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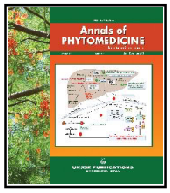

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Effect of dietary substitution of dried *Moringa oleifera* Lam. leaves on blood-serum proteins of Badri cattle

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Abstract

The present study was carried out on 45 Badri cattle of the age groups 6-12 months, >12-18 months and lactating animals. All the animals of different age-groups were divided into control (T_0), treatment 1 (T_1) and treatment 2 (T_2) groups where the treatment groups 1 and 2 were fed with concentrate and dried Moringa leaves @10 % and 20% substitutions, respectively, for a period of 12 months. The results showed that total serum protein and serum globulin were significant in all the age-groups of experimental animals, while significantly increased albumin/globulin ratio was observed in lactating animals from 0.94 ± 0.01 in T_0 group to 0.89 ± 0.01 in T_2 group, respectively. The study revealed that substitution of dried Moringa leaves in concentrate feed of Badri cattle improved haematological protein quality of the experimental animals, and hence, can be substituted by the farmers in field conditions.

1. Introduction

Livestock holds a crucial role in the Indian agricultural economy, serving as a means of livelihood and fostering economic independence for a significant portion of the population. The Badri cattle breed is native to Uttarakhand, a geographically diverse state with a rich biodiversity. These cattle are short-statured and weigh between 200 and 250 kg, with active and vigorous calves (Patoo *et al*., 2016). Badri cattle are found in three primary coat colours: grey, red, and black, featuring a small udder resulting in a modest daily milk yield of 1-1.5 kg (NBAGR, 2023; Kumar and Gaur, 2016). Adapted to hilly terrains, Badri cattle navigate uneven landscapes effortlessly with their small, straight legs and hard foot-pads. The pointed hooves aid in digging mountainous soil, facilitating the ascent of steep slopes. The *Moringa oleifera* Lam. tree, also known as the Miracle tree, Drumstick tree and Horseradish tree, thrives in the Tarai region of Uttarakhand, providing valuable benefits to the state's people and livestock (Joshi *et al.*, 2022). Every part of the Moringa tree, including leaves, bark, flowers, fruit, seeds, and roots, is utilized for medicinal purposes or consumed directly, offering various health benefits (Gandhi, 2018).

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Diabetes mellitus' complications like peripheral vascular disease, retinopathy, nephropathy, neuropathy and coronary heart disease has been a major problem in the livestock population of the country due to which a marked decrease in the levels of antioxidants in livestock have been reported causing conditions like cardiomyopathy (Nasri *et al*., 2015). Increase in blood cholesterol in livestock leads to increase in the risk of cardiovascular diseases after the cholesterol binds to low-density lipoproteins. This is where Moringa plant comes to the rescue as consumption of Moringa products have been associated with reduced blood sugar levels, lowered blood cholesterol and impressive antioxidant effects for both humans and large ruminants (Ali, 2017). According to Sarwatt *et al*. (2004), consuming Moringa foliage improved the milk yield and milk protein levels in cattle due to a positive effect on the rumen environment, leading to increased rumen microbial output and that the protein in Moringa had good rumen bypass characteristics. The past researches involving supplemen-tation or substitution of Moringa's green leaves as fodder or powder of its various parts in the concentrate feed of livestock are ample but those involving the use of dried and crushed Moringa leaves in the livestock feed are scarce. Therefore, the present study was conducted to assess the effect of dried *M. oleifera* leaves on blood-serum protein parameters of various age groups of Badri cattle.

2. Materials and Methods

Present study was conducted at Badri cattle unit of Instructional Dairy Farm, Nagla, College of Veterinary and Animal Sciences, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand. The place is located in foot hills of Himalayas at 29.5°N

latitudes and 79.30°E longitude at an altitude of 243.84 meters above mean sea level. Maximum temperature reaches up to 44°C in summers and minimum temperature up to 1°C in winters. The Badri cattle unit of dairy farm exclusively houses indigenous Badri breed of cattle of various age groups.

2.1 Selection of animals

The experimental trial was conducted on 45 animals already present at Badri cattle unit of Instructional Dairy Farm, Nagla, GBPUAT, Pantnagar. The animals selected were of 6-12 months, >12-18 months and lactation age-groups. The animals of the required age-groups were selected from the available Badri cattle herd and maintained in separate sheds to conduct the study. The selected experimental animals were dewormed as per the standard schedule at least 10 days before the beginning of the experimental feeding trial. The trial was conducted for a period of 12 months (from 2 April, 2021 to 1 April, 2022) where the animals were fed on green fodder, dry fodder and concentrate feed (both sole and mixed with dried *M. oleifera* leaves) as per NRC (2001) requirements which predicts that dietary crude protein contents between 16.5-17.5% of the dry matter supply the protein requirements of dairy cows in most conditions.

2.2 Grouping of experimental animals

45 animals were selected from the animals available at the Badri cattle unit of the dairy farm and then they were divided into the 3 broad groups of Control, Treatment 1 and Treatment 2 and the sub-groups of different ages keeping in mind the uniformity of average body weights of experimental animals in each group in the following manner (Table 1):

Table 1: Grouping of experimental animals

Age-groups	T_0	T_1	T_2
6-12 months	5	5	5
>12–18 months	5	5	5
Lactation	5	5	5
Total	**15**	**15**	**15**

All the 15 animals of different age-groups in the control (T_0) group were fed with sole concentrate feed as per the schedule of the dairy farm. The 15 animals of treatment 1 (T_1) group were fed with concentrate feed substituted with 10% of dried *M. oleifera* leaves and the remaining 15 animals of treatment 2 (T_2) group were fed with concentrate feed substituted with 20% of dried *M. oleifera* leaves.

2.3 Feed schedule

The exclusive concentrate feed and the substitute concentrate feed incorporated by *M. oleifera* leaves were fed twice daily (after milking time, *i.e.*, at 4 am and 4 pm) to the control and treatment animals, respectively, based on each experimental animal's body weight. Plastic tubs were used for individual feeding of all the experimental animals.

2.4 Procurement of Moringa leaves and concentrate feed and feed schedule

M. oleifera leaves were gathered from the vicinity of G.B. Pant University of Agriculture and Technology, Pantnagar, where there is an abundance of Moringa trees. These leaves were then spread out and sun-dried. The concentrate feed needed for doing the experimental *M. oleifera* substitution was obtained from the feed unit of the Instructional Dairy Farm (IDF) at GBPUAT, Pantnagar. Both green and dry fodder for all experimental animals was supplied *ad libitum* from the fodder unit of the Instructional Dairy Farm, GBPUAT, Pantnagar, following the farm's daily schedule.

2.5 Collection of blood sample and storage

The blood collection site was prepared aseptically by clipping the hair and using sterile gauze pieces and spirit. The blood sample (4 ml) was collected from jugular vein aseptically using disposable syringe with 18-gauge hypodermic needle early in morning at 8 am. The blood was transferred immediately to 4 ml clot activator vacutainer tube. After that, the blood samples were immediately transported to laboratory in a box containing ice packs. 45 blood samples from Badri cattles of 6-12 months, >12-18 months and lactating age-groups were collected each time in the beginning and after 3rd month, 6th month, 9th month and 12th month of the experimental trial for serum biochemical study. These blood samples were then stored at 4°C to preserve their integrity and characteristics till the separation of serum was done from them.

2.6 Separation of serum and analysis

The blood samples collected in 4 ml clot activator vacutainers and stored at 4°C were taken out of the refrigerator and these vacutainers were centrifuged at 3000 rpm for 10 min for clot retraction after refrigeration. The clot was separated by using spinal needle. The serum was collected in a 2 ml Eppendorf tube using a capillary pipette and stored at –20°C for biochemical studies. Serum analytes, *i.e.*, total serum proteins (by Biuret method), serum albumin (by BCG Dye method), serum globulin (by subtraction) and albumin: globulin ratio were analysed by UV-VIS spectrophotometer using diagnostic kits.

2.7 Statistical analysis

The experimental data obtained in the present study was analysed statistically applying one-way ANOVA by using SPSS software version 21 (Snedecor and Cochran, 1994). The significant mean difference was separated by Tukey post hoc analysis with significance level defined at $p< 0.05$.

3. Results

3.1 Total serum protein

The average values of total serum protein of each group of Badri cattle measured after every 3 months from the beginning till the end of experimental period is presented in Table 2. The average values of total serum protein concentration of T_2 sub-group in 6-12 months group were higher and statistically significant ($p<0.05$) than T_0 sub-group from the 3rd month till the end of the experimental trial. The overall observation of T_2 sub-group (7.51 ± 0.06 g/dl) was higher and statistically significant ($p<0.05$) than T_0 (7.27 ± 0.02 g/dl) and T_1 (7.42 ± 0.02 g/dl) sub-groups, respectively. The average values of total serum protein concentration of T_2 sub-group in >12-18 months group were higher and statistically significant ($p<0.05$) than T_0 and T_1 sub-groups from the beginning till the end of the experimental trial. The overall observation of T_2 sub-group (7.46 ± 0.02 g/dl) was higher and statistically significant ($p<0.05$) than T_0 (7.27 ± 0.01 g/dl) and T_1 (7.34 ± 0.02 g/dl) sub-groups, respectively.

Table 2: Average total serum protein (g/dl) of different groups of Badri cattle during the experimental period

Groups	Beginning	3 months	6 months	9 months	12 months	Overall
6-12 M						
T_0	7.26 ± 0.02	7.26 ± 0.02^{a}	7.27 ± 0.02^{a}	7.28 ± 0.02^{a}	7.29 ± 0.02^{a}	7.27 ± 0.02^{a}
T_1	7.36 ± 0.02	7.40 ± 0.02^{ab}	7.43 ± 0.02^{ab}	7.44 ± 0.02^{ab}	7.45 ± 0.03^{ab}	7.42 ± 0.02^{ab}
T_2	7.41 ± 0.07	7.49 ± 0.06^{b}	7.54 ± 0.07^{b}	7.55 ± 0.07^{b}	7.57 ± 0.08^{b}	7.51 ± 0.06^{b}
>12-18 M						
T_0	7.26 ± 0.01^{a}	7.27 ± 0.01^{a}	7.27 ± 0.01^{a}	7.27 ± 0.01^{a}	7.27 ± 0.01^{a}	7.27 ± 0.01^{a}
T_1	7.29 ± 0.01^{a}	7.32 ± 0.02^{a}	7.35 ± 0.02^{a}	7.37 ± 0.02^{b}	7.38 ± 0.03^{b}	7.34 ± 0.02^{a}
T_2	7.39 ± 0.02^{b}	7.43 ± 0.03^{b}	7.47 ± 0.03^{b}	7.49 ± 0.02^{c}	7.50 ± 0.03^{c}	7.46 ± 0.02^{b}
Lactation						
T_0	7.35 ± 0.04	7.36 ± 0.04^{a}	7.36 ± 0.04^{a}	7.36 ± 0.04^{a}	7.37 ± 0.04^{a}	7.36 ± 0.04^{a}
T_1	7.45 ± 0.01	7.47 ± 0.01^{b}	7.50 ± 0.01^{b}	7.52 ± 0.01^{b}	7.53 ± 0.01^{b}	7.49 ± 0.01^{b}
T_2	7.45 ± 0.02	7.50 ± 0.01^{b}	7.52 ± 0.00^{b}	7.54 ± 0.01^{b}	7.55 ± 0.01^{b}	7.51 ± 0.01^{b}

Values bearing different superscripts in the same column differ significantly ($p<0.05$).

The average values of total serum protein concentration of T_1 and T_2 sub-groups in lactating animals' group were higher and statistically significant ($p<0.05$) than T_0 sub-group from the 3rd month till the end of the experimental trial. The overall observation of T_1 (7.49 ± 0.01 g/dl) and T_2 (7.51 ± 0.02 g/dl) sub-groups were higher and statistically significant ($p<0.05$) than T_0 (7.36 ± 0.04 g/dl) sub-group, respectively.

3.2 Serum albumin

The average values of serum albumin of each group of Badri cattle measured after every 3 months from the beginning till the end of experimental period have been presented in Table 3. The average values of serum albumin concentration of T_2 sub-group in 6-12 months group were higher and statistically significant ($p<0.05$) than T_0 sub-group from the 6th month till the end of the experimental trial. The overall observation of T_2 sub-group (3.44 ± 0.01 g/dl) was higher but non-significant ($p>0.05$) than T_0 (3.35 ± 0.02 g/dl) and T_1 (3.39 ± 0.02 g/dl) sub-groups, respectively.

The average values of serum albumin concentration of T_2 sub-group in >12-18 months group were higher and statistically significant ($p<0.05$) than T_0 sub-group from the 9th month till the end of the experimental trial. The overall observation of T_2 sub-group (3.51 ± 0.01 g/dl) was higher but non-significant ($p>0.05$) than T_0 (3.48 ± 0.02 g/dl) and T_1 (3.46 ± 0.01 g/dl) sub-groups, respectively.

Table 3: Average serum albumin (g/dl) of different groups of Badri cattle during the experimental period

Groups	Beginning	3 months	6 months	9 months	12 months	Overall
6-12 M						
T_0	3.36 ± 0.03	3.35 ± 0.03	3.35 ± 0.03^{a}	3.35 ± 0.02^{a}	3.35 ± 0.03^{a}	3.35 ± 0.02
T_1	3.36 ± 0.03	3.38 ± 0.02	3.40 ± 0.03^{ab}	3.41 ± 0.02^{ab}	3.41 ± 0.02^{ab}	3.39 ± 0.02
T_2	3.39 ± 0.01	3.43 ± 0.01	3.45 ± 0.01^{b}	3.46 ± 0.01^{b}	3.48 ± 0.01^{b}	3.44 ± 0.01
>12-18 M						
T_0	3.48 ± 0.02	3.47 ± 0.02	3.48 ± 0.02	3.48 ± 0.02^{a}	3.48 ± 0.02^{a}	3.48 ± 0.02
T_1	3.44 ± 0.01	3.46 ± 0.01	3.49 ± 0.01	3.51 ± 0.01^{ab}	3.52 ± 0.01^{ab}	3.46 ± 0.01
T_2	3.46 ± 0.01	3.49 ± 0.01	3.52 ± 0.01	3.54 ± 0.01^{b}	3.55 ± 0.01^{b}	3.51 ± 0.01
Lactation						
T_0	3.48 ± 0.01	3.47 ± 0.01	3.47 ± 0.01^{a}	3.47 ± 0.01^{a}	3.47 ± 0.01^{a}	3.47 ± 0.01
T_1	3.47 ± 0.02	3.49 ± 0.02	3.51 ± 0.01^{ab}	3.51 ± 0.01^{ab}	3.52 ± 0.01^{ab}	3.50 ± 0.02
T_2	3.48 ± 0.02	3.51 ± 0.02	3.53 ± 0.01^{b}	3.55 ± 0.01^{b}	3.55 ± 0.01^{b}	3.52 ± 0.01

Values bearing different superscripts in the same column differ significantly ($p<0.05$).

The average values of serum albumin concentration of T_2 sub-group in 6-12 months group were higher and statistically significant ($p<0.05$) than T_0 sub-group from the 6th month till the end of the experimental trial. The overall observation of T_2 sub-group (3.52 ± 0.01 g/dl) was higher but non-significant ($p>0.05$) than T_0 (3.47 ± 0.01 g/dl) and T_1 (3.50 ± 0.02 g/dl) sub-groups, respectively.

3.3 Serum globulin

The average values of serum globulin of each group of Badri cattle measured after every 3 months from the beginning till the end of experimental period have been presented in Table 4. The average values of serum globulin concentration of T_2 sub-group in 6-12 months group were higher and statistically significant ($p<0.05$) than T_0 and T_1 sub-groups from the 6th month till the end of the experimental trial. The overall observation of T_2 sub-group (3.88 ± 0.03 g/dl) was higher and statistically significant ($p<0.05$) than T_0 (3.55 ± 0.04 g/dl) and T_1 (3.72 ± 0.03 g/dl) sub-groups, respectively. The average values of serum globulin concentration of T_2 sub-group in >12-18 months group were higher and statistically significant ($p<0.05$) than T_0 and T_1 sub-groups from the 6th month till the end of the experimental trial. The overall observation of T_2 sub-group (3.88 ± 0.03 g/dl) was higher and statistically significant ($p<0.05$) than T_0 (3.65 ± 0.01 g/dl) and T_1 (3.73 ± 0.02 g/dl) sub-groups, respectively.

Table 4: Average serum globulin (g/dl) of different groups of Badri cattle during the experimental period

Groups	Beginning	3 months	6 months	9 months	12 months	Overall
6-12 M						
T_0	3.53 ± 0.05^a	3.58 ± 0.05^a	3.54 ± 0.05^a	3.55 ± 0.05^a	3.56 ± 0.05^a	3.55 ± 0.04^a
T_1	3.64 ± 0.04^{ab}	3.67 ± 0.04^{ab}	3.74 ± 0.03^b	3.77 ± 0.03^b	3.78 ± 0.03^b	3.72 ± 0.03^b
T_2	3.77 ± 0.04^b	3.84 ± 0.03^b	3.90 ± 0.03^c	3.93 ± 0.04^c	3.95 ± 0.04^c	3.88 ± 0.03^c
>12-18 M						
T_0	3.65 ± 0.01	3.65 ± 0.00^a	3.65 ± 0.01^a	3.65 ± 0.01^a	3.65 ± 0.01^a	3.65 ± 0.01^a
T_1	3.67 ± 0.01	3.70 ± 0.02^a	3.74 ± 0.03^b	3.76 ± 0.03^b	3.77 ± 0.02^b	3.73 ± 0.02^b
T_2	3.74 ± 0.04	3.85 ± 0.01^b	3.92 ± 0.02^c	3.94 ± 0.02^c	3.96 ± 0.02^c	3.88 ± 0.02^c
Lactation						
T_0	3.65 ± 0.01^a	3.65 ± 0.01^a	3.66 ± 0.02^a	3.66 ± 0.02^a	3.66 ± 0.02^a	3.66 ± 0.02^a
T_1	3.69 ± 0.01^a	3.77 ± 0.02^{ab}	3.83 ± 0.03^b	3.85 ± 0.03^b	3.87 ± 0.04^b	3.80 ± 0.02^b
T_2	3.80 ± 0.04^b	3.88 ± 0.04^b	3.96 ± 0.06^b	3.99 ± 0.06^b	4.01 ± 0.07^b	3.93 ± 0.04^c

Values bearing different superscripts in the same column differ significantly ($p<0.05$).

The average values of serum globulin concentration of both T_1 and T_2 sub-groups in lactating animals' group were higher and statistically significant ($p<0.05$) than T_0 sub-group from the 6th month till the end of the experimental trial. The overall observation of T_2 sub-group (3.93 ± 0.04 g/dl) was higher and statistically significant ($p<0.05$) than T_0 (3.66 ± 0.02 g/dl) and T_1 (3.80 ± 0.02 g/dl) sub-groups, respectively. The overall observation of T_1 sub-group was also higher and statistically significant ($p<0.05$) than T_0.

Table 5: Average A/G ratio of different groups of Badri cattle during the experimental period

Groups	Beginning	3 months	6 months	9 months	12 months	Overall
6-12 M						
T_0	0.93 ± 0.03	0.92 ± 0.02	0.92 ± 0.02	0.92 ± 0.02	0.92 ± 0.02	0.92 ± 0.02
T_1	0.94 ± 0.01	0.94 ± 0.01	0.92 ± 0.01	0.91 ± 0.01	0.91 ± 0.01	0.92 ± 0.01
T_2	0.91 ± 0.02	0.90 ± 0.01	0.89 ± 0.01	0.88 ± 0.02	0.89 ± 0.01	0.89 ± 0.01
>12-18 M						
T_0	0.95 ± 0.01	0.95 ± 0.01^a	0.95 ± 0.01^a	0.95 ± 0.01^a	0.95 ± 0.01^a	0.95 ± 0.01
T_1	0.93 ± 0.01	0.94 ± 0.01^{ab}	0.94 ± 0.00^b	0.94 ± 0.01^b	0.94 ± 0.01^b	0.94 ± 0.01
T_2	0.94 ± 0.01	0.91 ± 0.01^b	0.90 ± 0.01^b	0.90 ± 0.01^b	0.90 ± 0.01^b	0.92 ± 0.01
Lactation						
T_0	0.95 ± 0.01	0.94 ± 0.01^a	0.94 ± 0.01^a	0.94 ± 0.01^a	0.94 ± 0.01^a	0.94 ± 0.01^a
T_1	0.94 ± 0.00	0.92 ± 0.01^{ab}	0.91 ± 0.01^{ab}	0.91 ± 0.01^{ab}	0.90 ± 0.01^a	0.92 ± 0.01^{ab}
T_2	0.91 ± 0.01	0.90 ± 0.01^b	0.89 ± 0.01^b	0.88 ± 0.02^b	0.87 ± 0.01^b	0.89 ± 0.01^b

Values bearing different superscripts in the same column differ significantly ($p<0.05$).

3.4 Albumin/Globulin (A/G) ratio

The average values of A/G ratio of each group of Badri cattle measured at every 3 months from the beginning till the end of experimental period have been presented in Table 5. The average values of A/G ratio of T_2 sub-group in 6-12 months group were lower and non-significant ($p<0.05$) than T_0 and T_1 sub-groups from the beginning

till the end of the experimental trial. The overall observation of T_2 sub-group (0.89 ± 0.01) was lower and non-significant (p>0.05) than T_0 (0.92 ± 0.02) and T_1 (0.92 ± 0.01) sub-groups, respectively. The average values of A/G ratio of T_2 sub-group in >12-18 months group were higher and statistically significant (p<0.05) than T_0 sub-group from the 6th month till the end of the experimental trial. However, the overall observation of T_2 sub-group (0.92 ± 0.01) was lower and non-significant (p>0.05) than T_0 (0.95 ± 0.01) and T_1 (0.94 ± 0.01) sub-groups, respectively.

The average values of A/G ratio of T_2 sub-group in lactating animals' group were higher and statistically significant (p<0.05) than T_0 sub-group from the 3rd month till the end of the experimental trial. The overall observation of T_2 sub-group (0.89 ± 0.01) was lower and statistically significant (p<0.05) than T_0 (0.94 ± 0.01) and T_1 (0.92 ± 0.01) sub-groups, respectively.

4. Discussion

The serum biochemical parameters provide useful information for the evaluation of health status of animals and reflect many metabolic alterations of organs and tissues. The experimental feeding period of 12 months in this research showed an overall increase in total serum protein which was in the normal range as that of Zebu cattle according to the findings of Kalyani *et al*. (2018). These also agreed with the findings of Muna *et al*. (2009) and Chandrashekhar *et al*. (2017) who found increased total serum protein level in local non-descript cattle and Deoni cattle, respectively. The increase in total serum protein in the treatment animals of the experiment as compared to the control group can be attributed to the different bioactive components (flavonoids, anthraquinones, *etc*.) in dried Moringa leaves which play a role in improved nutrient utilization in treatment animals. No overall significant effect was observed in the concentration of serum albumin, which may be due to negligible physiological conversion of protein from Moringa leaves'-mixed feed to serum albumin due to lower doses of Moringa substitution. The serum albumin concentration results of the experiment were not in line with the results of Cozzi *et al*. (2011) and Chandrashekhar *et al*. (2017) who observed between significant rise in albumin concentration in the serum of Holstein Friesian and Deoni cattle, respectively, which may be explained by the difference in the protein content of the Moringa leaves available for feeding due to climatic differences of their regions of growth or their growing-stage. Overall significant increase in serum globulin in the treatment animals of all the age-groups was in line with the results of Giri *et al*. (2017) who observed an overall rise in serum-globulin concentration in the dairy cows at high altitudes. The increased serum globulin in treatment animals may be attributed to higher nitrogen retention by the treatment animals fed the Moringa leaves'-mixed concentrate feed. The increase in serum globulin agreed to the observations of Meel *et al*. (2018) too. The A/G ratio showed a significant rise only in the treatment animals of the lactating group which was in agreement with the findings of Rasooli *et al*. (2004) and Chandrashekhar *et al*. (2017) who observed increase in A/G ratio in the local Iranian cattle and Deoni cattle, respectively. This can be attributed to numerical rise in the serum albumin concentration in lactating animals which was non-significant in itself when solely compared with observations of different groups. No negative effects like hyperproteinemia in blood of experimental animals were observed owing to proper dose of protein-rich Moringa leaves and the experimental feed fed to individual animals according to their daily dry matter intake (DMI) requirements.

5. Conclusion

Dried *M. oleifera* leaves mixed in the concentrate feed and fed to Badri cattle improved the blood-serum proteins' level. Thus, it can be concluded that dried *M. oleifera* leaves can be substituted at the rates of 10% and 20% in the concentrate feed of Badri cattle by the farmers in their households and field conditions for improving the physiological health of their animals.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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