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Analysing the Effect of Silage Feeding on Blood Metabolities of Different Producing *Murrah* Buffaloes

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Abstract

Background: Maize is well known as the important green fodder resource in dairy farming system across the globe. At the same time, farmers routinely make silage out of the surplus maize fodder for reducing their fodder crisis. Since maize is abundantly cultivated by the dairy farmers for dual purpose (green fodder and silage making), it is imperative to study their impact on animal production and various blood metabolites.

Methods: The current experiment was carried out on mid-lactation buffaloes, which were divided into four groups based on their body weight and milk yield. The first two classes are low-production animals that are fed non-legume feed, wheat straw and silage. Similarly, the final two are high-producing groups that are given the similar diet. The concentrate and silage ratios were fed at 40:60 in the form of Total Mixed Ration (TMR) for the II and IV groups. Similarly, the ratio of concentrates to green fodder was 40:60 in Groups I and II.

Result: Significant results were obtained for NEFA and BUN levels in between the high and low-producing groups (P<0.05). Various biochemical tests like glucose, protein, albumin, globulin and creatinine levels have shown no significant difference (P>0.05). It was seen that silage feeding in different producing groups maintained the concentration of various blood profiles up to the basal levels.

Key words: Biochemical, Cortisol, Hormonal, *Murrah*, Silage.

Introduction

The livestock system in India is crucial to both the socioeconomic development of millions of rural households and the country's economy, accounting for 26.8% of the agriculture sector's GDP in 2019-2020 (DAHD, 2019). Indian cattle productivity is low when compared to developed countries. The animals are fed crop waste and grasses from grazing areas.

The buffaloes, which are mostly raised in mixed agricultural systems, have a low input requirement and are well-known for their hardiness in surviving on subpar crop wastes. About 109.85 million buffaloes are thought to be in existence now in India, making up 20.45% of all cattle in the country (DAHD, 2019).

Buffaloes are the primary milk-producing animal in India, with a large body size and higher dry matter consumption. It is challenging to give animals wholesome, high-quality feed throughout the year. In low seasons, fodder is typically scarce. Both the animals' body weight and production declines as a result. Therefore, one way to combat the lack of fodder is to search for alternate feed sources. To meet animal needs and combat scarcity, maize silage might be very helpful. The primary feed that Indian dairy buffaloes consume is maize silage. Silages are thought to be the most economical feed material for the feeding of ruminants.

To address the energy needs of high-producing dairy cows, superior silages with higher fermentable concentrates and lower fibre content are typically utilized Xue *et al*. (2020). Roughage is a necessary part of ruminant diets to maintain a stable environment in the rumen, which promotes health and maximizes production Matthews *et al*. (2018). Diets commonly comprise relatively high quantities of concentrate and high-quality forages to meet the energy requirements of high-producing lactating dairy cows. Due to its excellent agronomic qualities, high nutrient production, good tensile quality and ease of incorporation into total mixed ration (TMR), maize silage is one of the most widely used forages given to dairy cows (Neylon and Kung, 2003). Beauchemin and Buchanan Smith, (1990) found that the majority of commercial dairy diets have high concentration levels and premium maize silages that are frequently chopped fine. Therefore, adequate particle length of forages is necessary for proper ruminal function as coarse particles stimulate chewing activity and hence increase saliva output.

The practice of feeding processed corn silage to lactating dairy cows has gained popularity recently. Evaluations of the effects of chop length and corn silage crop processing were conducted on milk production, intake

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and digestion (Beauchemin *et al*., 2003). To our knowledge, the scientific information about biochemical blood parameters of different producing buffaloes fed with maize silage is not sufficient. Therefore, this experiment aimed to investigate the effects of maize silage on the biochemical and hormonal parameters of different producing lactating buffaloes.

MATERIALS AND METHODS

Feed analysis

Commonly used feeds and fodder samples used in the trials were collected and then dried in hot air oven at 70°C for 24 hrs till a constant weight was attained. Then, the dried samples were ground through a 1 mm sieve using an electrically operated willy mill. The ground samples were stored in bottles of 200 ml capacity, labeled properly and kept for further analysis. The proximate analysis was done as per the procedure mentioned (AOAC, 2005).

Research trial and experimental design

In the present study, thirty-two lactating Murrah buffaloes were chosen from the LRC, NDRI and Karnal, to participate in a 120-day feeding trial using maize silage. The mid-lactation buffaloes were divided into four groups according to their body weight and milk output for the experiment. The first two groups of animals are low producers and their diet consists of silage, wheat straw and standard oat fodder. The latter two are also high-yielding groups that are fed silage and oat green fodder. For the II and IV groups, the concentrate and silage ration were fed at a ratio of 40:60 in the form of total mixed ration (TMR). Similarly, for groups I and II, the ratio of concentrates to traditional green feed was 40:60. Animals were offered *ad-libitum* fresh drinking water twice daily in the morning at 10:00 h and evening at 17:30 h.

Blood sampling

Blood samples (10 ml) were collected at zero days and then at every fortnight till 120 days of trial in sterile zheparinized vacutainer tubes from jugular vein puncture, posing minimum disturbance to the animal. Immediately after collection, samples were kept in the ice box and transported to the laboratory for further processing. The plasma was separated by centrifugation at 3000 rpm for 30 minutes and stored at 20°C in different aliquots for the analysis of various biochemical constituents.

The plasma concentration of urea was estimated by Berthlot method by using a diagnostic reagent kit provided by Recombigen Laboratories PVT. LTD (New Delhi)-110042. Total protein was estimated in blood plasma samples using a kit supplied by Recombigen Ltd. The peptide bonds of proteins react with cupric ion in the alkaline medium of the biuret reagent and produce a purple-colored complex, whose absorbance is proportional to the protein concentration, which is measured at 555 nm. Plasma concentration of albumin was estimated by using a diagnostic reagent kit provided by Recombigen Laboratories PVT.LTD (New Delhi)-110042. The total globulin concentration (g%) was calculated by subtracting the total albumin from the total protein. Plasma concentration of glucose was estimated method by using diagnostic reagent kits provided by Recombigen Laboratories PVT. LTD (New Delhi) - 110042. The plasma NEFA concentration was estimated through the copper soap solvent extraction method modified by Shipe *et al.* (1980). Blood cortisol was estimated through Bovine Cortisol ELISA KIT manufactured by yBioSMyBioSource company USA (Product no. #MBS2608983). Blood Triiodothyronine (T3) was estimated using a Bovine Triiodothyronine (T3) ELISA Kit manufactured by yBioSMyBioSource company USA (Product no #MBS2609823). Blood thyroxine T4 was estimated using the Bovine thyroxine, T4 ELISA Kit manufactured by MYBiosource company USA (Product no #MBS702370).

Statistical analysis

The data collected was analyzed by Multiple ANOVA Techniques using the PROC ANOVA Procedure of SAS 9.3. Homogenous subsets were separated using the Duncan-multiple-range test and the level of significance was declared *(P*<0.05).

RESULTS AND DISCUSSION

Chemical composition (% DM basis) of different feeds used in the feeding trial

The chemical compositions of various feedstuffs used in the feeding trials have been mentioned in Table 1. The DM % of different feeds ranged from 17.2 to 90.39 %. The OM % of different feeds ranged from 89.87 to 94.54 %. The CP % of different feeds ranged from 3.7% to 21.78%. The NDF % of different feeds ranged from 24.1 to 77.11%. The ADF

Table 1: Chemical composition (% DM basis) of different feeds used in the feeding trial.

Parameter (%DM)	Concentrate mixture	Wheat straw	Oats	Maize silage
DM	90.39±0.88	90.63±0.64	17.2±0.35	28.68±0.28
OM	94.54±0.43	89.87±0.22	90.68±0.59	90.7±0.89
CP	21.78±0.57	3.7±0.93	9.93±0.67	7.88±0.63
NDF	24.1±0.42	77.11±0.56	56.73±0.86	55.47±0.73
ADF	11.49±0.71	51.32±0.48	44.83±0.58	41.9±0.48
EE	3.9±0.37	1.3±0.45	2.82±0.53	2.83±0.35
TA	5.45±0.17	10.13±0.79	9.32±0.71	9.3±0.27

% of different feeds ranged from 11.49 to 51.32% with the highest value in wheat straw and the lowest in concentrate. The EE % of different feeds ranged from 1.3 to 3.9%. The total ash % of different feeds ranged from 5.45 to 10.13%. The chemical composition of various feedstuffs was within the previously reported normal range (Das *et al*., 2014; Prusty, 2015; Sharma, 2014).

Effects of silage feeding on different biochemical parameters in different producing groups

Effects of silage intervention on glucose levels in different production groups

The level of blood glucose (mg/dl) at the start of the experiment (0 day) was similar in all the groups and mean values were 51.17±1.45, 48.5±1.12, 51.67±1.71and 49.17±1.90 for T_1, T_2, T_3 and T_4 groups, respectively. There were non-significant differences (p>0.05) in blood glucose levels at different fortnights in both the silage and -green-fed groups. The non-significant results (p>0.05) for blood glucose levels at different fortnights were also obtained for both low and high-producing groups (Fig 1). There was a wide variation in glucose levels with no clear-cut trends in different fortnights. The overall mean of the blood glucose level in the T_1, T_2, T_3 and T_4 groups are 51.14 50.14, 50.54 and 50.52. There was non-significant difference (p>0.05) in the overall mean of the blood glucose level among all the groups (Table 2).

These values of the blood glucose levels were within the normal physiological range and comparable with the findings of a previous study (Rajvaidya, 2016). Glucose is necessary for all bodily cells to produce energy, which means that it is also necessary for all cells to continue to be healthy and repair themselves Giridharan *et al*. (2018). Propionate, which is created during rumen fermentation, is commonly considered to be the primary substrate for gluconeogenesis. The current study's propionate levels in rumen fluid varied from 16.7 to 18.3 mol/100 mol and were equivalent across diets (Gencoglu and Turkmen, 2006) suggesting that dietary modifications may not affect blood glucose levels. Similar results were obtained during silage feeding to calves Singh *et al*. (2022).

Blood urea nitrogen (BUN)

There were non-significant differences (p>0.05) for blood BUN levels at different fortnights in both the silage and-greened groups. The significant results (p<0.05) for blood BUN (mg/dl) levels at different fortnights were obtained for both the low and high-producing groups. There was a great variation in blood BUN levels with no clear-cut trend on different fortnights.

The overall mean of the blood BUN level in the T_1, T_2, T_3 and T_4 groups are 16.95±0.71, 17.66±0.63, 18.46±0.71 and 18.99±0.51 for T_1, T_2, T_3 and T_4 groups, respectively. There was a significant difference (p<0.05) in the overall mean of

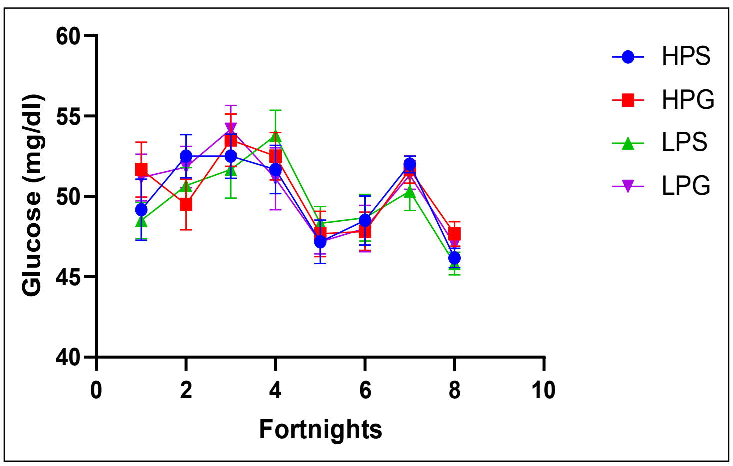

Fig 1: Effect of silage and green fodder feeding on glucose levels (mg/dl).

Table 2: Average blood biochemical levels in different production groups.

Parameters	LPG	LPS	HPG	HPS	SEM
Glucose (mg/dl)	51.14	50.14	50.54	50.52	0.77
Total protein (mg/dl)	7.66	7.79	7.58	7.88	0.22
Albumin (mg/dl)	4.14	4.12	4.15	4.19	0.35
Globulin (mg/dl)	3.52	3.67	3.32	3.70	0.24
BUN (mg/dl)	16.95[a]	17.66[a]	18.46[b]	18.99[b]	0.57
NEFA (mg/dl)	116.98[a]	118.15[a]	129.20[b]	127.53[b]	0.99

Means bearing different superscripts[a, b] in the same row differs significantly (p<0.05).

the blood BUN level among all the groups (Fig 2). The significant differences ($p<0.05$) were obtained in between the low and high-producing groups in the overall mean. However non-significant results for the overall mean were obtained by silage intervention in the treatment groups (Table 2). The numerical higher value for BUN level was obtained in the silage-fed groups.

Blood urea N (BUN) concentration is one marker of protein status in a group of animals that might be useful in finding problems with feeding regimens or modifying diets Kohn *et al*. (2002). Because the high-producing groups are fed higher protein diets to sustain production levels, the higher BUN levels are the result. In addition, low nutritional energy can increase BUN and rumen bacteria by inhibiting the synthesis of microbial proteins. These findings are consistent with (Rajvaidya, 2016; Singh *et al*., 2022) and fall within the normal physiological range.

Non-esterified fatty acids (NEFA)

The level of blood NEFA (mg/dl) at the day of the start of the experiment was similar and mean values were 127.13±2.88, 126.21±3.13, 122.30±2.89 and 119.54±3.04 for T_1, T_2, T_3 and T_4 groups, respectively. There were no significant differences ($p>0.05$) in blood NEFA levels at different fortnights in both the silage and -green-fed groups. Also, non-significant results ($p>0.05$) for blood NEFA (mg/dl) levels at different fortnights were obtained for both the low and high-producing groups. There was a large variation in blood NEFA levels with no clear-cut trend in different fortnights. The overall mean of the blood NEFA level in the T_1, T_2, T_3 and T_4 groups are 124.98, 122.15, 126.20 and 126.14 (Fig 3). The significant results ($p<0.05$) for the overall average were obtained in between the high and low-producing groups (Table 2) The non-significant results for NEFA levels were obtained while comparing the low and high producing groups separately.

These values of NEFA were within the normal physiological range in all the production groups and comparable with (Rajvaidya, 2016). The negative energy balance of animals is represented by the NEFA concentration in the blood. Due to higher movements of the body reserves toward milk production, high productive animals have greater NEFA values. Body reserves of low producing animals have reduced NEFA value due to fewer mobilizations

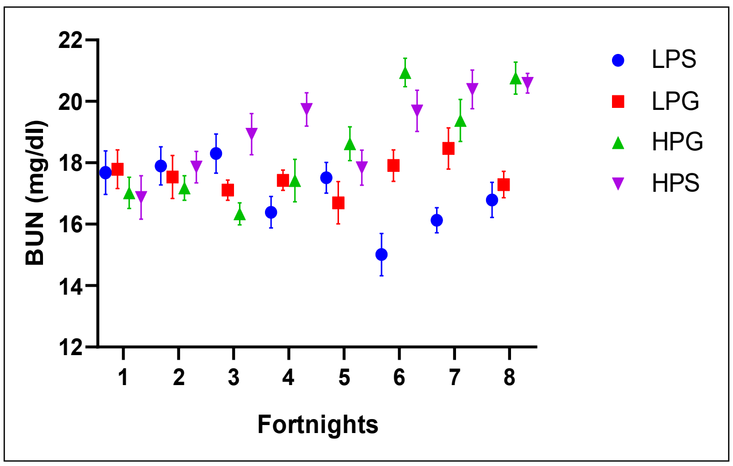

Fig 2: Effect of silage and green fodder feeding on BUN levels (mg/dl).

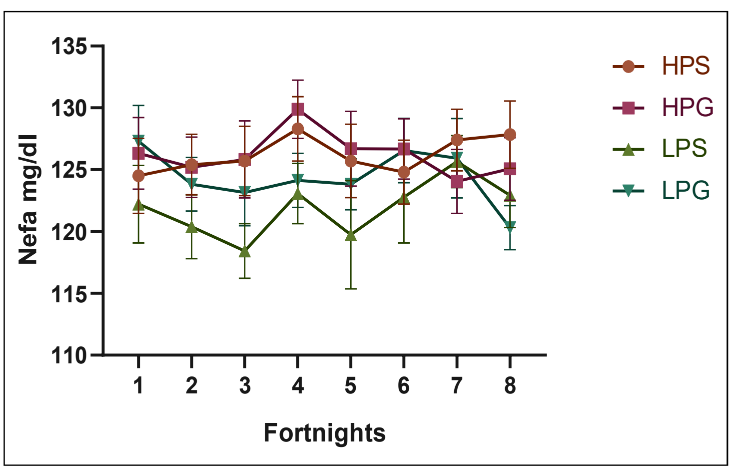

Fig 3: Effect of silage and green fodder feeding on NEFA levels (mg/dl).

of body reserves towards milk production. This could explain the significant differences in NEFA values in between the high and low-producing groups.

Total protein (TP)

The level of total protein (mg/dl) at the day of the start of the experiment was similar in all the groups and mean values were 7.72±0.46, 7.81±0.48, 7.41±0.48 and 8.02±0.46 for T_1, T_2, T_3 and T_4 groups, respectively. There were no significant differences (p>0.05) for total protein levels at different fortnights in both the silage and -green-fed groups. Also, non-significant results (p>0.05) for total protein levels at different fortnights were obtained for both the low and high-producing groups (Fig 4). There was a great variation in total protein levels with no clear-cut variation in different fortnights. There was no significant difference (p>0.05) in the overall mean of the total protein level among all the groups (Table 2).

There were non-significant differences between treatment groups because of protein intake that was the same across all treatments. When more protein is consumed than is necessary for growth and maintenance, protein concentration rises. These values are within the normal physiological range and are comparable (Rajvaidya, 2016; Singh *et al*., 2022).

Albumin

The level of albumin (mg/dl) at the day of the start of the experiment was similar in all the groups and mean values were 4.01±0.33, 3.64±0.35, 3.95±0.3 and 3.58±0.37 for T_1, T_2, T_3 and T_4 groups, respectively. There were no significant differences (p>0.05) for albumin levels at different fortnights in both the silage and -green-fed groups. Also, non-significant results (p>0.05) for albumin levels at different fortnights were obtained for both the low and high-producing groups (Fig 5). There was a great variation in albumin levels with no clear variation in different fortnights. The overall mean of the albumin level in the T_1, T_2, T_3 and T_4 groups are 4.14, 4.12, 4.15 and 4.19 (Table 2). There was a non-significant difference (p>0.05) in the overall mean of the albumin level among all the groups and the values are within normal physiological range and comparable with the findings (Rajvaidya, 2016).

Adequate amounts of nitrogen and nutrients are necessary for the synthesis of albumin. Wingfield *et al*. (2015) concluded that the shortage of amino acids for protein

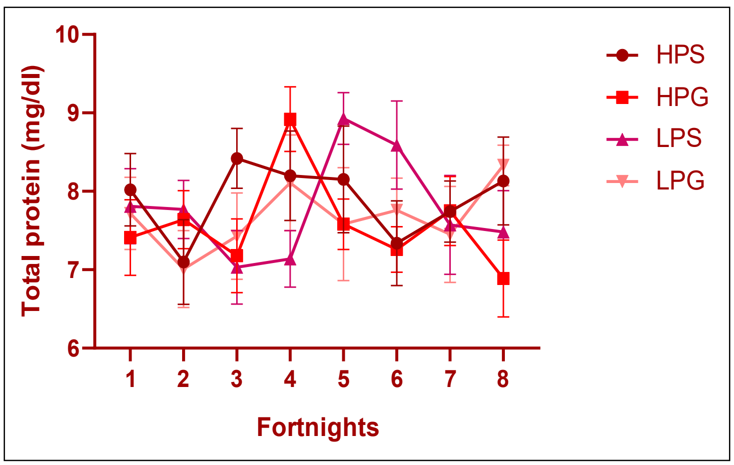

Fig 4: Effect of silage and green fodder feeding on protein levels (mg/dl).

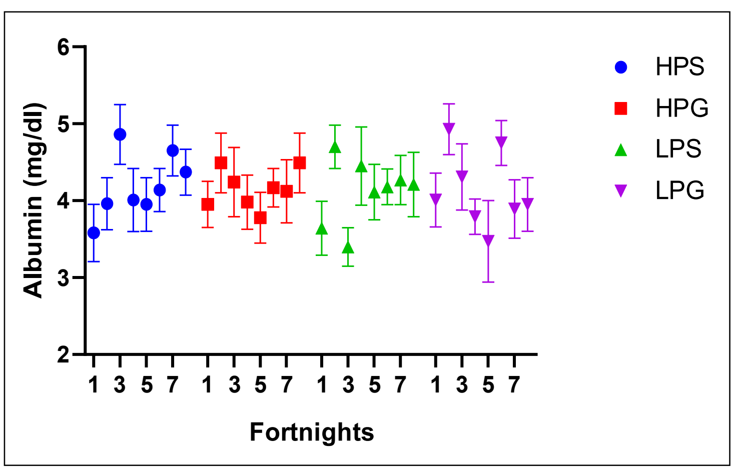

Fig 5: Effect of feeding silage and green fodder on albumin levels (mg/dl).

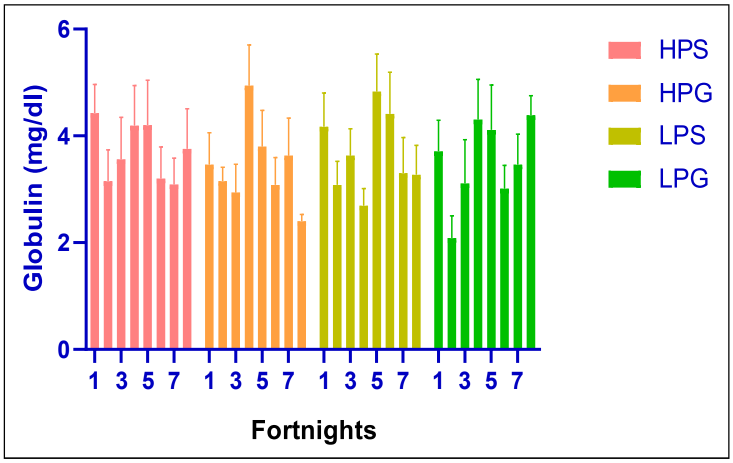

Fig 6: Effect of feeding silage and green fodder on globulin levels (mg/dl).

Table 3: Effects of silage feeding on hormonal parameters in different producing groups.

Parameters	LPG	LPS	HPG	HPS
Cortisol (ng/ml)	4.37[a]±0.13	4.93[a]±0.21	5.18[a]±0.18	5.87±0.23
T3 (ng/ml)	0.95[a]±0.08	1.02[a]±0.14	0.98[a]±0.10	1.38±0.19
T4 (ng/ml)	48.38[a]±2.56	47.3[a]±3.56	49.32[a]±1.28	46.65±2.18

synthesis and/or inadequate nutritional absorption *via* the intestinal lumen as a result of illness can both lower the liver's ability to make albumin. There was a non-significant difference (p>0.05) in the overall mean of the albumin level among all the groups and the values are within the normal physiological range and comparable with the findings (Rajvaidya, 2016). So, it proves that feeding silage has provided the required nutrients for albumin synthesis.

Globulin

The level of globulin (mg/dl) at the day of the start of the experiment was similar in all the groups and mean values were 3.71±0.58, 4.17±0.63, 3.46±0.6 and 4.43±0.53 for T_1, T_2, T_3 and T_4 groups, respectively. There were non-significant differences in globulin levels at different fortnights in both the silage and -green-fed groups. Also, non-significant results for globulin levels at different fortnights were obtained for both the low and -and high-producing groups (Fig 6). There was a great variation in globulin levels with no clear-cut trend in different fortnights (Table 2). There was no significant difference in the overall mean of the globulin level among all the groups (Table 2). These values are within the normal physiological range and comparable with the findings (Rajvaidya, 2016).

Globulin plays an important role in liver function, blood clotting and fighting infection. The normal level indicates no negative effect on globulin levels. The normal range indicates no effect on the normal globulin levels.

Effects of silage feeding on hormonal parameters in different producing groups

The cortisol levels were not affected in all the producing groups and similar levels were seen in all the producing groups (Table 3). Cortisol is a steroid hormone that is produced by the adrenal glands. Cortisol levels in the blood are an important stress indicator in evaluating stress levels (Ferguson and Warner, 2008). Thus, it informs that it do not affect stress hormone levels in the animals.

T_3 and T_4 are known thermoregulators in animals, involved in the nutritional and environmental metabolic activities of animals. These hormones regulate the energy balance and protein metabolism, thermoregulation Kim *et al*. (2008). Thus, the normal range of these hormones is very important for maintaining the proper basal metabolic rate and as well proper functioning of various vital functions of the body. In our study, silage has maintained them within the normal range and hence contributing to homeostasis and positive energy balance (Table 3). Similarly, (Khare *et al*., 2016; Chandra *et al*., 2016) also reported a normal range of T3 and T4 levels in their experiments.

CONCLUSION

From the present research, it may be concluded that the inclusion of silage in different producing groups has no adverse effects on various blood metabolites. They may affect the biochemical parameters but the differences observed were very small. It has helped in maintaining the concentration of various critical biochemical and hormonal profiles up to the basal levels. Hence *silage* can be used as a green fodder source during scarcity in nearby future to bridge the demand and supply gap of nutrients and provide better productivity.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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