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Effect of replacement of concentrate mixture with *Moringa oleifera* leaf meal on haemato-biochemical parameters in Barbari goats

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Abstract
The experiment was conducted on ten Barbari goats to evaluate the feeding effect of *Moringa oleifera* on hematobiochemical parameters. The goats were supplemented with *Moringa oleifera* for a period of 180 days to study the effects and divided into two groups of five animals in each, as control and treatment. The goats of control group were fed with concentrate mixture and Hybrid Napier (CO_3) as per ICAR requirements and in treatment group, 50% concentrate mixture was replaced by *M. oleifera* leaf meal on dry matter basis along with Hybrid Napier.Plasma glucose (mg/dl), total protein (g/d), albumin (g/d), globulin (g/d) levels were found similar between the groups and are in normal range. Blood urea Nitrogen was higher (p<0.05) in treatment group (42.63 mg/dl) compared to control group, though the value found within the normal range for goats. Blood IgG1 value found to be significantly higher (p<0.05) in treatment group compared to control (11.08 mg/dl). There was no effect on the blood mineral profile with the replacement of concentrate with *M. oleifera* leaf meal. Based on above results, it may be concluded that, replacing 50% of concentrate mixture with *M. oleifera* improves the hematobiochemical parameters in Barbari goats and significantly improve the immune status of the treatment groups.

Keywords: *Moringa oleifera*, hybrid Napier, hematobiochemical, blood minerals, immune status

Introduction
Goats are widely distributed across all agro-climatic regions of the country, and many people in future may shift from agriculture to goat rearing because goats can survive in harsh environment and still provide good profit (Devendra, 2015) [7]. The major constraint in goat rearing is the low productivity compared to their actual genetic potential because of poor management and feeding. Feed resources are the major limitations to meet the demand and there exist large gap between requirements and availability of animals' feeds and fodders (IGFRI vision-2030) [13]. The cost of feeding accounts for about 70% of the total cost of goat rearing, and most of the farmers depending on goat farming for their liveability are the small and marginal farmers in India. Smallholder livestock producers are not capable of providing adequate supplements because of high cost and availability of the feeds like concentrates, cereal grains, or their by-products. The incorporation of feed stuffs from non-conventional resources (NCFR) in the goat ration can be one such solution to overcome feed scarcity in India. The use of top feeds (foliage from trees and shrubs) can improve the utilization of poor-quality feed resources during the lean season and meet the fodder supply when green fodder availability is decreased. Tree foliage, usually has high nitrogen content and can serve as a supplement, increasing dietary protein levels, improving the intake and digestibility of the ingested food (Elaidy *et al*., 2017) [8]. Moringa is one of the best NCFRs available in India which belongs to the family of Moringaceae, commonly known as 'drumstick' 'horseradish tree' and sahjan in local language (Debela and Tolera, 2013) [6]. Moringa has good copping ability, as well as good potential for forage production. The use of *M. oleifera* as a feed supplement for ruminants has several advantages, which not only ensure green fodder availability round the year but also reduce the feed cost. It has the potential to replace the protein source in a diet due to its high protein content. The crude protein content of *Moringa oleifera* leaf ranges between 23 to 30.3% on dry matter basis (Wu D *et al*., 2013) [24]. In the recent years *M. oleifera* has dragged all the attentions of researchers in animal husbandry because of its very good nutritional, anti-oxidative and medicative properties (Bryant *et al*., 2014). *M. oleifera* leaf is rich in vitamins, flavonoids, phenols, and carotenoids (Pakade *et al*.,

2013) [21]. There are few studies on M. oleifera feeding in Barbari goats. The present study is aimed to evaluate the effects of replacement of concentrate with *M. oleifera* leaf meal on blood biochemical parameters in Barbari goats.

Materials and Methods

The experiment was conducted at Integrated Farming System Unit of Livestock Research Centre, National Dairy Research Institute (N.D.R.I.), Karnal, Haryana, India. The ICAR-NDRI, Karnal is located on 29° 43' N latitude and 76° 58' E longitudes at an altitude of 245 meters above the mean sea level in the Indo-Gangetic alluvial plain. The minimum ambient temperature falls to near freezing point in winter in December/ January and maximum goes approximately up to 45 °C in May/June in summer. The annual rainfall is about to 700 mm, most of which is received from July to September. A subtropical climate prevails in the area.

Moringa oleifera leaf meal preparation

M. oleifera was collected from the campus of NDRI. The leaves were collected and chopped into small pieces averaging about 2 cm in diameter and were dried under shade. Then the leaves were ground to powder form in a grinding machine, weighed and packed for feeding.

Selection of animals and feeding

Ten pregnant Barbari goats were selected from Dairy based Integrated farming system project, NDRI, Karnal and divided into two groups of 5 animals each on the basis of expected production ability and body weight (average of control and treatment groups are 19.8 and 20.2 kg, respectively). The selected animals for study were free from any anatomical, physiological and infectious disorders. The experiment was conducted as per the guidelines of institutional ethical committee and the animals were kept in pakka floor in the shed.

The animals were fed as per ICAR, 2013 [12] standards. The animals had free access to clean drinking water throughout the day and night. The animals under treatment groups were fed with *Moringa oleifera* leaf meal (50% of concentrate mixture). Dried leaves of *M. oleifera* was mixed in concentrate and fed to the experimental animals.

Design of experiment

Experiment was conducted over a period of six months from December to May. Each animal was kept under observations during the whole experimental days. The animals were assigned as T_1 and T_2. T_1 was taken as control and T_2 as treatment where feeding practice was as follows:

- T_1- Hybrid Napier grass + Concentrate Mixture as per ICAR, standard (2013) [12].
- T_2- Hybrid Napier grass + 50% of concentrate mixture replaced with *Moringa oleifera* leaf meal on DM basis as per ICAR, standard (2013) [12].

A metabolic trial was conducted in the end of experimental period for 5 days collection to determine the digestibility of nutrients and nitrogen balance. Animals were shifted to the metabolism shed after 12 weeks of preliminary feeding to the metabolism trial for their adaptation to the surroundings. Animals were weighed before and after the trial consecutively for two days. Feeds were offered in feeding buckets and water in plastic bucket. Fresh drinking water was provided *ad. lib* and the quantity was measured each time to calculate the total water intake.

Sampling and hematobiochemical analysis of blood plasma

Samples were collected from all the animals before commencement of real feeding and thereafter blood samples were collected from the animals by jugular puncture in heparinised vacutainer in the morning at monthly intervals and mixed well by rotating the tubes between palms to ensure proper mixing of blood and anticoagulants. Then samples were brought to the laboratory after placing in ice box carefully without giving any sudden jerks and vigorous movements. Fresh blood sample about 0.5mL was immediately processed for routine haematological parameters. Rest samples were centrifuged at 3000 rpm for 20 min to separate plasma. The plasma samples were stored at -20 °C for estimation of Albumin, total protein, blood urea nitrogen, glucose, IgG1. Biochemical parameters were evaluated by using Fully Automatic Biochemistry Analyser Model. IgG1 was estimated by using special goat IgG1 kit. Plasma minerals (Ca, Fe, Cu, Zn, and Mn) were also measured by using automatic atomic spectrophotometry (AAS).

Statistical Analysis

Analysis of data was done by SPSS software using ANOVA. Mean and standard error were calculated and comparisons between groups were made.

Results

Plasma glucose (mg/dL)

The findings of blood plasma glucose level (mg/dL) in control and treatment group have been presented in the Table 1. The overall mean of plasma glucose values (mg/dL) was 65.24±0.51 and 64.56±0.73 in control and treatment group, respectively. The result showed no significant difference between the two group (p>0.05). The initial plasma glucose level found to be 65.27±0.60 and 65.76±2.17 mg/dl in control and treatment group, respectively. The final glucose level (mg/dL) was 65.6±1.79 and 64.1±1.53 in control and treatment group, respectively.

Table 1: Blood plasma glucose level (mg/dL) in different groups fed with *Moringa oleifera* leaf meal replacing concentrate mixture as a protein source in Barbari goats

Group		
Month	**Control**	**Treatment**
0	65.27±0.60	65.76±2.17
1	64.76±1.41	63.48±2.73
2	63.44±1.51	60.78±1.93
3	65.49±1.26	67.24±0.89
4	65.52±1.30	65.48±1.80
5	66.58±1.81	65.14±1.85
6	65.6±1.79	64.1±1.53
Overall Mean± SE	65.24±0.51	64.56±0.73

The values are Mean ± SE of observations on five animals in each

Plasma total protein (g/dL)

The result of blood plasma total protein level (g/dL) in control and treatment groups have been presented in Table 2. The overall mean of plasma total protein level (g/dL) value was 7.43±0.12 and 7.53±0.11 in control and treatment group, respectively. The result showed no significant difference between the two group (p>0.05).

Plasma albumin (g/dL)

Table 2: Blood plasma total protein level (g/dL) in different groups fed with *Moringa oleifera* leaf meal replacing concentrate mixture as a protein source in Barbari goats

Group		
Month	**Control**	**Treatment**
0	6.98±0.23	6.98±0.20
1	7.82±0.42	7.45±0.25
2	7.13±0.31	7.48±0.30
3	7.45±0.41	7.52±0.27
4	7.49±0.26	7.33±0.51
5	7.60±0.26	8.17±0.12
6	7.54±0.30	7.81±0.10
Overall Mean± SE	7.43±0.12	7.53±0.11

The values are Mean ± SE of observations on five animals in each

The result of blood albumin level (g/dL) have been presented in Table 3. The mean plasma albumin level (g/dL) values were 3.10±0.04and 3.14±0.03 in control and treatment group, respectively. The result showed no significant difference between the groups (p>0.05). The initial plasma albumin level found to be 2.70±0.18and 2.86±0.10 g/dL in control and treatment group, respectively.

Table 3: Blood plasma albumin level (g/dL) in different groups fed with *Moringa oleifera* leaf meal replacing concentrate mixture as a protein source in Barbari goats

Group		
Month	**Control**	**Treatment**
Initial	2.70±0.18	2.86±0.10
1	3.25±0.09	3.15±0.09
2	3.08±0.06	3.05±0.04
3	3.06±0.09	3.10±0.12
4	3.07±0.11	3.24±0.05
5	3.20±0.07	3.25±0.10
6	3.31±0.03	3.34±0.07
Overall Mean± SEM	3.10±0.04	3.14±0.03

The values are Mean ± SE of observations on five animals in each

Plasma globulin (g/dL)

The result of blood globulin level (g/dL) have been presented in Table 4.The mean plasma globulin level (g/dL) value was 4.36±0.13 and 4.55±0.10 in control and treatment group, respectively. The result showed no significant difference between the groups (p>0.05). The initial plasma globulin (g/dL) found to be 4.46±0.38 and 4.75±0.27 in control and treatment group, respectively.

Table 4: Blood plasma globulin level (g/dL) in different groups fed with *Moringa oleifera* leaf meal replacing concentrate mixture as a protein source in Barbari goats

Group		
Month	**Control**	**Treatment**
0	4.46±0.38	4.75±0.27
1	4.57±0.47	4.30±0.26
2	4.05±0.35	4.43±0.27
3	4.39±0.40	4.42±0.16
4	4.42±0.35	4.36±0.44
5	4.39±0.27	4.92±0.18
6	4.23±0.31	4.72±0.21
Overall Mean± SE	4.36±0.13	4.55±0.10

The values are Mean ± SE of observations on five animals in each

Blood urea nitrogen (mg/dL)

The findings of blood urea nitrogen level (mg/dL) in control and treatment group have been presented in Table 4.5. The overall mean of blood urea nitrogen (mg/dL) value was 40.48±0.96and 42.63±1.47 in control and treatment group, respectively. The overall mean BUN level was significantly higher in treatment group compared to control. The initial blood urea nitrogen level (mg/dL) found to be 39.6±0.97 and 39.6±2.22in control and treatment group, respectively.

Table 5: Blood urea nitrogen level (mg/dL) in different groups fed with *Moringa oleifera* leaf meal replacing concentrate mixture as a protein source in Barbari goats

Month	**Control**	**Treatment**
0	39.6±0.97	39.6± 2.22
1	39.6±2.03	39.20±1.95
2	40.4^{a}±0.39	46.40^{b}±2.92
3	42.6±2.40	46.40±2.99
4	42.0±2.82	41.20±0.79
5	38.8±2.33	40.40±2.39
6	40.4±2.48	45.20±2.80
Overall Mean± SE	40.48^{a}±0.96	42.63^{b}±1.47

Means bearing different superscripts in a row differ significantly ^{ab} (p<0.05)

Plasma IgG1 (mg/dL)

The findings of blood IgG1 levels have been presented in Table 6. The mean values of IgG1 (mg/mL) were 8.89±0.69 and 11.08±0.73 in control and treatment group, respectively. There was significantly higher (p<0.05) IgG1 in treatment group compared to control. In the first, second and third month, there was significantly higher values of IgG1 (p<0.05) in treatment compared to control group.

Table 6: Blood plasma IgG-1 (mg/mL) in different groups fed with *Moringa oleifera* leaf meal replacing concentrate mixture as a protein source in Barbari goats

Month	**Control**	**Treatment**
Initial	7.93±2.11	10.3±2.11
1	9.55±2.03	12.3±1.93
2	8.89±2.09	12.4±1.94
3	8.99±1.23	12.3±2.17
4	8.51±2.49	10.6±2.41
5	8.45±1.90	9.46±1.71
6	9.91±2.01	10.2±2.03
Overall Mean ±SE	8.89^{a}±0.69	11.08^{b}±0.73

Means bearing different superscripts in a row differ significantly ^{ab} (p<0.05)

Effect of replacement of concentrate with *Moringa oleifera* leaf meal on blood mineral profile in Barbari goats

The findings of blood plasma mineral level of experimental goats have been presented in Table 7. The macro and micro mineral concentrations in blood plasma did not differ significantly (p>0.05). The overall Ca (%) was 10.64±0.68 and 10.77±0.21 in control and treatment group, respectively. Numerically higher value of Ca concentration was observed in treatment group compared to control group. The P concentration (%) was 5.63±0.25 and 5.73±0.25 in control and treatment group, respectively. The Cu concentration (ppm) were 0.94±0.01 and 0.96±0.01 in control and treatment group, respectively. Numerically higher Iron content (ppm) was observed in treatment group (1.31±0.05) than control (1.43±0.07). Zn concentrations (ppm) were 1.27±0.01 and 1.29±0.01 in control and treatment group, respectively. Mn concentration (ppm) were 0.93±0.03 and 0.94±0.06 in control and treatment group, respectively.

Table 7: Effect of replacement of concentrate with *Moringa oleifera* leaf meal on Blood minerals of Barbari goats

Minerals	Control	Treatment	P value
Ca (%)	10.64±0.28	10.77±0.21	0.50
P (%)	5.63±0.25	5.73±0.25	0.90
Cu (ppm)	0.94±0.01	0.96±0.01	0.20
Fe (ppm)	1.31±0.05	1.43±0.07	0.09
Zn (ppm)	1.27±0.01	1.29±0.01	0.89
Mn (ppm)	0.93±0.03	0.94±0.06	0.511

The values are Mean ± SE of observations on five animals in each

Discussion

The concentration of plasma glucose in goats was observed within the normal range (60.75-70.75 mg/dL) (Mohammed *et al*., 2016) [18]. The result is in agreement with Meel *et al*. (2018b) [16], Damor *et al*. (2017) [5] and (2017) who reported no significant difference in serum glucose values in goats fed with Moringa leaves by replacing concentrate mixture in the diet. However, Babekar and Abdalbagi (2015) [2] reported that goats fed 20% Moringa included showed a significant decrease in serum glucose level. Non-significant difference in total protein value between the groups under concentrate mixture and Moringa diet was also reported by Ali *et al*. (2017) [25] and Kumar *et al*. (2020) [26]. However, Meel *et al*. (2018b) [16], Babeker and Abdalbagi (2015) [2] and Damor *et al*. (2017) [6] reported significant changes among different goats fed with different levels of Moringa leaves replacing concentrate mixture. Observed albumin values are under the normal range (2.72-3.72 g/dL) given by (Mohammed *et al*., 2016) [18]. The values of albumin are in agreement with Ali *et al*. (2017) [25] who also reported non-significant difference between control and Moringa diet. However, Meel *et al*. (2018b) [16], Babeker and Abdalbagi (2015) [2] and Damor *et al*. (2017) [5] reported significantly higher values of blood albumin among different levels of Moringa leaves replacing concentrate mixture in goats. The value of globulin of goats are in agreement with Ali *et al*. (2017) who reported non-significant difference between control and Moringa diet. On contrary to this result, Meel *et al*. (2018b) [16], Babeker and Abdalbagi (2015) [2] and Damor *et al*. (2017) [5] reported significant increase in blood globulin values among different levels of Moringa leaves replacing concentrate mixture in goats. Increased blood urea concentration in treatment group, may be due to the improved immunoregulatory functions of Moringa diet. Higher level of dietary protein in the supplemented feed suggested the higher blood urea concentration which is positively correlated with serum antioxidant activities (Marciniak *et al*., 2005) [27]. The elimination of excess reactive oxygen molecules that may impair their immune functions helps in boosting of immunity which can be solved by the antioxidant action of urea. There is elevation of oxidative stress if blood urea concentration decreases which eventually lead to different diseases (Simoyi *et al*., 2002) [22]. Suckow *et al*. (2012) [23] reported that blood urea indicates the health status for normal kidney and liver functions, thus it shows that Moringa has no harmful effect on kidney and liver.

The values observed in the present study are within the normal range (10.92±0.84 mg/mL) of IgG1 level (mg/mL) in goats given by Micusan and Berduas (1977) [17]. High IgG1 level is the biomarker of immunity indicating that goats under treatment groups had greater immunity than control. IgG is one of the major immunoglobulins helping in complement activation, opsonization and neutralization of toxins (Mahajan and Mehta, 2010) [15]. Ojeka *et al*. (2016) [20] who reported increased serum IgG level in Wistar rats after administration of aqueous leaf extract of *M. oleifera* @ 80 mg/ kg BW.

Damor *et al*. (2017) [5] who reported significantly higher Ca level in Moringa supplemented group compared to control group.Dried Moringa leaves has a depository of minerals (Moyo *et al*., 2011) [19]. Al-kharusi *et al*. (2009) [1] reported that Moringa mineral profile plays important role in nutritional, medicinal and therapeutic values. Copper helps in stimulating body defence system, due to its active involvement in neutrophil production and affects phagocyte killing ability. It is essential for the development of antibody and lymphocyte replication (Moyo *et al*., 2011) [19]. For oxygen transport and cellular processes of growth and division, Iron is a necessary (Kozat, 2007) [14]. Hassan *et al*. (2011) [10] reported that Iron helps in normal functioning of the central nervous system and in the oxidation of carbohydrates, proteins and fats. Zn is an essential mineral element for the synthesis of DNA, RNA, insulin and function and structure of several enzymes (Brisibe *et al*., 2009) [4] along with anti-viral, antibacterial, anti-fungal and anti-cancer properties (Brisibe *et al*., 2009) [4].

Conclusions

Inclusion of *Moringa oleifera* leaf meal in the goat ration proved to be effective. Replacement of concentrate mixture @ 50% with *M. oleifera* leaf meal significantly increased haematological parameters. Blood plasma IgG1 level also increased significantly in the Moringa fed goats indicating a better immunity profile. *Moringa oleifera* leaf meal may be effectively used as a good protein substitute replacing up to 50% of the concentrate mixture without affecting the health status of goats.

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