was fed routinely.

Approximately 5 ml of blood was collected from each experimental animal by jugular vein puncture on the day 0 (before treatment), every weekly (at 7 days interval) up to 42days of the experimental feeding for estimation of haematological and biochemical parameters.

### 2.1 Estimation of haematological parameter

Estimation of haematological parameters like Hb, PCV, RBC, WBC were done with the help of Auto analyser (MS4Se, MSF J0308) by following standard procedures as per the manufacturer's protocol.

### 2.2 Estimation of blood biochemical parameter

Estimation of blood biochemical parameters like Calcium, Phosphorus, BUN (Blood Urea Nitrogen), Glucose, Total protein, Albumin, NEFA (Non non-esterified Fatty Acid) and GGT (Gamma-Glutamyl Transferase) were estimated manually by standard protocol using commercially available diagnostic kits.

### 2.3 Productive parameters estimation

#### 2.3.1 Kidding traits

The pregnant does were separated from the flock 7 days before the expected date of parturition. The course of parturition was divided into three stages for recording the signs of parturition. Each animal was observed closely from two days before the expected date of kidding up to the period of placental expulsion. The does that revealed the signs of onset of labour were observed continuously for recording the observation during the parturition. .

### **2.3.2 Birth weight of Kids.**

Birth weight of kids was recorded within one hour of kidding. Digital weighing balance was used to record the birth weight of kids.

### **2.3.3 30 Days milk yield**

30 days Milk yield was recorded by isolating the kids from their mothers overnight and measuring their bodyweight before letting them suckle in the morning.

## **2.4 Reproductive parameters estimation**

### **2.4.1 Time taken for the expulsion of the foetus and foetal membrane**

In the kidding behaviour, any abnormality or difficulty that occurred during the parturition and the time taken for the expulsion of the foetus and expulsion of the foetal membrane was observed.

### **3.4.2 Incidence of peripartum diseases**

There was no incidence of any peripartum diseases in both the group during the experimental period.

## **2.4 Statistical Analysis**

With the use of SPSS software (ver. 20), data were evaluated by one way analysis of variance using a general linear model. The post-hoc Tukey test was used to compare mean values that showed significant differences.

## **3. RESULTS AND DISCUSSION**

### **3.1 Haematological parameters**

#### **3.1.1 Total Leukocyte Count (TLC)**

In the present study, the mean TLC (K/ $\mu$ L) was found to be ranging from 15.76 $\pm$ 2.30 to 17.82 $\pm$ 4.07 in T - 0 group, and 15.59 $\pm$ 2.57 to 19.19 $\pm$ 6.91 in T - 1 group. Paired T-test (assuming equal variance) revealed no significant difference in the mean values of TLC between the groups on different days of observation. The TLC levels were within the reference range as given by Feldman *et al.* (2002) in both groups.

**TABLE 1: MEAN $\pm$ SE LEVEL OF TOTAL LEUKOCYTE COUNT (K/ $\mu$ L) IN CONTROL (T - 0) AND SUPPLEMENTED (T - 1) GROUP AT DIFFERENT DAYS OF THE TRANSITION PERIOD**

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Groups	Pre-parturient day			Day of kidding	Post-parturient day		
	-21	-14	-7	0	7	14	21
T - 0	16.92±4.21	15.91±2.05	15.76±2.30	16.41±2.33	17.14±2.38	17.82±4.07	16.68±3.03
T - 1	15.59±2.57	17.25±4.83	18.4±4.64	19.19±6.91	18.04±3.51	18.25±4.38	17.34±4.56

The leucocyte count peaked on the day of parturition in both groups and was found to be on the higher side of the normal reference range during the periparturient period, it might be due to the transitional period stress upon the animals or any underlying subclinical metabolic abnormalities or infections.

A similar trend of WBC counts was reported by Akraeim *et al.* (2021) in pregnant ewes. Supplementation of bypass fat and bypass protein in the present study had no significant effect on total leucocyte count. Similar results in Murrah heifers and lactating buffaloes supplemented with bypass fat and bypass protein was reported by Katiyar *et al.* (2019) and Rajneesh *et al.* (2020). Contrary to our findings, Moty *et al.* (2012) reported significantly higher ( $p<0.05$ ) WBC count after supplementing bypass fat (calcium salts of palm oil fatty acids - CSPFA) in buffaloes.

**Comment [13]:** What do you think the reason behind for opposite results between your finding and this author's?

### 3.1.2 Neutrophil Count

The mean neutrophil count ( $K/\mu L$ ) in the present study was found to be ranging from 10.19±1.53 to 11.05±2.51 in T - 0 group, and 9.93±1.75 to 12.35±3.80 in T - 1 group. Paired T-test (assuming equal variance) revealed no significant difference in the mean neutrophil values between the groups. The neutrophil count in both groups was higher than the reference range as given by Feldman *et al.* (2002). Neutrophils not only participate in infection control but also as an inflammatory response.

As the goats approach kidding, which is the most stressful event during the transition period, they evidence higher levels of stress hormones especially corticosteroids due to the incurred stress and neuroendocrine changes leading to neutrophilia (Lee and Kehrli, 1998), which might be the reason for the increased neutrophil count in the overall study population. In the present study, supplementation of bypass fat and bypass protein had no significant effect on neutrophil count which was in accordance with the findings of Invernizzi *et al.* (2016) in goats supplemented with fish oil and stearic acid. A similar trend was also reported by Movaliya *et al.* (2013), Katiyar *et al.* (2019) and Rajneesh *et al.* (2020) in buffaloes supplemented with bypass fat and bypass protein.

**TABLE 2: MEAN±SE LEVEL OF NEUTROPHIL COUNT ( $K/\mu L$ ) IN CONTROL (T - 0) AND SUPPLEMENTED (T - 1) GROUP AT DIFFERENT DAYS OF THE TRANSITION PERIOD**

Groups	Pre-parturient day			Day of kidding	Post-parturient day		
	-21	-14	-7	0	7	14	21
<b>T - 0</b>	11.05±2.51	10.19±1.53	10.24±1.50	10.85±2.09	10.97±1.75	10.92±2.47	10.53±2.13
<b>T - 1</b>	9.93±1.75	10.57±2.01	11.51±3.04	12.35±3.80	10.58±2.54	10.72±2.20	10.85±2.09

### 3.1.3 Haemoglobin (Hb)

The mean haemoglobin (Hb) levels (g/dl) in the present study were found to be ranging from 8.52±0.97 to 9.45±1.21 and 8.17±0.61 to 9.08±1.20 in T - 0 and T - 1 group respectively. Paired T-test (assuming equal variance) revealed no significant difference in the mean values of haemoglobin between the groups. The values of hemoglobin levels recorded in the present study were observed to be within the normal range (Feldman *et al.*, 2002).

Haemoglobin levels were estimated to evaluate the health status of the goats and any improvements thereof after supplementing with bypass fat and bypass protein. However, the present study revealed no significant effect on haemoglobin levels after supplementation of bypass fat and bypass protein during the transition period. A similar finding was reported by Invernizzi *et al.* (2016) in Alpine goats supplemented with fish oil and stearic acid.

**TABLE 3: MEAN±SE LEVEL OF HAEMOGLOBIN (g/dL) IN CONTROL (T - 0) AND SUPPLEMENTED (T - 1) GROUP AT DIFFERENT DAYS OF THE TRANSITION PERIOD**

Groups	Pre-parturient day			Day of kidding	Post-parturient day		
	-21	-14	-7	0	7	14	21
<b>T - 0</b>	9.45±1.21	9.08±1.18	9.22±1.70	8.85±0.93	8.77±0.52	8.73±0.96	8.52±0.97
<b>T - 1</b>	9.02±1.34	9.08±1.07	8.98±0.7	9.08±1.20	8.25±0.57	8.17±0.61	8.18±0.73

## 3.2 BLOOD BIOCHEMICAL PARAMETERS

### 3.2.1 Serum Calcium

The mean serum calcium levels (mg/dl) in the present study were found to be ranging from 7.62±2.07 to 8.85±0.9 in T - 0 group, and 6.54±1.14 to 9.48±0.65 in T - 1 group. In the present study, serum calcium levels were detected within the reference range (8.9 -11.7 mg/dL) as reported by Batmaz (2013). Paired T-test (assuming equal variance) revealed no significant difference in the mean values of serum calcium between the groups. Calcium levels were highest from day -21 to day -14 of prepartum and lowest at one week (day -7) after parturition in both the groups, a similar pattern in serum calcium levels was observed by Krajnikova *et al.* (2003) in goats. They found a trend of decreasing calcium levels near term, which reached its lowest on day 3 postpartum. A decrease in calcium levels just after the parturition is a characteristic of the puerperal period which is related to milk production.

**TABLE 4 MEAN±SE LEVEL OF SERUM CALCIUM (mg/dL) IN CONTROL (T - 0) AND SUPPLEMENTED (T - 1) GROUP AT DIFFERENT DAYS OF THE TRANSITION PERIOD**

Groups	Pre-parturient day			Day of kidding	Post-parturient day		
	-21	-14	-7	0	7	14	21
T - 0	8.85±0.9	8.21±1.42	7.67±1.92	8.57±2.40	7.62±2.07	8.23±2.64	8.09±2.31
T - 1	9.32±0.79	9.48±0.65	8.64±1.53	7.78±0.71	6.54±1.14	7.83±1.42	7.49±0.70

Bypass fat and bypass protein supplementation had no significant effect on serum calcium levels in the present study. The reason might be due to strong homeostatic control of the animal on blood calcium levels as increase in calcium requirements at the tissue level is met by increased absorption from the gastrointestinal tract (Wadhwa *et al.* (2012), Nirwan *et al.* (2019) and Ranaweera *et al.* (2019) observed similar results in cross-bred cows by supplementing bypass fat.

### 3.2.2 Serum Phosphorous

In the present study, the mean serum phosphorous levels (mg/dl) were found to be ranging from 2.92±0.84 to 3.32±0.56 and 2.94±0.63 to 3.07±0.72 in T - 0 and T - 1 groups respectively. Paired T-test (assuming equal variance) revealed no significant difference in the mean values of serum phosphorus between the groups. Phosphorus levels were detected below reference values (4.2-9.1 mg/dL) in both groups as given by Batmaz (2013). Depressed dry matter intake during the periparturient period might lead to hypophosphatemia which is justified by the strong positive correlation between dietary phosphorus intake and serum phosphorus levels.

**TABLE 5 MEAN±SE LEVEL OF SERUM PHOSPHORUS (mg/dL) IN CONTROL (T - 0) AND SUPPLEMENTED (T - 1) GROUP AT DIFFERENT DAYS OF THE TRANSITION PERIOD**

Groups	Pre-parturient day			Day of kidding	Post-parturient day		
	-21	-14	-7	0	7	14	21
T - 0	3.13±0.43	3.23±0.48	3.16±0.56	3.32±0.56	3.23±0.48	3.12±0.67	2.92±0.84
T - 1	2.94±0.63	3.01±0.6	3.00±0.50	3.07±0.72	3.01±0.60	3.02±0.44	2.96±0.50

Bypass fat and bypass protein supplementation showed no significant effect on serum phosphorous levels in the present study, which was in accordance with the findings of Nirwan *et al.* (2019) and Ranaweera *et al.* (2019) in crossbred cows supplemented with bypass fat.

### 3.2.3 Serum Glucose

The mean serum glucose levels (mg/dL) in the present study were found to be ranging from 46.76±4.57 to 52.64±9.34 in T - 0 group and 47.35±6.60 to 54.04±9.04 in T - 1 group. The present findings were in concordance with the findings of Khan and Ludri (2002), Gurgoze *et al.* (2009) and Akraeim *et al.* (2021) who reported lower blood glucose levels in pregnant does and ewes as compared to non-pregnant ones. The lower level of serum glucose might be due to reduced dry matter intake during the transition period, and increased utilization of glucose by the fetus and for lactation.

**TABLE 6: MEAN±SE LEVEL OF SERUM GLUCOSE (mg/dL) IN CONTROL (T - 0) AND SUPPLEMENTED (T - 1) GROUP AT DIFFERENT DAYS OF THE TRANSITION PERIOD**

Groups	Pre-parturient day			Day of kidding	Post-parturient day		
	-21	-14	-7	0	7	14	21
T - 0	46.76±4.57	51.88±3.85	52.64±9.34	50.05±5.59	50.21±2.97	48.00±2.94	51.00±2.09
T - 1	47.35±6.60	52.90±6.87	54.04±9.04	51.68±6.79	51.66±5.95	48.00±6.28	52.30±5.53

In the present study paired T-test (assuming equal variance) revealed no significant difference in the mean values of serum glucose between the groups. Blood glucose level is not a sensitive indicator of energy status in ruminants as the homeostatic mechanism of the animal's body doesn't allow the applicable changes in serum glucose levels (Katiyar *et al.*, 2019). The present study results were in

agreement with the findings of Ranjan *et al.* (2012), Shelke *et al.* (2012) and Katiyar *et al.* (2019) in buffaloes, and Manriquez *et al.* (2019) in cows supplemented with bypass fat.

### 3.2.4 Serum non-esterified fatty acids (NEFA)

The mean Serum NEFA levels (mmol/L) in the present study were found to be ranging from 0.45±0.04 to 0.88±0.05 in T - 0 group, and 0.38±0.04 to 0.75±0.09 in T - 1 group. During the prepartum period, the serum NEFA levels were high and peaked near parturition and steadily decreased afterward. A similar pattern was also observed by Sadjadian *et al.* (2012), Soares *et al.* (2018) and Akkaya *et al.* (2020) in goats during the periparturient period. These increased NEFA concentrations might be related to hormonal changes and lipolysis which is triggered by the energy requirements for the development of the fetus, mammary gland and milk production after the birth of the kid (Cheng *et al.*, 2007). Serum NEFA level is an indicator of negative energy balance which represents the mobilization of free fatty acids from the adipose tissue to other parts of the body (Adewuyi, 2005).

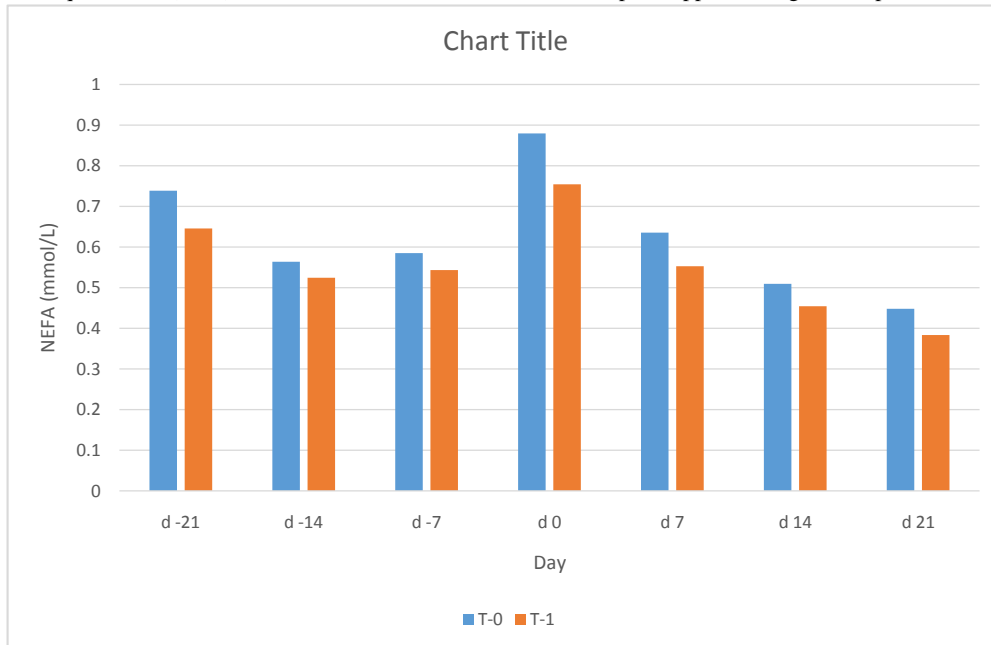
In the present study, paired T-test (assuming equal variance) revealed a significant ( $p < 0.05$ ) difference in the mean values of serum NEFA between the groups on the day of kidding (day 0) and day 21 of postpartum, indicating that the impact of negative energy balance was less in the supplemented group (T - 1) in comparison with the control (T - 0) group.

**TABLE 7: MEAN±SE LEVEL OF SERUM NEFA (mmol/L) IN CONTROL (T - 0) AND SUPPLEMENTED (T - 1) GROUP AT DIFFERENT DAYS OF THE TRANSITION PERIOD**

Groups	Pre-parturient day			Day of kidding	Post-parturient day		
	-21	-14	-7	0	7	14	21
T - 0	0.74 <sup>a</sup> ±0.12	0.56 <sup>a</sup> ±0.10	0.59 <sup>a</sup> ±0.10	0.88 <sup>a</sup> ±0.05	0.64 <sup>a</sup> ±0.12	0.51 <sup>a</sup> ±0.06	0.45 <sup>a</sup> ±0.04
T - 1	0.65 <sup>a</sup> ±0.12	0.52 <sup>a</sup> ±0.08	0.54 <sup>a</sup> ±0.07	0.75 <sup>b</sup> ±0.09	0.55 <sup>a</sup> ±0.17	0.45 <sup>a</sup> ±0.08	0.38 <sup>b</sup> ±0.04

The lower NEFA levels might be due to reduced lipolysis of fat reserves in the bypass fat-supplemented group. Similar findings were reported by Invernizzi *et al.* (2016), Nirwan *et al.* (2019) and

Manriquez *et al.* (2019) who found lower serum NEFA levels upon supplementing rumen-protected fat.



**Fig. 1 GRAPHICAL REPRESENTATION OF MEAN±SE LEVEL OF SERUM NEFA (mmol/L) IN CONTROL (T - 0) AND SUPPLEMENTED (T - 1) GROUP AT DIFFERENT DAYS OF THE TRANSITION PERIOD**

### 3.2.5 Total Protein

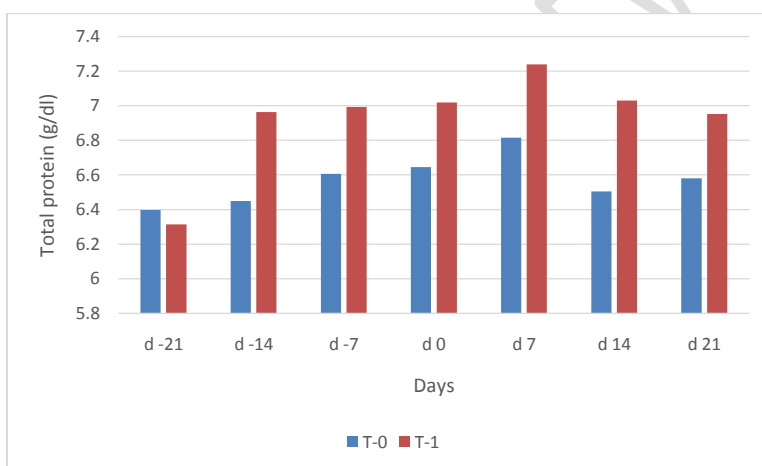
In the present study, the mean total protein levels (g/dL) were found to be ranging from  $6.40 \pm 0.21$  to  $6.82 \pm 0.41$  and  $6.31 \pm 0.21$  to  $7.24 \pm 0.28$  in T - 0 and T - 1 groups. The total protein values were within the reference range (3.50–13.00 g/dL) as given by Batmaz (2013). Paired T-test (assuming equal variance) revealed a significant ( $p < 0.05$ ) difference in the mean values of total protein between the groups on day -14, day -7 of prepartum, on the kidding (day 0), day 7, 14 and 21 of postpartum. The consistently higher level of serum total protein in the T-1 group during the peri-parturient period might be due to the supplementation of protected protein. The protection of dietary proteins from rumen degradability resulted in higher concentrations of proteins available for absorption from the lower part of the gastrointestinal tract simultaneously leading to high levels of plasma protein. These results were in accordance with the findings of Singh *et al.* (2014) on Barbari goats, Katiyar *et al.* (2019) in Murrah buffaloes and Kumari *et al.* (2021) in buffalo heifers by feeding bypass protein, whereas Moty *et al.* (2012) found improvement in total protein after supplementation of bypass fat alone.

**TABLE 8: MEAN±SE LEVEL OF TOTAL PROTEIN (g/dL) IN CONTROL (T - 0) AND SUPPLEMENTED (T - 1) GROUP AT DIFFERENT DAYS OF THE TRANSITION PERIOD**

Groups	Pre-parturient day			Day of kidding	Post-parturient day		
	-21	-14	-7	0	7	14	21
<b>T - 0</b>	6.40 <sup>a</sup> ±0.21	6.45 <sup>a</sup> ±0.14	6.61 <sup>a</sup> ±0.23	6.65 <sup>a</sup> ±0.19	6.82 <sup>a</sup> ±0.41	6.51 <sup>a</sup> ±0.10	6.5 <sup>a</sup> ±0.15
<b>T - 1</b>	6.31 <sup>a</sup> ±0.21	6.96 <sup>b</sup> ±0.21	6.99 <sup>b</sup> ±0.25	7.02 <sup>b</sup> ±0.26	7.24 <sup>b</sup> ±0.28	7.03 <sup>b</sup> ±0.12	6.95 <sup>b</sup> ±0.15

Means in the same column having different superscripts are significantly ( $P < 0.05$ ) different

Ranjan *et al.* (2012) and Wadhwa *et al.* (2012) reported that there was no improvement in total protein levels by supplementation of rumen-protected fat alone. Movaliya *et al.* (2013) stated that there was no significant increase in the serum protein levels after supplementing bypass methionine and lysine.



**Fig. 2 GRAPHICAL REPRESENTATION OF MEAN±SE LEVEL OF TOTAL PROTEIN (g/dL) IN CONTROL (T - 0) AND SUPPLEMENTED (T - 1) GROUP AT DIFFERENT DAYS OF THE TRANSITION PERIOD**

### 3.2.6 Serum albumin

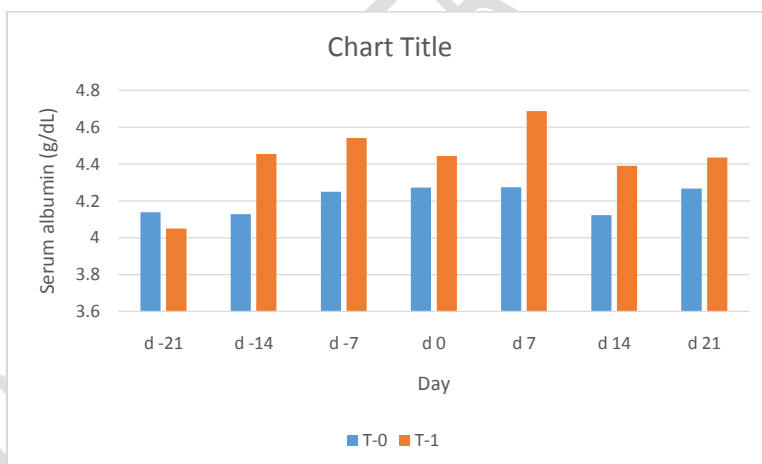
The mean serum albumin levels (g/dL) in the present study were found to be ranging from 4.12±0.19 to 4.27±0.38 in T - 0 group, and 4.05±0.21 to 4.69±0.23 in T - 1 group. Albumin levels were found to be within the reference range (0.5-5 g/dL) given by Batmaz (2013) in both groups. Significantly higher ( $p < 0.05$ ) albumin levels were found in the T - 1 group on day -14, day 7 and day 21 over the T - 0 group. The serum albumin concentration is a reflection of the animal's ability to synthesize and store proteins.

**TABLE 9: MEAN±SE LEVEL OF ALBUMIN (g/dL) IN CONTROL (T - 0) AND SUPPLEMENTED (T - 1) GROUP AT DIFFERENT DAYS OF THE TRANSITION PERIOD**

Groups	Pre-parturient day			Day of kidding	Post-parturient day		
	-21	-14	-7	0	7	14	21
<b>T - 0</b>	4.14 <sup>a</sup> ±0.23	4.13 <sup>a</sup> ±0.19	4.25 <sup>a</sup> ±0.25	4.27 <sup>a</sup> ±0.28	4.27 <sup>a</sup> ±0.38	4.12 <sup>a</sup> ±0.19	4.27 <sup>a</sup> ±0.16
<b>T - 1</b>	4.05 <sup>a</sup> ±0.21	4.46 <sup>b</sup> ±0.23	4.54 <sup>a</sup> ±0.24	4.44 <sup>a</sup> ±0.12	4.69 <sup>b</sup> ±0.23	4.39 <sup>a</sup> ±0.11	4.44 <sup>b</sup> ±0.27

Means in the same column having different superscripts are significantly (P< 0.05) different

In the present study, rumen-protected protein supplementation resulted in higher concentrations of plasma proteins and albumin levels. Similar findings were reported by Katiyar *et al.*(2019) in Murrah buffaloes by supplementing bypass fat and bypass protein. Various other workers reported improvement in albumin levels after supplementing dietary bypass protein (Ghani *et al.*, 2011 in Sohagi lambs and Singh *et al.*,2014 in Barbari goats).



**Fig. 3 GRAPHICAL REPRESENTATION OF MEAN±SE LEVEL OF SERUM ALBUMIN (g/dL) IN CONTROL (T - 0) AND SUPPLEMENTED (T - 1) GROUP AT DIFFERENT DAYS OF THE TRANSITION PERIOD**

In contrast to our study, Movaliya *et al.* (2013) and Kumari *et al.* (2021) reported that there was no effect on albumin levels when supplemented with bypass protein-rich feeds in buffalos. Ranjan *et al.* (2012); Wadhwa *et al.* (2012) reported that there was no improvement in albumin levels by supplementation of rumen-protected fat.

### 3.2.7 Serum Globulin

The mean serum globulin levels (g/dL) in the present study were found to be ranging from 2.26±0.14 to 2.54±0.26 in T - 0 group and 2.31±0.16 to 2.53±0.25 in T - 1 group. Paired T-test (assuming equal variance) revealed no significant difference in the mean serum globulin values between the groups. Globulins are major blood proteins produced mostly by liver and immune system. Comparatively lower level of globulin in the present study during the prepartum period (day -21 to day 0) indicates moderately reduced immune response in both the groups, which might be due to transitional stress. The present study could not find any significant effect on serum globulin levels by supplementing bypass fat and bypass protein during the transition period. Non-significant variations in serum globulins level were reported by Katiyar *et al.* (2019) in Murrah buffaloes supplemented with bypass protein and Wadhwa *et al.* (2012) in crossbred cow supplemented with rumen-protected fat (150–200 g of calcium salts of rice bran fatty acid oil).

**TABLE 10: MEAN±SE LEVEL OF GLOBULIN (g/dL) IN CONTROL (T - 0) AND SUPPLEMENTED (T - 1) GROUP AT DIFFERENT DAYS OF THE TRANSITION PERIOD**

Groups	Pre-parturient day			Day of kidding	Post-parturient day		
	-21	-14	-7	0	7	14	21
T - 0	2.26±0.14	2.32±0.16	2.36±0.11	2.37±0.20	2.54±0.26	2.38±0.22	2.31±0.11
T - 1	2.31±0.16	2.37±0.23	2.34±0.10	2.39±0.19	2.53±0.25	2.43±0.26	2.33±0.10

### 3.2.8 Blood Urea Nitrogen (BUN)

The mean BUN levels (mg/dl) in the present study were found to be ranging from 26.83±2.91 to 44.91±1.44 in T - 0 group and 23.98±2.98 to 42.08±1.45 in T - 1 group (Table 11). Paired T-test (assuming equal variance) revealed a significant ( $p < 0.05$ ) difference in the mean values of serum BUN between the supplemented and non-supplemented groups on the day of kidding (day 0), day 7 and day 21 of postpartum.

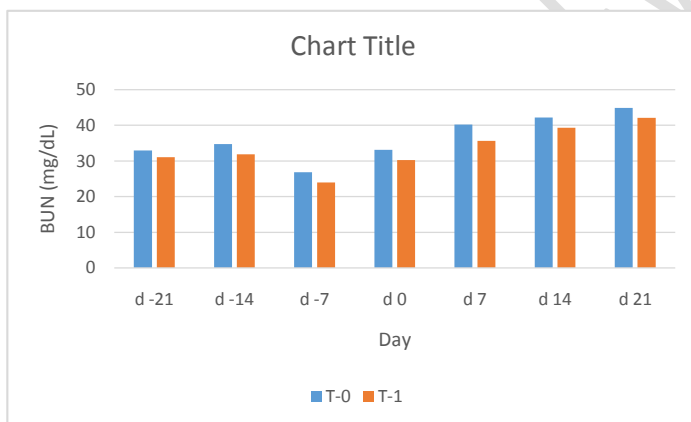
**TABLE 11: MEAN±SE LEVEL OF BUN (mg/dL) IN CONTROL (T - 0) AND SUPPLEMENTED (T - 1) GROUP AT DIFFERENT DAYS OF THE TRANSITION PERIOD**

Group s	Pre-parturient day	Day of kidding	Post-parturient day

	-21	-14	-7	0	7	14	21
<b>T - 0</b>	32.90 <sup>a</sup> ±2.6 7	34.75 <sup>a</sup> ±4.4 2	26.83 <sup>a</sup> ±2.9 1	33.1 <sup>a</sup> ±2.2 5	40.25 <sup>a</sup> ±3.3 6	42.18 <sup>a</sup> ±3.4 2	44.91 <sup>a</sup> ±1.4 4
<b>T - 1</b>	31.03 <sup>a</sup> ±1.0 3	31.88 <sup>a</sup> ±4.4 8	23.98 <sup>a</sup> ±2.9 8	30.25 <sup>b</sup> ±1.74	35.62 <sup>b</sup> ±1.5 8	39.31 <sup>a</sup> ±3.1 4	42.08 <sup>b</sup> ±1.4 5
Means in the same column having different superscripts are significantly (P< 0.05) different							

BUN values were found to be in lower range at prepartum observation days (day -21 and 7) till kidding (day 0) in both the groups. Significantly (p<0.05) lower BUN level was observed in T-1 group on day 0, 7 and 21 as compared to T-0 group. Similar pattern of reduced BUN levels in goats during transition period was observed by Sadjadian *et al.* (2012) and Akkaya *et al.* (2020).

**Comment [14]:** Reason out why BUN decreased till parturition day and increased thereafter??



**Fig. 4 GRAPHICAL REPRESENTATION OF MEAN±SE LEVEL OF BUN (mg/dL) IN CONTROL (T - 0) AND SUPPLEMENTED (T - 1) GROUP AT DIFFERENT DAYS OF THE TRANSITION PERIOD**

The quality and quantity of protein intake is reflected by the serum BUN level. The higher concentration of rumen degradable protein will increase the ammonia levels in the rumen which will be absorbed by the blood and converted into urea in the liver (Kumari *et al.*, 2021). In the present study, serum BUN levels were significantly (p<0.05) lower in the T - 1 group indicating efficient utilization of bypass protein. When the amount of rumen degradable protein is constant BUN level is negatively correlated with dietary intake of energy, this might be a reason for higher BUN level in T - 0 group. The results in the present study were in accordance with the results of Ghani *et al.* (2011) in lambs, Hassan

and Saeed (2012) in lambs, Shelke *et al.* (2012) in buffaloes, Movaliya *et al.* (2013) in buffaloes and Singh *et al.* (2014) in kids supplemented with bypass protein.

### 3.2.9 Serum GGT activity

The mean serum GGT levels (IU/L) in the present study were found to be ranging from 26.69±1.34 to 30.91±0.55 in T - 0 group, and 26.75±1.30 to 31.10±2.28 in T - 1 group (Table 12). In the present study, GGT activity was found to be in the normal range according to the reference value (0-30 IU/L) given by Batmaz (2013). GGT which is also termed as membrane bound enzyme due to its location at hepatocytes and biliary epithelium has a major role in cell detoxification and its elevated serum level is an indicator of hepatobiliary disease. Senturk, (2013) has reported that higher GGT activity was an indicator of negative energy balance associated with fatty liver in ruminants. In the present study no significant difference in GGT activities was observed in both the groups indicating better liver health of the study population with minimal or no effect of negative energy balance on hepatobiliary system. It might be due to the type of breed used in the study, as Assam Hill Goat is a meat type of animal and there is minimal lactational stress post kidding.

**TABLE 12: MEAN±SE LEVEL OF SERUM GGT ACTIVITY (IU/L) IN CONTROL (T - 0) AND SUPPLEMENTED (T - 1) GROUP AT DIFFERENT DAYS OF THE TRANSITION PERIOD**

Groups	Pre-parturient day			Day of kidding	Post-parturient day		
	-21	-14	-7	0	7	14	21
T - 0	28.24±1.01	28.63±0.148	29.37±0.58	30.26±0.73	30.91±0.55	26.69±1.34	28.65±1.43
T - 1	27.68±1.79	28.08±1.16	29.99±1.41	29.93±1.60	31.10±2.28	26.75±1.30	28.86±1.19

## 3.3 PRODUCTIVE PARAMETERS

### 3.3.1 Birth weight of Kids

The 12 does in the control group (T - 0) gave birth to 17 kids and the 12 does in the supplemented (T - 1) group gave birth to 19 kids. The mean values of the birth weight (Kg) of the kids produced by the does of T - 0 group was found to be 1.37±0.37 kg (ranging from 0.85 to 1.65 kg) while that of the T - 1 group was 1.49±0.45 kg (ranging from 0.8 to 1.75 kg). Numerically higher birth weight was obtained in supplemented group; however, the difference was statistically not significant. Significantly (p<0.05) increased birth weight of kids after bypass fat supplementation was reported by Mahboub *et al.* (2011) in does supplemented during the last stage of pregnancy. Their study revealed negative correlation between litter size and birth weight of kids ( $r = - 0.658, P<0.01$ ). During the transition period the dry matter intake was reduced due to physical fill limitation for the rumen and drift in the endocrine and metabolic profile. Maternal protein deficiency is more important during this period than that of energy in terms of fetal growth or birth weight. The deficiencies impact the in-utero fetal

development, fetal thermogenic capacity and quality colostrums. The comparatively better birth weight in the bypass fat and bypass protein supplemented group in the present study might be due to availability of the quality protein and its better bioavailability for fetal growth. Similar higher birth weight was reported by El- Shabrawy (2006) in Zariabi goats fed formaldehyde soybean meal (F-SBM) and heat-treated soybean seed (H-SBS) diets and Ghoniem *et al.* (2020) in Sufflok and Ossimi cross-ewes supplemented with rumen-protected fat. Hosam *et al.* (2022) found that supplementation of rumen-protected methionine in Awassi ewes at 3 and 5 per cent level gave birth to kids weighing 4.47 and 4.05 kg respectively, which were significantly ( $p < 0.05$ ) higher than the kids parturied by the control group (3.34 kg).

Comment [15]: Well explained.

### 3.3.2 Milk Yield

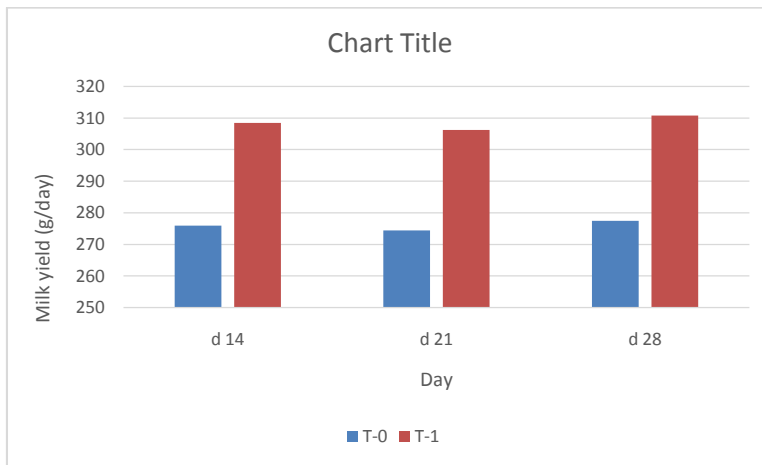
The mean values of milk yield (ml) were found to be  $271.94 \pm 21.37$ ,  $274.38 \pm 35.22$  and  $277.5 \pm 29.62$  in the T - 0 group and  $303.5 \pm 21.16$ ,  $306.25 \pm 19.67$  and  $310.75 \pm 23.93$  in the T - 1 group at 14<sup>th</sup>, 21<sup>st</sup> and 28<sup>th</sup> day after kidding respectively. The T-test (assuming equal variance) revealed significant ( $p < 0.05$ ) difference in the mean values of milk yield between the supplemented (T-1) and non-supplemented (T-0) groups on day 14, 21 and 28 of postpartum. Optimum body condition of does during the transition period is of paramount importance for ensuing productive and reproductive performances after kidding. In the present study better health and energy status of the animal in the supplemented group might have led to a higher milk yield.

**TABLE 13 MEAN $\pm$ SE MILK YIELD (g/day) IN CONTROL (T - 0) AND SUPPLEMENTED (T - 1) GROUP AT DIFFERENT DAYS OF OBSERVATION POSTKIDDING**

Groups	Days after kidding		
	14	21	28
T - 0	$271.94^a \pm 21.37$	$274.38^a \pm 35.22$	$277.50^a \pm 29.62$
T - 1	$303.50^b \pm 21.16$	$306.25^b \pm 19.67$	$310.75^b \pm 23.93$

Means in the same column having different superscripts are significantly ( $P < 0.05$ ) different.

Bypass fat supplements help in effectively converting the gross energy and digestible energy to net energy for lactation and helps saving glucose from oxidation which is otherwise used for lactose synthesis. Bypass protein supplementation provides the necessary amino acids used for milk synthesis. Higher milk yield post-supplementation of either bypass fat or bypass protein during transition period in various milch animals were reported by several workers (Shelke *et al.*, 2012 in buffaloes; Moty *et al.*, 2012 on buffaloes; Wadhwa *et al.*, 2012 in crossbred cattle; Mobeen, 2019 in Nili-Ravi buffaloes and Sahiwal cows and Ranaweera *et al.*, 2019 in crossbred cattle).



**Fig. 5 GRAPHICAL REPRESENTATION OF MEAN $\pm$ SE MILK YIELD (g/day) IN CONTROL (T - 0) AND SUPPLEMENTED (T - 1) GROUP AT DIFFERENT DAYS OF OBSERVATION**

### 3.4 REPRODUCTIVE PARAMETERS

#### 3.4.1 Time taken for the expulsion of the foetus and foetal membrane

In the kidding behaviour, any abnormality or difficulty that occurred during the parturition and the time taken for the expulsion of the foetus and expulsion of the foetal membrane was observed.

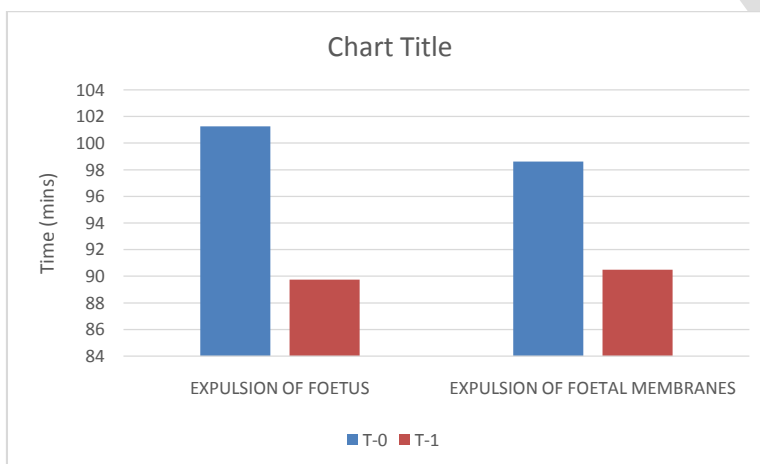
In the present study, the mean duration between the onset of restlessness to the expulsion of the foetus was found to be  $101.25 \pm 9.35$  and  $89.75 \pm 11.84$  minutes, and expulsion of the foetus to the expulsion of the foetal membrane was found to be  $98.63 \pm 8.83$  and  $90.5 \pm 5.86$  minutes in T - 0 and T - 1 group respectively. T-test (assuming equal variance) revealed significantly ( $p < 0.05$ ) lower mean values of time taken for the expulsion of foetus and expulsion of foetal membrane in supplemented group (T-1) as compared to the non-supplemented group (T-0). Time taken for expulsion of foetus and foetal membrane is influenced by factors like body structure of the doe and its ability to distend the pelvic region *i.e.*, primiparous does take more time for expulsion of foetus and consequently, placenta (Grandinson, 2005). Physiological release of oxytocin also influences the time taken for expulsion of foetus and placenta. In the present study, reduction in the time taken for expulsion of foetus and foetal membrane in supplemented group might be due to better energy status of animals at the time of parturition (Hafez, 2000). Similar finding was also reported by Khalil *et al.* (2012) in 3-5 per cent bypass fat supplemented cows.

**TABLE 14: AVERAGE TIME TAKEN (Mins) IN CONTROL (T - 0) AND SUPPLEMENTED (T - 1) GROUP FOR EXPULSION OF FOETUS AND FOETAL MEMBRANES**

Groups	Time taken for the expulsion of the foetus	Time taken for the expulsion of foetal membrane

<b>T - 0</b>	101.25 <sup>a</sup> ±9.35	98.63 <sup>a</sup> ±8.83
<b>T - 1</b>	89.75 <sup>b</sup> ±11.84	90.5 <sup>b</sup> ±5.86

Means in the same column having different superscripts are significantly (P< 0.05) different.



**FIG 6 GRAPHICAL REPRESENTATION OF AVERAGE TIME TAKEN (Mins) IN CONTROL (T - 0) AND SUPPLEMENTED (T - 1) GROUP FOR EXPULSION OF FOETUS AND FOETAL MEMBRANES**

### 3.4.2 Incidence of peripartum diseases

There was no incidence of any peripartum diseases in both the group during the experimental period. Assam Hill Goat being a meat type of animal, the nutrient partitioning for milk production might be less, hence lower the impact of negative energy balance and better periparturient reproductive health.

No incidence of periparturient diseases in the present study indicates better managerial plan (especially the nutrition and the reproductive health plan) of the herd and comparatively less susceptibility of Assam Hill Goat towards the reproductive diseases.

## 4. CONCLUSION

It can be concluded that the supplementation of bypass fat (10g/day/animal) and bypass protein (5g/day/animal) during the transition period in AHG has no significant effect on TLC, Neutrophil count, haemoglobin, Ca, P, globulin and glucose levels. Supplementation reduced the effect of negative energy balance and facilitated quicker recovery from NEB. Significantly

higher serum protein and albumin levels along with reduced BUN levels in the supplemented group indicated better absorption and reduced wastage of dietary protein by the microbial breakdown. Supplementation had no significant effect on the birth weight of the kids however; significant improvement in milk yield was recorded. There was a significant reduction in the time taken for the expulsion of foetus and expulsion of foetal membranes in the bypass fat and bypass protein supplemented group. The present study could not find any significant effect on the immune response of the animals by supplementing bypass fat and bypass protein.

## 8. DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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UNDER PEER REVIEW

