



Effect of *Moringa oleifera* Leaf Meal on Milk of Lactating Goats

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Effect of Varying Levels of *Moringa oleifera* Leaf Meal on Nutrient Utilization, Milk Production and Composition of Milk in Lactating Goats

Gursimran Singh^{1*}, J.S. Lamba¹, R.S. Grewal¹, Jasmine Kaur¹, Shashi Nayyar² and Mandeep Singla³

¹Department of Animal Nutrition, College of Veterinary Sciences, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India

²Department of Veterinary Physiology and Biochemistry, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India

³Department of Livestock Production Management, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India

* Correspondence: gursimran.saini26@gmail.com

ABSTRACT

The effect of substituting crude protein (CP) in soybean meal with *Moringa oleifera* leaf meal (MLM) supplementation on milk production parameters and nutrient utilization in lactating goats were studied. To determine the best level of MLM for the *in vivo* trial, an *in vitro* experiment was undertaken with MLM inclusion rates of 0, 10, 20, 30, 40 and 50% of the CP of soyabean meal replacement. Net gas production, total volatile fatty acid and propionic acid concentrations were significantly higher in 30% MLM total mixed ration (TMR). Twelve lactating goats were randomly assigned to control group (soybean meal as a main protein source) and MLM (CP of soybean was replaced by 30% MLM) group for the *in vivo* trial with six animals in each group. Animals were fed a TMR of 60:40 fodder and concentrate respectively. Feeding of MLM resulted in significantly higher digestibility of ADF, Cellulose, OM and NFC. But, DM intake and nutrients (CP, EE, NDF and ADF) intake (% BW) were found to be similar. Supplementation of MLM had no significant ($P < 0.05$) effect on average milk yield, milk composition (protein, SNF, lactose and fat) and ECM. Fatty acid (FA) profiles of milk revealed that the levels of saturated, monounsaturated and polyunsaturated FA were similar in both the groups. Hence, it can be concluded that MLM can replace 30% CP of soyabean meal in the ration of lactating goats.

KEYWORDS: Digestibility, Fatty acid profile, Milk composition, *Moringa oleifera* leaf meal, Nutrient intake

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INTRODUCTION

Agriculture plays a crucial role in Indian economy. Almost half of the country's population depends on agriculture for livelihood. Majority of farmers in our country are marginal and small farmers so they cannot depend completely on farming. By 2050, India's population is predicted to grow by 34% and the livestock sector would need to produce 186.2 million tonnes of milk and 18.7 million tonnes of meat per year to meet the dietary recommendations of the Indian Council for Medical Research (ICMR, 2010) for a population of 1.7 billion people. This indicates that present milk and

meat production levels would need to be increased by 1.5 and 3 times, respectively (NIANP, 2013). To meet this need, large amounts of feed resources will be required, posing a challenge to the feed production systems. At present the estimated annual availability of total concentrate feed in the country is only 61 million tonnes against a demand of 96 million tonnes, indicating a deficit of 36%. Total green fodder availability is 734 million tonnes against a demand of 827 million tonnes, resulting in a deficit of 11.24%. Total dry fodder availability is 326.4 million tonnes against a demand of 426.1 million tonnes with a deficit of 23.4% (Roy et al., 2019).

Although the concentrate can be used with low-quality roughage, rising ingredient prices have necessitated the search for an unconventional feed source that is both inexpensive and contains a high amount of protein with balanced amino acids. Tree leaves are low-cost protein sources for ruminants (Kholif et al., 2015). *Moringa oleifera* is a great alternative that fits all of the above characteristics. It is a multipurpose, non-leguminous tree that is widely grown and adapts to a variety of environmental conditions. The presence of fatty acids, protein, vitamins, and minerals in the leaves of *M. oleifera* suggests that it could be used as a feed supplement (Becker, 1995).

MATERIALS AND METHODS

The samples of MLM used in the *in vitro* experiment were obtained from Animal Nutrition Department of GADVASU, Ludhiana and were sun dried. In a hammer mill, dried MLM samples were ground into a fine powder and sieved through a mesh of 1-mm. A total of 6 concentrate rations were made, substituting 10, 20, 30, 40 and 50% of the CP of soybean with moringa. All of the prepared rations were isonitrogenous in composition and contained around 19.5% CP. The chemical composition of feeds and fodder was carried out according to AOAC (2005).

In-vitro study

Rumen liquor was obtained from the fistulated buffalo bulls in the morning and strained across muslin cloth into a thermo-flask maintained at 39°C and transferred to the experiment laboratory. A 24 hr *in vitro* gas production experiment was carried out as stated by Menke and Steingass (1988). The metabolizable energy (ME) value was calculated using the amount of net gas generated (NGP). The Neutral Detergent Fibre (NDF) content in the tube was also tested. NDF degradability, OM degradability, true digestibility, microbial biomass production (MMP), total degradable sample (TDS), and partition factor (PF) are additional metrics. Further parameters like NDF degradability, OM

degradability, true digestibility (% TD), microbial mass production (MMP), Total degradable sample (TDS) and partition factor (PF) were estimated as per Makkar (2004). The method given by Cottyn and Boucque (1968) was used to estimate volatile fatty acids using a Netchrom 9100 gas chromatograph with a flame ionisation detector.

In-vivo study

The *in vivo* research was carried out in a Goat sheep rabbit farm/ Department of Livestock Production Management, GADVASU, Ludhiana, Punjab (India) for 90 days. Twelve lactating goats were chosen and randomly divided into two groups based on age, milk production, parity, and body weight. The experimental diets used in the study were, a control (soybean meal as a supplemental protein source) and 30% moringa leaf meal (the CP of soybean was replaced by 30% *M. oleifera* leaf meal) diet. The lactating goats were fed a 60:40 TMR of fodder and concentrate. Animals were fed in accordance with ICAR (2013) feeding guidelines. The animals were fed twice a day. The daily record of feed intake and leftover was recorded.

The milk yield was noted twice in a week and composition of milk was determined weekly. Direct trans-esterification method given by O'Fallon et al. (2007) was used to measure Fatty acids in the milk. The thrombogenic and atherogenic indices were measured as per Bryszak et al. (2019). The energy content of milk was evaluated by the formula given by Tyrell and Reid (1965) and Energy corrected milk (ECM) was computed as per Sjaunja et al. (1991).

The animals were weighed on weekly basis. At the end of the experiment, blood samples from individual animals (4 hrs after feeding) were obtained in heparinized tubes from the jugular vein. After centrifugation at 2000 rpm for 15 minutes, the plasma was obtained and stored at 20°C until it was analysed for various biochemical components. The data were analysed using SPSS (2012) version 21 and a basic ANOVA as recommended by Snedecor and Cochran (1994). Tukeys B was used to test the mean difference.

RESULTS AND DISCUSSION

In-vitro gas production and degradability of substance

The net gas production (NGP) was significantly higher ($P<0.05$) in 30% TMR however, it was similar among all the other TMRs. The varying quantities of moringa leaf meal in TMR had no significant effect on the true degraded substrate (TDS), OMD, NDFD and MMP. The partition factor (PF) was significantly ($P<0.05$) higher in 10% TMR

but comparable with rest of the TMRs. The EMMP was significantly ($P<0.05$) lowest in 20% TMR and higher in 10% TMR. The 30% TMR had produced significantly higher ($P<0.05$) SCFA while it was similar in rest of the TMRs. Metabolizable energy (ME) was significantly ($P<0.05$) higher in 20% moringa TMR but varied non-significantly in other TMR. The quantity of fermentable methane and fermentable carbon dioxide produced were similar in all the TMR but were numerically lower in 30% TMR.

Table 1. Effect of various concentrations of MLM based TMR on nutrient utilization in *in-vitro* study

Parameters	TMR 1 (0%)	TMR 2 (10%)	TMR 3 (20%)	TMR 4 (30%)	TMR 5 (40%)	TMR 6 (50%)	SEM
NGP (ml)	65.5 ^a	64.7 ^a	65.0 ^a	68.0 ^b	65.2 ^a	65.5 ^a	0.33
TDS (mg)	332.6	333.1	332.0	333.0	332.0	331.6	0.16
PF	4.01 ^a	4.18 ^b	3.94 ^a	4.07 ^{ab}	4.03 ^{ab}	4.03 ^{ab}	0.02
OMD (%)	78.8	81.2	80.7	79.5	79.3	79.6	0.31
NDFD (%)	58.5	60.1	61.3	59.9	59.7	58.8	0.47
MMP (mg)	118.5	128.2	118.4	122.0	120.0	120.0	1.16
EMMP (mg)	45.2 ^{ab}	47.3 ^b	44.1 ^a	46.0 ^{ab}	45.5 ^{ab}	45.4 ^{ab}	0.31
TD (%)	79.4	81.7	81.0	79.7	79.8	80.1	0.31
SCFA (mmol)	0.76 ^a	0.76 ^a	0.76 ^a	0.80 ^b	0.76 ^a	0.77 ^a	0.03
ME (MJ/Kg) DM	8.35 ^a	8.36 ^a	8.60 ^b	8.35 ^a	8.35 ^a	8.38 ^a	0.02
CO ₂ (mmol)	0.23 ^a	0.23 ^a	0.22 ^a	0.21 ^a	0.22 ^a	0.21 ^a	0.003
CH ₄ (mmol)	0.65 ^a	0.63 ^a	0.63 ^a	0.63 ^a	0.63 ^a	0.65 ^a	0.003

NGP- Net gas production, TDS- Truly Degraded Substrate, OMD- Organic matter degradability, NDFD- Neutral Detergent Fibre degradability, MMP- Microbial mass production, EMMP- Efficiency of microbial mass production, TD- True digestibility, SCFA- Short chain fatty acid, ME- Metabolizable energy, SEM- Standard error of the mean

Means of the rows with different superscripts differ significantly ($P<0.05$)

Volatile fatty acid in *in-vitro* study

The TVFA and acetic acid concentrations were significantly higher ($P<0.05$) in 30% TMR and 50% TMR but TVFA was lowest in control and acetic acid was lowest in control and 10% TMR. The relative percentage of acetate and propionate were significantly ($P<0.05$) higher and acetic acid to

propionic acid ratio was lower in 30% moringa based TMR. The percentage of iso butyrate was not affected by MLM. The relative percentage of iso valerate and butyrate were significantly higher in 40% moringa supplemented total mixed rations. However, butyrate percentage was significantly lower in 30% MLM TMR.

Table 2. Effect of various concentrations of MLM based TMR on *in-vitro* volatile fatty acid concentration and relative proportion

Parameters(m mol/L)	TMR 1 (0%)	TMR 2 (10%)	TMR 3 (20%)	TMR 4 (30%)	TMR 5 (40%)	TMR 6 (50%)	SEM
Acetate	4.14 ^a	4.17 ^a	4.40 ^{bc}	4.51 ^b	4.31 ^{ab}	4.39 ^b	0.08
Propionate	1.03 ^{ab}	1.00 ^a	1.08 ^b	1.15 ^c	1.06 ^{ab}	1.06 ^{ab}	0.01
Iso-butyrate	0.12 ^b	0.13 ^b	0.11 ^{ab}	0.10 ^a	0.13 ^b	0.13 ^b	0.004
Butyrate	0.29 ^a	0.32 ^{ab}	0.30 ^{ab}	0.27 ^a	0.35 ^b	0.35 ^b	0.01
Iso-valerate	0.01 ^a	0.009 ^a	0.01 ^a	0.009 ^a	0.02 ^b	0.02 ^{ab}	0.006
TVFA	5.60 ^a	5.64 ^{ab}	5.91 ^{bc}	6.05 ^c	5.88 ^{abc}	5.95 ^c	0.07
Relative proportion, %							
Acetate	73.8 ^b	73.9 ^b	74.4 ^{bc}	74.6 ^c	73.2 ^a	73.8 ^b	0.24
Propionate	18.4 ^b	17.7 ^a	18.2 ^{ab}	19.0 ^b	18.0 ^{ab}	17.8 ^a	0.15
Iso butyrate	2.27	2.40	1.99	1.65	2.29	2.18	0.08
Butyrate	5.23 ^{bc}	5.71 ^{bcd}	5.16 ^b	4.52 ^a	5.97 ^d	5.87 ^{cd}	0.16
Iso valerate	0.018 ^a	0.012 ^a	0.014 ^a	0.012 ^a	0.032 ^b	0.023 ^a	0.002
AA:PA ratio	4.00 ^{ab}	4.17 ^c	4.07 ^{bc}	3.91 ^a	4.04 ^{abc}	4.14 ^c	0.027

TVFA- Total volatile fatty acids, AA:PA- Acetic acid to Propionic acid ratio, SEM- Standard error of the mean Means of the rows with different superscripts differ significantly ($P < 0.05$)

Table 3. Chemical and ingredient composition of MLM and TMR fed to lactating goats

Ingredients	TMR 1 (Control)	TMR 2 (30% MLM)	Chemical composition (% DM)	<i>Moringa oleifera</i>
Maize fodder	60	60	Ash	11.3
Maize grain	14	14	Organic matter	88.7
Soyabean meal	8	5.6	Crude protein	27.0
Moringa leaf meal	0	4	NDF	22.7
Wheat bran	7.8	7.2	ADF	16.9
Deoiled rice bran	8.2	7.32	Hemicellulose	5.80
Mineral mixture	0.8	0.8	Ether extract	6.23
Common salt	0.4	0.4	Cellulose	14.1
Urea	0.2	0.28		
By-pass fat	0.6	0.4		
Chemical composition (% DM)				
	TMR 1		TMR 2	
Dry matter	91.0		91.1	
Ash	11.3		11.2	
Organic matter	88.7		88.8	
Crude protein	14.12		14.21	
NDF	46.30		44.65	
ADF	25.8		24.90	
Hemicellulose	20.50		20.4	
ADL	11.12		11.35	
Ether extract	2.58		2.65	
Cellulose	13.20		12.5	

MLM- *Moringa oleifera* leaf meal, NDF- Neutral detergent fiber, ADF- Acid detergent fiber, ADL- Acid detergent lignin

Digestibility of nutrients and feed intake

The dry matter intake was similar in both the groups in terms of BW% indicating that moringa leaf meal, when incorporated at 30% had no negative impact on the voluntary dry matter intake of experimental goats. Since both groups consumed the same amount of total dry matter, the intake of CP, OM, NDF, EE, ADF, cellulose and hemicellulose were likewise similar. The findings of this study correspond with those of Priya et al. (2020) and

Damor et al. (2017), who found no statistically significant differences in average daily dry matter consumption in goats. Furthermore, Tona et al. (2014) found that the dry matter consumption of concentrate diets including MLM increased as the level of MLM increased. Li et al. (2019) reported similar CPI in control and moringa group but Fadiyimu et al. (2010) reported increase in CPI with increase in inclusion of Moringa in the ration. They also reported similar OMI in both the groups.

Table 4. Effect of feeding MLM based TMR on nutrients digestibility parameters and feed intake(% DM) basis

Parameters	Control	Treatment	SEM	P value
Intake (%BW)				
Dry matter	3.96	3.95	0.07	0.91
Organic matter	1.97	2.09	0.53	0.26
Crude protein	0.64	0.63	0.41	0.89
Ether extract	0.12	0.12	0.002	0.95
Neutral detergent fiber	1.82	1.80	0.04	0.88
Acid detergent fiber	1.00	1.01	0.02	0.90
Cellulose	0.67	0.67	0.02	0.97
Hemicellulose	0.86	0.84	0.02	0.74
Digestibility of nutrients (g/kg)				
Dry matter	72.5	75.1	0.81	0.12
Organic matter	73.6	77.0	0.80	0.03
Neutral detergent fiber	67.5	69.8	0.81	0.17
Acid detergent fiber	53.4	59.8	1.55	0.03
Ether extract	77.5	76.0	0.70	0.29
Cellulose	58.2	64.8	1.54	0.03
Hemicellulose	78.8	81.9	0.83	0.06
Crude protein	82.7	83.8	0.49	0.27
Non-fiber carbohydrate	88.3	91.4	0.66	0.02

When compared to the control group, the MLM group had significantly higher digestibility of OM, ADF, cellulose and NFC. On the other hand, digestibility of DM, NDF, EE, CP and hemicellulose were observed to be similar among both the groups. The current findings are consistent with findings of improved *in vivo* digestibility published by Kholif et al. (2015), who found a significant increase in organic matter and fibre digestibility of feed containing moringa leaves. Fadiyimu et al. (2010)

reported that CF and EE digestibility was highest when fed at 25 percent *M. oleifera* level.

Milk yield and composition

The milk yield, composition and components yield of lactating goats fed with MLM are shown in Table 5. It was observed that average milk yield and milk components (fat, lactose, protein and SNF), were not affected by feeding of MLM in lactating goats. However, higher values of fat, protein and

SNF were observed. Both groups had similar milk components and total milk solid yield, which can be linked to similar milk yield in control and MLM groups. Moreover, milk energy, ECM, milk yield/kg DMI and milk solid yield (gm)/kg DMI were also similar in both the groups.

These observations for milk yield were in agreement with Kekana et al. (2019) and Sun et al.

(2017) as they reported no effect of MLM on milk yield but Kholif et al. (2019) and Bashar et al. (2020) reported higher milk yield in group fed with MLM. Sun et al. (2017) and Olvera et al. (2020) also reported no significant effect of feeding MLM on milk composition. Milk energy and milk energy output was higher than what Kholif et al. (2015) had reported.

Table 5. Effect of feeding MLM based TMR on milk yield and composition of lactating goats

Parameters	Control	Treatment	SEM	P values
Milk production				
Initial (kg/day)	0.95	0.97	0.05	0.86
Average yield (kg/day)	0.96	0.95	0.03	0.81
ECM (kg/day)	1.13	1.13	0.05	0.93
Milk composition (%)				
Fat	5.15	5.42	0.26	0.62
SNF	9.24	9.36	0.10	0.58
Protein	3.45	3.55	0.05	0.35
Lactose	5.09	5.07	0.05	0.82

ECM- Energy corrected milk, SNF- Solid not fat, DMI- Dry matter intake, SEM- Standard error of the mean

Fatty acid profile of milk

The fatty acid profile of milk revealed that feeding of MLM diet had no significant difference for total SFA, total MUFA and total USFA but individual fatty acids like C4:0 was significantly higher in control group and C13:0 which was significantly higher in MLM fed group. The levels of total PUFA were also not significant among groups but the MLM fed group had numerically higher (up to 26%) levels of PUFA in milk. MLM feeding resulted in decrease in ω -6/ ω -3. A lower ω -

6/ ω -3 fatty acid ratio is better for lowering the likelihood of many chronic diseases that are common in both Western and developing countries (Simopoulos, 2002). Atherogenicity index and thrombogenicity index were statistically similar but numerically lower values were observed with feeding of MLM. Lowering the atherogenicity index is one of the important markers of increased milk quality as a human food product because of its beneficial effects in lowering the cardiovascular risk (Kholif et al., 2019).

Table 6. Effect of feeding MLM based TMR on milk fatty acid constituents

Saturated fatty acid (m mol/L)	Control	Treatment	SEM	P values
Carbon 4:0	0.08	0.003	0.02	0.04
Carbon 6:0	1.54	1.26	0.17	0.47
Carbon 8:0	2.73	1.80	0.36	0.22
Carbon 10:0	9.23	5.19	1.44	0.187
Carbon 11:0	0.056	0.046	0.004	0.35
Carbon 12:0	4.09	3.46	0.29	0.33
Carbon 13:0	0.076	0.15	0.02	0.05
Carbon 14:0	11.14	11.67	0.27	0.39
Carbon 15:0	0.996	1.1	0.06	0.42
Carbon 16:0	31.42	35.43	1.69	0.28
Carbon 17:0	0.636	0.603	0.02	0.51
Carbon 18:0	11.71	13.28	0.59	0.21
Monounsaturated fatty acid				
Carbon 14:1 (ω -5)	0.44	0.75	0.10	0.16
Carbon 17:1	0.18	0.19	0.007	0.27
Carbon 18:1 (ω -9)	21.52	21.86	0.16	0.36
Carbon 20:1 (ω -9)	0.696	0.346	0.09	0.02
Carbon 22:1 (ω -9)	0.0067	0.020	0.004	0.12
Carbon 24:1 (ω -9)	0.05	0.043	0.013	0.84
Polyunsaturated fatty acid				
Carbon 18:2 (ω -6)	2.36	3.07	0.20	0.06
Carbon 18:3 (ω -6)	0.236	0.256	0.01	0.26
Carbon 18:3 (ω -3)	0.133	0.186	0.01	0.11
Carbon 20:3 (ω -6)	0.076	0.0133	0.02	0.09
Fatty acid class				
ω -3	0.133	0.186	0.02	0.01
ω -6	2.67	3.34	0.19	0.71
ω -6/ ω -3	20.67	18.19	1.81	0.55
Total USFA	26.65	26.20	0.19	0.41
Total SFA	73.73	74.02	0.17	0.46
Total MUFA	22.9	23.22	0.18	0.43
Total PUFA	2.80	3.53	0.20	0.06
Fatty acid ratio				
SFA/USFA	2.79	2.84	0.03	0.42
SFA/MUFA	3.22	3.18	0.03	0.63
SFA/PUFA	26.89	21.47	1.58	0.07
Atherogenicity index	3.29	3.03	0.11	0.27
Thrombogenicity index	4.51	3.95	0.19	0.19

SFA- saturated fatty acids, MUFA- monounsaturated fatty acids, PUFA- polyunsaturated fatty acids, USFA- unsaturated fatty acids, SEM- Standard error of the mean, Atherogenic index- $[(C14:0 + C16:0 \times C12:0 + 4)/(MUFA + PUFA(\hat{u}-6) \text{ and } (\hat{u}-3))]$, Thrombogenic index- $(C14:0 + C16:0 + C18:0)/[0.5 \times MUFA + 0.5 \times PUFA(\hat{u}-6) + 3 \times PUFA(\hat{u}-3) + (\hat{u}-3)/(\hat{u}-6)]$.

CONCLUSION

From the present study it may be inferred that digestibility of nutrients (ADF, Cellulose, OM and TCHO) was improved with feeding of MLM based TMR. Milk yield, milk fatty acid profile, milk composition and milk composition yield were not affected. Therefore, on basis of milk yield and digestibility it was concluded that MLM could replace the protein of soybean meal up to 30% in the concentrate mixture of lactating goats without any negative consequences on palatability, digestibility of nutrients and milk parameters.

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