



Effect of feeding *Moringa oleifera* based complete pellet feed on milk yield, composition, fatty acids, somatic cell count and cell mediated immunity in lactating goats

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ABSTRACT

Lactation cum feeding trial was conducted on 12 female Barbari goats (Age approx. 2-3 years and mean body weight 34.72 ±1.28 kg) for 120 days after approximately one month of kidding and effect on milk production, milk constituents, somatic cell count, milk fatty acid profile and cell mediated immunity was studied. Animals were divided into two groups (Gr G and Gr M) of six each as per completely randomized design. Two types of complete pellet (*Moringa oleifera* and *Cicer arietinum*) based was formulated containing sundried Moringa biomass and Gram straw along with conventional feed ingredients. Lactating goats of Gr G was fed with gram straw based complete pellet feed while goats of Gr M was fed with Moringa based complete pellet feed. Both the complete pellet feeds were iso nitrogenous. No significant difference in body weight changes was recorded between groups. The milk production (g/day) was higher (Avg. 14.54%) in Gr M as compared to Gr G at different fortnight. No significant difference was reported in the milk composition. Milk fat, SNF, Protein and lactose was similar in both the groups. Milk Somatic cell count ($\times 10^3/\text{ml}$) was significantly ($P < 0.05$) lower in lactating goats fed with Moringa based feed. Cell-mediated immune response assessed through delayed-type hypersensitivity (DTH) reaction against phyto haemagglutinin (PHA-P) was more pronounced in Gr M at 72 and 96 hrs. Higher concentration of unsaturated fatty acids (about 16.19%) and lower saturated fatty acids (about 5.49%) was reported in Gr M. Milk fatty acid profile indicators like Index of atherogenicity (IA) was lower while hypo/hyper cholesteremic ratio and health promoting index was higher in milk of moringa based pellet fed goats. Present study concluded that Barbari goats fed with Moringa based complete pellet feed produced higher milk yield with healthier fatty acid profile with low somatic cell counts.

Keywords: Cell mediated immunity, Fatty acids, Lactating goats, Milk production, Somatic cell counts

Goats are predominantly being reared on community pastures under grazing system with little supplementation during productive phase of life. But due to shrinkage of grazing land and pressure of urbanization the system of goat rearing is changing to intensive system. Intensive system of goat production recommended upon limited or lack of grazing resources and suitable under urban locations where higher returns from goat products expected. The goats should receive a meal that satisfies the need in terms of quality and quantity. Complete pellet feed is a technique provided synchronized nutrient availability in the rumen of ruminant animals with higher nutrient use efficiency for production functions (Kumar 2022). *M. oleifera* as animal feed has attracted interest owing to its rich nutrients like

high protein (23-30% in leaf) (Su and Chen 2020), a high biomass production ranging from 43 to 115 t/ha annually (Kholif *et al.* 2016) and low anti nutrient content along with many plant secondary metabolites having potential health benefits (Makkar and Becker 1997). This makes it an ideal feed resource as well as a good and cheap protein source for goats. India is the largest producer of Moringa and a yield up to 650 metric tonnes of green leaves per hectare can be achieved using optimum conditions for the cultivation (Rajangam *et al.* 2001). Moringa has been evaluated *in vitro* (Chaudhary *et al.* 2006) as well as *in vivo* (Jadhav *et al.* 2018) in the animal ration mainly as a substitutes for traditional protein source or as a supplements. We have evaluated Moringa as green biomass (Kumar *et al.* 2022 a, b) mainly in the growing goats. In lactating goats Kholif *et al.* (2016) reported that feeding *M. oleifera* diets resulted in higher ($P < 0.05$) milk yield, energy-corrected milk and milk contents of protein and lactose than for the control diet. *M. oleifera* feeding also increased ($P < 0.05$) total unsaturated fatty acids and total conjugated linoleic

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acid of milk and decreased ($P < 0.05$) saturated fatty acids. *M. oleifera* leaf meal supplementation improved growth performance, as well as milk output and the quality in cows and goats (Babiker *et al.* 2017 and Sanchez *et al.* 2006). Limited studies have been conducted on utilization of dried Moringa biomass based complete pellet feed in lactating goats. So present experiment was conducted to study the effect of moringa-based pellet feeding on the milk yield, composition, fatty acids, somatic cell count and cell mediated immunity in lactating goats.

MATERIALS AND METHODS

Cultivation and preparation of Moringa based complete pellet feed: Moringa (PKM-2 variety) were grown at Agriculture farm of ICAR-CIRG, Makhdoom. The green biomass containing leaves and small branches of Moringa plant were harvested from the field and cut into small pieces of 2-3 cm with the help of chaff cutter. This chaffed biomass was dried in the sunlight. The dried biomass was mixed with other feed ingredients of complete feed and pellet was prepared with the help of pellet making machine. Similarly, Gram straw based pellet was prepared by pelletizing gram straw and other feed ingredients with the help of pellet making machine. The size of this pellet was 8 mm in diameter with 2-3 cm length.

Animal management and rations: Lactation-cum-feeding trial was conducted on 12 female Barbari goats (Age approx. 2-3 years and mean body weight 34.72 ± 1.28 kg) for 120 days after approximately 1 month of kidding and effect on milk production, milk constituents, somatic cell count and milk fatty acid profile was studied. Animals were divided into two groups (Gr G and Gr M) of six each as per completely randomized design. Lactating goats of Gr G were fed with gram straw based complete pellet feed, while goats of Gr M were fed with Moringa based complete pellet feed. Both the complete pellet feed were iso-nitrogenous.

Experimental procedure: Weighed half of quantities of respective pellet were offered to both the group of goats at 08:00 AM and 2.00 PM daily. *Ad lib.* water was provided and was changed twice daily throughout the experimental period. Pellet were offered and residues were sampled fortnightly for subsequent analysis of DM to determine DM intake. Fortnightly body weight of the goats and milk yield of each of the animals was recorded. Milk samples were collected at 30, 60 and 90 days of experimental feeding from all the animals of both the groups by individual milking. For milk constituents and somatic cell counts, milk samples collected from each animal during morning and evening were pooled individually and the representative milk samples of individual animal were analysed using automatic milk scanner.

Milk fatty acids analysis: Milk of individual goats from the both groups at 60 days of experimental feeding was used for fatty acid study. Fatty acid methyl esters (FAME) of milk samples were prepared as per O'Fallon *et al.* (2007). One ml milk sample was taken and placed in 50 ml centrifuge tubes to which 0.7 ml of 10N KOH in

water and 5.3 ml of methanol (Sigma Aldrich) were added. The tubes were incubated in a 55°C water bath for 90 min with vigorous shaking for 5 s every 20 min. After cooling to room temperature in tap water, 0.58 ml of 24N H₂SO₄ was added. The tubes were again incubated at 55°C for 90 min in a water bath with shaking for 5 s every 20 min. The tubes were cooled in tap water. Three ml of hexane (Sigma Aldrich) was added and the tubes were vortexed for 5 min on a multi-tube vortex. The tubes were centrifuged for 10 min at 2000 g and the hexane layer was taken out and placed into Eppendorf tubes stored at -20°C till analysis in the GC-MS/MS analysis. Fatty acids were identified by comparing their retention time with the fatty acid methyl standards (Supelco 37) and were expressed as a percentage of total fatty acids.

Cell-mediated immune response: The cell-mediated immune response was assessed through *in vivo* delayed-type hypersensitivity (DTH) reaction against phytohaemagglutinin-P (PHA-P). An area of about 1 cm² was encircled with a marker pen, on both sides of the neck region. The thickness of the skin was measured with the help of Vernier callipers, for basal (0 h) value. All the animals were then injected intra-dermally with 125 µl of either PBS as a negative control on one side and PHA-P (20 µg/125 µl PHA-P solution) on the other side of neck region. The thickness of the skin was subsequently measured at 12, 24, 48, 72 and 96 h.

Feed analysis: Feed offered to does during experimentation was analysed for their proximate composition as per AOAC (2012). Dry matter of feed sample was estimated by drying in hot air oven at $100 \pm 1^\circ\text{C}$ until constant dry weight was achieved. Organic matter (OM) was determined by decarbonization and then ashing at 550°C for 5 h in muffle furnace. Crude protein (CP) content ($N \times 6.25$) was measured as per the method described by AOAC (2012), method 984.13 (A-D). Ether extract (EE) was estimated using method 920.39 (A). Neutral detergent fibre (NDF) and Acid detergent fibre (ADF) were discerned by the methods of Van Soest *et al.* (1991) and expressed inclusive of residual ash. Cellulose was calculated as: ADF-Acid detergent lignin (ADL), while hemicellulose was calculated as: NDF-ADF.

Statistical analysis: The data recorded during the experimental trial were analyzed by independent sample t-test as described by Snedecor and Cochran (1989). All the data was analysed by SPSS software.

RESULTS AND DISCUSSION

Chemical composition of feed: The dry matter (g/kg) of Moringa and gram straw pellet was 912.2 and 955.8, respectively. Crude protein (g/kg) was 150.6 for moringa and 150.1 for gram straw pellet, both being iso-nitrogenous. Fibre fractions were nearly similar (Table 1).

Body weight changes and milk attributes: Fortnightly body weight changes were recorded and no significant difference was found. Mean body weight (kg) at the start of experimental feeding was 35.50 for Gr G and 33.94

Table 1. Composition of feeds (on % DM basis)

Attribute	Moringa pellet	Gram straw pellet
<i>Ingredients (%)</i>		
Sun dried moringa biomass	70.0	
Gram straw		60.0
Barley	23.8	21.8
Linseed (<i>Linum usitatissimum</i>) cake		12.0
Cotton seed (<i>Gossypium</i>) cake	5.0	5.0
Mineral mixture	0.8	0.8
Salt	0.4	0.4
<i>Chemical composition (% of feed)</i>		
Dry matter	91.22	95.58
Crude protein	15.06	15.01
Ether extract	6.94	4.37
Total ash	8.35	12.76
Organic matter	91.65	87.24
Neutral detergent fibre	43.29	46.31
Acid detergent fibre	30.68	35.22
Hemi cellulose	12.61	11.09

for Gr M which changed to 32.06 and 33.14 at the end of experimental feeding, respectively. Moringa biomass is a good source of protein with moderate fibre fractions along with many bioactive compounds. Both the complete pellet feed were iso-nitrogenous to meet the nutrient requirements of the lactating Barbari medium sized goats. The mean daily dry matter intake (kg) was significantly ($P<0.05$) higher in Gr M (1.81) as compared to Gr G (1.38) during the experimental feeding trial. The higher intake of Moringa pellet indicates its higher palatability and acceptability by goats, however, many factors like nutrient composition in the diet, fibre digestion and digesta flow rate can affect the dry matter intake (Wankhede *et al.* 2022). Kholif *et al.* (2015) also observed improved palatability of Moringa relative to sesame meal in the diets of lactating goats.

A trend of decrease in milk production was recorded with the advancement of lactation. The milk production (g/day) was higher (Avg. 14.54%) in Gr M as compared to Gr G (Table 2) at different fortnight. On an average, around 15% improvement in milk was reported in Gr M as

Table 2. Milk production (g/d) of lactating goats in different groups

Collection day	Gr G	Gr M	P-value
0	1000±98.4	1006±99.6	0.87
15	1061±199	1182±158	0.58
30	951±209	1044±131	0.72
45	808±135	908±75	0.54
60	591±149	812±51.6	0.20
75	660±94	820±63	0.19
90	570±67	592±86.4	0.84
105	393±72.5	411±54.3	0.85
120	356±75.3	401±28.4	0.40

Gr G, goats fed with gram straw based pellet; Gr M, goats fed with Moringa based pellet.

compared to Gr G. Previous studies in lactating goats on feeding different forms of Moringa also reported increased milk yield (10% to 15%) with improved milk composition (Kholif *et al.* 2015, 2016). They attributed increased milk production to the higher feed intake and digestibility, undegraded feed protein, and improved rumen environment and fermentation. Higher energy and ruminal propionate levels of MO diets may be another probable reason for the higher milk production. Propionate plays a vital role as a precursor in gluconeogenesis and lactogenesis, and thus increases milk yield (Vanhatalo *et al.* 2003). Milk composition in terms of milk fat, SNF, protein and lactose were similar in both the groups (Table 3). However, Kholif *et al.* (2018) reported increased milk fat % and milk fat yield by 20% and 11% by replacement of berseem clover with Moringa in lactating goats.

Somatic cell count: Milk was collected and studied for SCC at 30, 60, 90 days of experimental feeding. Somatic cell count ($\times 10^3/\text{ml}$) was statistically ($P<0.05$) lower in Gr M as compared to Gr G (Supplementary Table 1). This indicated that incorporation of Moringa due to its antioxidants and health promoting effect improved the udder health of animals. Somatic cells are indicators of both resistance and susceptibility of animals to mastitis. It is a useful predictor of intra-mammary infection (IMI), and therefore, an important component of milk in assessment of aspects of quality, hygiene and mastitis control. Similarly, Liu *et al.* (2023) reported that diet supplemented with 50 mg/BW Moringa leaf flavonoids significantly reduced the number of somatic cells, improved antioxidants

Table 3. Milk constituents of lactating goats in different groups

Attribute (%)	Gr G	Gr M	P-value
<i>30 d of sampling</i>			
Total Solids	13.7±0.85	12.6±0.37	0.06
Fat*	5.06±0.84	4.63±0.33	0.03
Solid Not Fat	8.69±0.05	8.03±0.09	0.09
Protein	3.05±0.05	2.95±0.04	0.70
Lactose	4.60±0.07	4.41±0.05	0.31
Salts	0.69±0.00	0.66±0.00	0.75
<i>60 d of sampling</i>			
Total Solids	14.1±2.03	12.8±1.29	0.49
Fat	5.12±0.75	4.90±0.38	0.34
Solid Not Fat	8.95±1.29	7.92±1.02	0.71
Protein	3.26±0.47	2.89±0.37	0.71
Lactose	4.90±0.70	4.34±0.55	0.71
Salts	0.73±0.10	0.65±0.08	0.76
<i>90 d of sampling</i>			
Total Solids	15.8±0.74	16.5±0.49	0.50
Fat	5.27±0.52	5.54±0.35	0.52
Solid Not Fat	10.5±0.25	11.0±0.19	0.43
Protein	3.84±0.09	4.03±0.07	0.45
Lactose	5.77±0.14	6.05±0.10	0.46
Salts	0.86±0.02	0.90±0.01	0.37

*, differ significantly ($P<0.05$); Gr G, goats fed with gram straw based pellet; Gr M, goats fed with Moringa based pellet,

Table 4. Fatty acid profile of milk of lactating goats in different groups

Fatty acid	Gr G	Gr M	P-value
Butyric acid (c4:0)	0.98±0.06	0.78±0.19	0.16
Caproic acid (c6:0)	2.20±0.09	2.08±0.05	0.21
Caprylic acid (c8:0)	3.49±0.15	3.15±0.17	0.80
Capric acid me (c10:0)	13.5±0.68	11.8±0.82	0.40
Lauric acid me (c12:0)	6.04±0.67	5.55±0.53	0.90
Myristic acid me (c14:0)	14.2±0.53	10.1±0.63	0.30
Myristoleic acid me (c14:1)	1.01±0.05	0.67±0.27	0.00
Pentadecanoic acid me (c15:0)	1.21±0.07	1.49±0.06	0.61
cis-10-Pentadecanoic acid me (c15:1)	0.00±0.00	0.53±0.40	0.06
Palmitic acid methyl ester (c16:0)	28.9±1.38	28.5±0.85	0.56
Pamitolic acid (16:1)	0.93±0.07	1.10±0.08	0.62
Heptadecanoic acid me (c17:0)	0.27±0.16	0.15±0.15	0.79
cis-10-heptadecanoic acid me (c17:1)	0.00±0.00	0.13±0.13	0.05
Stearic acid (c18:0)	5.21±0.37	6.27±0.77	0.19
(C18:1n9c) Oleic acid	19.1±1.23	22.8±1.60	0.53
C18:2n6c Linoleic acid	2.85±0.11	3.38±0.18	0.21
C18:3n3 alpha linolenic acid	1.02±0.03	0.41±0.25	0.00
Total SFA	75.9±1.16	71.8±1.50	0.83
Total MUFA	20.1±1.24	24.0±1.64	0.69
Total PUFA	3.85±0.12	3.79±0.37	0.34
Total FA	99.9±0.15	99.6±0.31	0.30
PUFA/SFA ¹	0.05±0.00	0.05±0.00	0.09
Index of atherogenicity IA ²	3.85±0.27	3.00±0.26	0.92
Hypo/ Hypercholesteremic ratio HH ³	0.47±0.03	0.58±0.03	0.67
Health promoting index ⁴ HPI	0.26±0.01	0.34±0.02	0.46

*, differ significantly (P<0.05); Gr G, goats fed with gram straw based pellet; Gr M, goats fed with Moringa based pellet. ¹Polyunsaturated fatty acid/saturated fatty acid ratio (Σ PUFA/ Σ SFA); ²Index of atherogenicity [C12:0 + (4 × C14:0) + C16:0]/ Σ UFA; ³Hypocholesterolemic /hypercholesterolemic ratio (cis-C18:1 + Σ PUFA)/(C12:0 + C14:0 + C16:0); ⁴Health-promoting index Σ UFA/[C12:0+(4 × C14:0) + C16:0].

and immunity capacity in dairy cows. Improved cell-mediated immunity as measured through delayed-type hypersensitivity (DTH) reaction against phytohaemagglutinin (PHA-P) was also reported in this study. The secondary metabolites present in moringa leaves have improved the immunity and reduced the somatic cell count in the lactating goats. Moringa contains various flavonoids in the leaves, roots, flowers, and seed coats. The contents of flavonoids vary depending upon the geographic origins of Moringa. The most common flavonoids in Moringa leaves are kaempferol, quercetin, isorhamnetin, and apigenin (Milugo *et al.* 2013).

Milk fatty acid analysis: The fatty acid profile of milk produced from goat from Gr G and Gr M is presented in Table 4. There was no statistically significant difference in the individual milk fatty acid profile except C18:3n3

alpha linolenic acid which was significantly higher in milk from gram pellet fed group. However total saturated fatty acids were lower and total MUFA was higher in the milk of moringa pellet fed group. Nutritional indices for assessing fatty acids were calculated as per Chen and Liu (2020). Index of atherogenicity (IA), Hypo/Hypercholesteremic ratio (HH) and Health promoting index (HPI) was better with milk of moringa pellet fed group. Fatty acid composition of milk is quite variable depending upon the dietary modification which can affect the biohydrogenation in the rumen. Higher concentration of unsaturated fatty acids (about 16.19%) and lower saturated fatty acids (about 5.49%) were reported in lactating goats fed with Moringa based complete pellet feed. This modification in the fatty acid profile might be due to effect of secondary metabolites present in Moringa on the number of rumen microbes and their activity. Polyphenols and flavonoids present in Moringa decrease the number and the activity of ruminal bacteria involved in the biohydrogenation of dietary unsaturated fatty acids leading to increased absorption of unsaturated fatty acids from rumen and secretion in the milk. Chilliard and Ferlay (2004) reported that most (about 60%) of milk fatty acids originate from plasma uptake, whereas the rest are synthesized in the mammary gland. Unsaturated fatty acids are precursors of long chain n-3 PUFA, which are important bioregulators of many cellular processes of immune system (Cozma *et al.* 2013). Therefore these PUFA help in improvement of immune activity of the animals consuming the Moringa feed as well the PUFA secreted in the milk will improve the health of consumer taking the milk. So, the fatty acid profile indicator like Index of atherogenicity (IA) was lower while Hypo/Hypercholesteremic ratio and Health promoting index was higher in milk of moringa based pellet fed goats. The fatty acid composition is in focus nowadays due to the impact of different fatty acids in human nutrition and their relation with various diseases. This showed that goats fed with Moringa pellet were healthy and gave nutritious milk.

The cell-mediated immune response was assessed through delayed-type hypersensitivity (DTH) reaction against phytohaemagglutinin (PHA-P) and the results were expressed in terms of total skin thickness (mm) (Supplementary Table 2). There was no statistically significant difference between groups except at 96 h. However, the effect was more pronounced in Gr M at 72 and 96 h showing better immune response in Gr M. Unsaturated fatty acids are precursors of long chain n-3 PUFA, which are important bioregulators of many cellular processes of immune system (Cozma *et al.* 2013). Higher PUFA in the diet of Gr M might be the reason of immune activity.

Present study concluded that Barbari goats fed with Moringa based complete pellet feed produced higher milk yield along with reduced somatic cell count and better cell-mediated immunity. The milk fat of Moringa fed goats had healthier unsaturated fatty acids with higher health promoting index.

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