

IMPACT OF MORINGA OLEIFERA DRIED LEAVES AND A PHYSICAL FORM OF A FORAGE SOURCE ON THE PRODUCTION PERFORMANCE AND METHANE EMISSION OF DAIRY COWS

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ABSTRACT

The present study assessed the influence of *Moringa oleifera* (MO) dried leaves and a physical form of a forage source on the production performance and methane emission of dairy cows. Thirty Holstein-Friesian (10 primiparous and 20 multiparous) mid-lactating (DIM 181 ± 17 , mean \pm SD; 563 ± 25 kg of BW) cows were blocked (based on the parity) into groups of 6 and randomly assigned to each of five treatments; C= TMR (control diet), AP= TMR+4 Kg alfalfa pellets, AH= TMR+4 Kg alfalfa hay, M2=TMR+2 Kg MO-dry leaves, M4= TMR+4 Kg Moringa dry leaves. The results indicated a higher intake of Dry matter (DM) (22.2 Kg/d) and Crude protein (CP) (3.20 kg/d) in AP-supplemented cows. The results showed increased NDF (neutral detergent fiber) (11.24 Kg/d) and ADF (acid detergent fiber) (9.41 Kg/d) intakes in cows fed with the AH diet, and lower NDF (9.40 Kg/d) and ADF (7.35 Kg/d) intakes were observed in the control group cows. Moreover, the highest DM digestibility (68.4%) was observed in cows offered the M4 supplementation. Whereas, the digestibility of CP (65.9%) was greater in cows receiving AP-augmented diets. The results revealed enhanced NDF (57.2%) and ADF (52.8%) digestibilities in cows incorporated with AH in their diet. Our observations reflected higher milk production (27.22 Kg/d) in cows provided with the dietary addition of AH. While, increased milk production efficiency (MY/DMI) was found in cows fed with the dietary inclusion of M2 (1.36). Moreover, the higher 3.5% FCM (27.32 Kg/d) and milk efficiency (FCM/DMI) were noticed in M4-supplemented cows (1.35). Similarly, the milk composition % (protein, fat, TS, and SNF) was also improved in cows supplemented with M4 in their ration. Furthermore, a reduction in methane emission (CH₄/kg DMI per day and CH₄ g/kg 3.5% FCM) in M4 and M2 supplemented cows (CH₄ g per day and CH₄ g/ kg milk) was noticed. In conclusion, the dietary incorporation of MO-dried leaves in combination with a forage source improves milk production efficiency, milk composition, and decreases enteric methane emission in dairy cows.

Keywords: Tree leaves, *Moringa oleifera*, Forage, dairy cows, performance, methane

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INTRODUCTION

It is well established that the world's population is growing rapidly, at a rate of 1.8% per year, and is projected to reach 9.8 billion by 2050 (United Nations, 2017). Therefore, the livestock sector is essential for global food production and supports the livelihoods of billions of individuals globally (Kucukoflaz *et al.*, 2024; Melasari *et al.*, 2024). Ruminants (e.g., beef and dairy cows) are particularly significant because of their distinctive digestive system, which allows them to develop low-quality plant-based feeds into high-quality feed (Jiajun *et al.*, 2024; Al-Galiby *et al.*, 2023; Lileikis *et al.*, 2023). Nevertheless, enteric fermentation in ruminants produces a greenhouse gas, methane (CH₄),

which exacerbates global warming (Maña *et al.*, 2023). The FAO reported that enteric CH₄ production accounts for approximately 44% of the greenhouse emissions from the livestock industry (Gerber *et al.*, 2013). Additionally, the emissions of CH₄ from ruminants signify a substantial loss of dietary energy that could otherwise be allocated to meat or milk synthesis (Patra and Yu, 2015).

Hence, enteric methane mitigation from ruminants has emerged as a critical issue, ascribed to its environmental implications and contribution to climate change (Ardani *et al.*, 2024; Brenda *et al.*, 2024). Efforts are currently underway to develop strategies that reduce CH₄ emissions from ruminants by incorporating various feed sources into their diets (Haque, 2018). Dietary manipulation primarily entails altering the ratios of

roughage to concentrates or supplying high-quality forages (Kholif *et al.*, 2017). High-quality forages (i.e., grasses and legumes) possess higher protein levels and readily digestible carbohydrates, which support the improved performance of animals (Aluwong and Wuyep, 2011). Moreover, the legumes also contain secondary metabolites (i.e., saponins), which may impact the methanogenic archaea, consequently, decreasing methane production (Morgavi *et al.*, 2011). It has been reported that forages are an important source of fibre, i.e., neutral detergent fiber (NDF) and acid detergent fiber (ADF) (Truong and Thu, 2023). However, the proportions of NDF and ADF are affected by the stage of forage maturity (Bo *et al.*, 2023; Huifen *et al.*, 2023; Pan *et al.*, 2023). Alterations in the fiber contents of forages modulate ruminal fermentation (Yue *et al.*, 2023; Zhou *et al.*, 2024), which can contribute to greater methanogenesis (Kholif *et al.*, 2017), because of the release of H₂ associated with the fibrous feeds (Loza *et al.*, 2021). The pelleting of the forage source, such as alfalfa pellets, is also considered one of the essential feeding strategies for minimizing methane production in dairy cows (Beauchemin *et al.*, 2022). It has been well-documented that the pelleting of forages reflected the improved efficiency of feed utilization (Hironaka *et al.*, 1996).

Moreover, the researchers are also striving to develop alternative dietary strategies that employ agricultural by-products and tree leaves to provide sufficient nutrients and modify feed composition (Boudalia *et al.*, 2024). The utilization of tree leaves as feed constituents serves as a viable mitigation strategy, as the leaves possess greater nutritive value compared to grasses, making them more desirable to livestock (Bryant *et al.*, 2014). Thus, *Moringa oleifera* (MO) is considered an excellent source of nutrients (Saleemi *et al.*, 2023; Kholif *et al.*, 2015; Bashir *et al.*, 2024), attributed to the ideally balanced mix of amino acids and fatty acid profiles, along with a good digestibility of protein content (Babiker *et al.*, 2017). Several authors have described that dietary supplementation with MO leaves has been shown to enhance the DMI, milk yield, and milk composition in dairy animals (Kholif *et al.*, 2018; Kholif *et al.*, 2019; Foidl *et al.*, 2001). Moreover, MO leaves not only possess nutritional properties but also serve as sources of bioactive compounds, including phenolic acids and flavonoids (Kousar *et al.*, 2024; Kekana *et al.*, 2019). These compounds positively impact feed utilization and lessen CH₄ production (Kholif *et al.*, 2015).

To our knowledge, there is scarce information available regarding the impact of a physical form of a forage source, along with the supplementation of MO-dried leaves, on the sustainable performance of dairy cows. We hypothesized that the dietary inclusion of a

physical form of a forage source (hay vs pellet), along with the MO dried leaves, could be a valuable strategy to improve the dairy cow's performance while impacting their methane emission. Therefore, the current study was planned to assess the effect of supplementing lactating dairy cows with the physically processed forage source and MO-dried leaves on intake, milk production, milk composition, and methane emission.

MATERIALS AND METHODS

This experiment was conducted at Al-Rahman Dairy Farm, Dunya Pur, Pakistan. The experimental protocols were reviewed and approved by the ethical committee at the University of Agriculture, Faisalabad (No. Agron/3095).

Experimental design and diets: Thirty Holstein-Friesian (10 primiparous and 20 multiparous) mid-lactating (DIM 181 ± 17, mean ± SD; 563 ± 25 kg of body weight) cows were blocked (based on the parity) into groups of 6 and randomly assigned to each of five treatments; C= TMR (control diet), AP= TMR+4 Kg alfalfa pellets, AH= TMR+4 Kg alfalfa hay, M2=TMR+2 Kg Moringa dry leaves, M4= TMR+4 Kg Moringa dry leaves. The experimental diets were prepared in line with the nutritional requirements of the cows (NRC, 2001). The experimental rations were fed twice a day at 08:00 and 20:00 hours during the trial for 12 weeks following a two-week adjustment period. All the experimental animals were provided with fresh and clean water *ad libitum*. The nutrient composition of the treatment diets (Table 1) was evaluated before the experimentation. The refusals were recorded daily, and the diet adjustment was made following a feed refusal rate 5% weekly.

Sampling procedure: The treatment diet samples were collected daily, whereas the refusals were quantified, recorded, and obtained following the same protocols. The samples collected from each experimental unit (animal) were combined (composite), and a representative sample was taken for the chemical assessment (Chishti *et al.*, 2024). The samples were maintained at -20°C, stored in the encased plastic bags, and properly labelled for identification.

Chemical analysis: The samples (feed+faecal) were analyzed for the DM, CP, EE, ash, NDF, and ADF. The samples were dried in an oven at 60°C before processing for subsequent analysis. The collected samples were further analyzed for EE-method 920.39, CP-method 984.13, N×6.25; Kjeldahl, and ash-method 942.05 following AOAC (2005) guide. Whereas the NDF and ADF concentrations were assessed per the procedure outlined by Van Soest *et al.* (1991).

Table 1: Chemical composition of dietary treatments

Ingredients (%)	Experimental diets					
	C	AH	AP	M2	M4	
#						
AH	-	4	-	-	-	
AP	-	-	4	-	-	
M2	-	-	-	2	-	
M4	-	-	-	-	4	
CSM	4.5	3	3.5	6.5	6.5	
Maize	10	12	10	13.5	13	
Wheat bran	14.5	15	15	15.5	15	
SFM	8.5	8	9.5	3.5	3.5	
SBM	1.5	1.5	0	1.5	1.5	
Canola meal	9.5	9.5	11	10.5	9.5	
Maize Silage	39.5	35	35	35	35	
Molasses	8	8	8	8	8	
DCP	1	1	1	1	1	
Mineral premix	1	1	1	1	1	
Vit. Premix	1	1	1	1	1	
Toxin binder	1	1	1	1	1	
	Chemical composition (%)					
CP	14.61	14.67	14.8	14.84	14.88	
EE	2.57	2.6	2.5	2.64	2.61	
NDF	35.41	35.5	34.2	33.74	33.7	
ADF	22.5	22.7	21.6	21.07	21.1	
ME (Mcal/Kg)	1.945	1.952	1.97	1.988	2.01	

C-Control (TMR); AH- TMR + Alfalfa Hay 4 Kg; AP- TMR + Alfalfa pellets 4Kg; M2- TMR + Moringa 2 Kg; M4- TMR + Moringa 4 kg; CSM- cotton seed meal; SFM-sunflower meal; SBM- soybean meal; CP-crude protein; EE-ether extract; NDF-neutral detergent fiber; ADF-acid detergent fiber; ME- metabolizable energy

Milk production: All experimental cows were hand milked twice a day at 06:30 hours and 18:30 hours. The milk output of each experimental animal (cow) was recorded, and milk samples were collected bi-daily. The samples were subjected to treatment with 2-bromo-2 nitropropane-1, 3-diol and were maintained at 4°C until analysis (Johnson *et al.*, 2002). The milk composition encompassing protein, fat, SNF, and TS was analysed utilizing infrared methods (i.e., Lacto-Scan) weekly. The FCM of 3.5% was determined following the NRC (2001) formula: $FCM = 0.432 \times \text{milk yield} + 16.23 \times \text{fat yield}$. The production efficiency was determined by calculating the daily milk production by the daily DMI.

Digestion trial: The digestibility trial was conducted in the last week of the study. Digestibility was assessed using the marker (i.e., chromium oxide) method. The marker (20g) was incorporated into each cow's diet immediately post-feeding and thoroughly mixed. Faecal grab sampling was conducted, with sampling performed every 3 hours throughout the day (Sarwar *et al.*, 1992). Samples were combined following the treatment diets, and a representative sample was taken for chemical evaluation. The samples of dietary treatments, refusals, and faeces were evaluated for the DM, CP, NDF, and ADF.

Enteric methane evaluation: Two respiratory chambers established at Al-Rahman Dairy Farm, Dunya Pur, Pakistan, were used to assess the enteric methane emissions of cows under each treatment. Classical chambers equipped with air conditioning are primarily utilized for the evaluation of methane to minimize the possibility of air loss to the environment (Storm *et al.*, 2012). Because of the expense of the classical chambers, less costly systems have been devised for methane estimation (Castelan-Ortega *et al.*, 2020). During our study, we have used these low-cost chambers for methane estimation. The chambers were 1.8 m (width) \times 2.5 m (height) \times 3.8 m (length) with a volume of approximately 17m³. The estimation for methane was based on the mean rate of methane production/min. and flow rate/unit time. The evaluation of CH₄ was simultaneously done in the two chambers, followed by a pause while following the methodology of Tedeschi *et al.* (2022).

Statistical Analysis: The experimental data were analyzed following a randomized complete block design while using the Generalized Linear Model (GLM) procedure (SAS, 2009). The means for dietary treatments were compared using Tukey's test. Statistical differences were considered significant when P values were below 0.05 or 0.01.

RESULTS AND DISCUSSION

Nutrient Intake and digestibility of cows fed treatment diets: Nutrient intake and digestibility of cows fed treatment diets are presented in Table 2. The dietary inclusion of MO-dried leaves along with a forage source has significantly affected ($p < 0.05$) the nutrient intake and digestibility in the experimental animals. These findings indicated a higher DMI (22.2 Kg/d) and CPI (3.20 kg/d) in AP-supplemented cows. These results are linked with the findings of Hironaka *et al.* (1996), who reported that pelleting of the roughage-based diets may lead to enhanced DMI. Likewise, it has also been demonstrated that the intake of feed is higher when forage undergoes some processing, i.e., grinding and pelleting (Uden, 1988). Pelletizing of forages changes the physical form of feedstuff, which reduces particle size and makes it easier for animals to consume more feed while lowering the effective fiber content (Knaus *et al.*, 1999). Moreover, the improvement in forage intake following pelleting also exhibits higher nutrient digestibility (Van Soest, 1994).

The current study showed increased NDFI (neutral detergent fiber intake) (11.24 Kg/d) and ADFI (acid detergent fiber intake) (9.41 Kg/d) in the cows fed with AH-incorporated diet, and lower NDFI (9.40 Kg/d) and ADFI (7.35 Kg/d) were observed in the control group cows. It has been established that forage NDF exhibits a significant influence on feed intake and rumen fill in high-producing cows (Kendall *et al.*, 2009). Waldo (1986) indicated that the NDF content of the diet serves as the most effective indicator of the feed intake in dairy cows. Moreover, Oba and Allen (1999) reported a positive correlation between NDF digestibility and intake. Improved fiber digestibility in alfalfa-based diets leads to higher NDFI and ADFI. However, Mertens (1987) stated that the voluntary feed intake of animals is impacted by digestive tract fill when high-NDF feeds (i.e., crop residues) are provided.

Nutrient Digestibility: The nutrient digestibility (%) in all of the experimental animals was also influenced by the treatment diets (Table 2). The results showed the highest DM digestibility (68.4%) in cows with the M4 supplementation, and the lowest (57.3%) was found in the control group cows. The higher DM digestibility is linked with the lower fiber levels and secondary metabolites of MO-leaves that improve rumen microbial fermentation, which may lead to improved feed utilization in cows. Whereas the digestibility of CP (65.9%) was greater in cows receiving AP-augmented diets. Pelleting involves grinding and compressing the forage into dense, smaller particles and breaks open plant cell walls, which could increase the surface area available to rumen microflora (Ishaq *et al.*, 2019). The increased surface area may stimulate greater microbial colonization, which may lead to improved nutrient

utilization and enhanced protein degradation/digestion (Norrappoke *et al.*, 2025). However, contrasting results were also reported because pelleting reduces fiber particle size, which may lead to an increased rate of ruminal passage (Van-Soest, 1994). The small particles of pelleted forages pass faster than the larger particles of chopped forages, which could result in decreased nutrient digestibility (Waldo *et al.*, 1986) because of the lower ruminal retention rate.

The results revealed enhanced NDF (57.2%) and ADF (52.8%) digestibility in cows provided with the inclusion of AH in their ration. Moreover, the reduced proportions of NDF (43.8%) and ADF (41.8%) digestibility were found in the control group. High fiber diets may theoretically slow ruminal passage due to the increased requirement for microbial degradation; however, the more digestible fiber provided by alfalfa stimulates longer ruminal retention time, facilitating enhanced breakdown by microbes and nutrient utilization (Oba and Allen, 1999), resulting in higher fiber digestibility. Moreover, the fiber in alfalfa promotes chewing and saliva secretion, which aids in buffering rumen pH and sustaining optimal fermentation conditions, thereby enhancing fiber degradation (Jiang *et al.*, 2017).

Milk yield and milk composition of cows fed treatment diets: The current observations showed that the supplementation of graded levels of MO-dried leaves with a forage source (hay vs pellet) considerably affected the milk yield (Kg/d) and milk production efficiency (Table 3) of dairy cows. The results revealed higher milk production (27.22 Kg/d) in cows provided with the dietary addition of AH compared with the control group (23.51 Kg/d). These results agree with the findings of Beauchemin and Buchanan-Smith (1989), who exhibited higher milk production because of the dietary supplementation of hay. The increased milk production of hay-fed animals could be attributed to the increased fibre digestibility (Saleena *et al.*, 2024). Increased fiber digestion can arise from changes in substrate utilization by rumen microorganisms because of the greater concentration of the cellulolytic organisms, which may affect the rates of digesta passage. Similarly, it was also found that the incorporation of hay in the forage-based diet (Chishti *et al.*, 2022) stabilized the ruminal fluid pH, which may lead to an increase in milk production without affecting milk composition (Woodford *et al.*, 1986). Furthermore, Zebeli *et al.* (2012) demonstrated that enhanced fiber digestion provides metabolizable energy, which supports milk production.

Moreover, milk yield was reported to increase primarily due to higher intake of digestible NDF (Kendall *et al.*, 2009). At the same time, increased milk production efficiency (MY/DMI) was found in cows fed with the dietary inclusion of M2 (1.36). Moreover, the higher 3.5% FCM (27.32 Kg/d) and milk production efficiency

(FCM/DMI) were noticed in M4-supplemented cows (1.35). However, lower production efficiencies based on MY (1.18) and FCM (1.13) production were observed in cows offered the dietary incorporation of AP. The variations in milk production outcomes across studies can primarily be attributed to breed type, age, production stage, and the dietary inclusion of MO leaves (Amad and Zentek, 2023).

The improvement in milk production efficiency could be assumed because of the presence of phytochemicals in *Moringa oleifera* leaves associated

with the enhanced fermentation efficiency (Shankhpal *et al.*, 2019). Moreover, the fat yield and efficacy of milk production could be improved by the plant secondary metabolites (i.e., phenols and tannins) in moringa interlinked with the reduced rumen methanogenesis (Shaani *et al.*, 2016; Dong *et al.*, 2019). Additionally, MO leaves are considered a valuable source of protein/amino acids, which can also improve the performance of dairy cows by facilitating the utilization of dietary nitrogen (Bashar *et al.*, 2020).

Table 2: Nutrient Intake and digestibility of cows fed treatment diets

Parameters	C	M2	M4	AH	AP	SEM	P-value
Nutrient intake, kg/d							
DMI (Kg/day)	18.5 ^c	18.9 ^c	20.1 ^b	20.8 ^b	22.2 ^a	0.1757	<0.0001
CPI (Kg/day)	2.69 ^c	2.776 ^c	2.98 ^b	3.13 ^a	3.20 ^a	0.0197	<0.0001
NDFI (Kg/day)	9.40 ^d	9.648 ^{cd}	10.55 ^b	11.24 ^a	9.79 ^c	0.0728	<0.0001
ADFI (Kg/day)	7.35 ^c	7.544 ^c	8.10 ^b	9.41 ^a	7.69 ^{bc}	0.1016	<0.0001
Nutrient digestibility, %							
DMD	57.3 ^b	62.8 ^{ab}	68.4 ^a	67.8 ^a	62.1 ^{ab}	1.4959	0.0021
CPD	61.3 ^{ab}	56.3 ^b	59.4 ^{ab}	62.1 ^{ab}	65.9 ^a	1.8902	0.0500
NDFD	43.8 ^c	48.1 ^{Bc}	53.1 ^{ab}	57.2 ^a	51.2 ^{abc}	1.6919	0.0024
ADFD	41.8 ^b	44.4 ^b	47.4 ^{ab}	52.8 ^a	44.2 ^b	1.6651	0.0079

^{abcd}Values with different superscripts in a row differ significantly.

Abbreviations: C-Control (TMR); M2- TMR + Moringa 2 Kg; M4- TMR + Moringa 4 Kg; AH- TMR + Alfalfa Hay 4 Kg; AP- TMR + Alfalfa pellets 4Kg; **DMI**- dry matter intake; **CPI**- crude protein intake; **NDFI**- neutral detergent fiber intake; **ADFI**- acid detergent fiber intake; **DMD**- dry matter digestibility; **CPD**- crude protein digestibility; **NDFD**- neutral detergent fiber digestibility; **ADFD**- acid detergent fiber digestibility; **SEM**- standard error of the mean

Milk Composition: The effect of supplementation of MO-dried leaves and a forage source on the milk composition of experimental animals is exhibited in Table 3. The milk quality (composition) was significantly affected because of the dietary incorporation of MO-dried leaves along with a forage source (p<0.05). The improved milk composition % (protein, fat, TS, and SNF) was observed in cows supplemented with M4 in their ration. These results are linked with the observations of

Zhang *et al.* (2018) and Novianti *et al.* (2021), which documented that MO has been used to improve milk composition. Moringa has been shown to enhance milk fat content while decreasing somatic cell count and oxidative stress levels (Kekana *et al.*, 2019). Dong *et al.* (2019) also demonstrated that the dietary inclusion of MO-leaves in the diet of goats improved the fat content of milk; ultimately, this may result in an improvement in milk lipid and milk solids content.

Table 3: Milk yield and milk composition of cows fed treatment diets

Parameters	C	M2	M4	AH	AP	SEM	P-value
DMI, (Kg/day)	18.5 ^c	18.9 ^c	20.1 ^b	20.8 ^b	22.2 ^a	0.1757	<0.0001
Milk Yield (Kg/day)	23.51 ^d	25.65 ^c	26.1 ^{bc}	27.22 ^a	26.42 ^b	0.12	<0.01
3.5% FCM	23.3 ^c	25 ^b	27.32 ^a	27.29 ^a	25.1 ^b	0.103	<0.01
ME	1.27 ^c	1.36 ^a	1.3 ^b	1.31 ^b	1.18 ^d	0.004	<0.0001
ME FCM	1.26 ^c	1.33 ^b	1.35 ^a	1.32 ^b	1.13 ^d	0.003	<0.0001
Milk Composition (%)							
Protein	3.01 ^b	3.06 ^b	3.22 ^a	3.05 ^b	3.11 ^{ab}	0.0295	0.0001
Fat	3.59 ^b	3.81 ^b	4.23 ^a	4.07 ^a	3.7 ^b	0.0578	<0.01
TS	10.63 ^b	11.02 ^a	11.11 ^a	10.89 ^{ab}	10.9 ^{ab}	0.0908	0.0071
SNF	8.51 ^c	8.80 ^{ab}	8.88 ^a	8.59 ^c	8.67 ^{bc}	0.0496	<0.01

^{abcd}Values with different superscripts in a row differ significantly.

Abbreviations: C-Control (TMR); M2- TMR + Moringa 2 Kg; M4- TMR + Moringa 4 Kg; AH- TMR + Alfalfa Hay 4Kg; AP- TMR + Alfalfa pellets 4Kg; **DMI**- Dry matter intake; **FCM**- Fat corrected milk; **ME**- Milk production efficiency; **TS**- Total solids; **SNF**- Solids not fat; **SEM**- Standard error of the mean

Methane production: The findings revealed that the production of methane in cows is affected due to the dietary incorporation of MO-dried leaves in addition to the physical processing of a forage source (Table 4). The results showed greater methane production (CH₄/kg DMI per day and CH₄ g/ kg 3.5% FCM) in the control group cows, whereas a reduction in methane concentration was found in cows fed with the M4 inclusion. Moreover, the decreased emission of methane (CH₄ g per day and CH₄ g/ kg milk) was noticed in M2 cows. These results are aligned with the study of Leitathem *et al.* (2022), who concluded that the supplementation of MO-leaves in feed reduces methane emissions. Similar results for reduced

methane production in cows were found by Dey *et al.* (2014) and Soliva *et al.* (2005) on a forage-based diet. Likewise, the emissions from the rumen were decreased with the dietary addition of leaves or extracts of Moringa, resulting from active modulation of the rumen microbiome (Sarkar *et al.*, 2016; Ebeid *et al.*, 2020). The effects of these foliage plants on methane inhibition may be attributed to secondary metabolites, including alkaloids, flavonoids, and tannins. These compounds have the potential to interact with microbes in the rumen and alter ruminal fermentation patterns, resulting in a suppression of methanogenesis (Akanmu and Hassen, 2018; Dong *et al.*, 2019; Ku-Vera *et al.*, 2020).

Table 4: Methane production of cows fed treatment diets

Parameters	C	M2	M4	AH	AP	SEM	P-value
CH ₄ (1 kg ⁻¹ DM day ⁻¹)	20.804 ^a	18.633 ^c	17.700 ^c	19.746 ^b	18.233 ^c	0.2663	<0.01
CH ₄ (g/day)	343.03 ^b	314.90 ^c	320.97 ^c	379.22 ^a	356.28 ^b	4.7943	<0.01
CH ₄ (g/kg milk)	15.237 ^b	13.954 ^c	13.999 ^c	16.211 ^a	15.670 ^{ab}	0.2237	<0.01
CH ₄ (g/ kg 3.5% FCM)	14.986 ^a	12.815 ^b	12.369 ^b	14.660 ^a	15.122 ^a	0.2635	<0.01

^{abcd}Values with different superscripts in a row differ significantly.

Abbreviations: C-cotrol (TMR); M2- TMR + Moringa 2 Kg; M4- TMR + Moringa 4 Kg; AH- TMR + Alfalfa Hay 4 Kg; AP- TMR + Alfalfa pellets 4Kg; SEM- standard error of the mean

Conclusion: The current study showcased the impact of MO dried leaves and a physical form of a forage source on the production performance and methane emission of dairy cows. In conclusion, the dietary incorporation of MO dried leaves in combination with a forage source improves milk production efficiency Kg/Kg (MY/DMI=1.36 in M2 and FCM/DMI=1.35 in M4), milk composition, and decreases enteric methane emission in dairy cows.

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Authors' Contribution: MSIZ and MAUR- project administration and conceptualization; MQB, MR approval of the experimental procedures; MAUR-supervision, and finalization of the manuscript draft; MFAC- data analysis, manuscript preparation; MHS and W. Ali- data collection; UF, WA assisted in lab work; MFK and RM manuscript reviewing and editing.

REFERENCES

- Akanmu, A. M. and A. Hassen (2018). The use of certain medicinal plant extracts reduced in vitro methane production while improving in vitro organic matter digestibility. *Anim. Prod. Sci.* 58(5): 900-908. <https://doi.org/10.1071/AN16291>
- Al-Galiby, M.K., I.A. Al-Hassnawi and M.M. Al-Hassnawi (2023). Effect of different levels of probiotics on rumen environment and microbial condition in local goat kids. *J. Glob. Innov. Agric. Sci.* 11(2):147-152. <https://doi.org/10.22194/JGIAS/23.1076>
- Allen, M. S. (2000). Effects of diet on short-term regulation of feed intake by lactating dairy cows. *J. Dairy Sci.* 83(7):1598–1624. [https://doi.org/10.3168/jds.S0022-0302\(00\)75030-2](https://doi.org/10.3168/jds.S0022-0302(00)75030-2)
- Aluwong, T. and P.A. Wuyep (2011). Livestock-environment interactions: Methane emissions from ruminants. *Afr. J. Biotechnol.* 10(8):1265-1269. <https://doi.org/10.5897/AJB10.1117>
- Amad, A.A. and J. Zentek (2023). The use of Moringa oleifera in ruminant feeding and its contribution to climate change mitigation. *Front. Anim. Sci.* 4: 1137562. <https://doi.org/10.3389/fanim.2023.1137562>
- AOAC. 2005. Official Methods of Analysis (18th Ed). Association of Official Analytical Chemists, Inc., Suite 400, 2200. Wilson Boulevard Arlington, Virginia 22201, USA.
- Ardani, L.R., M. Zain, E. Elihasridas, R. Pazla, Y. Utami, R.R. Sari, M.T. Hadiwijaya, E.M. Putri, and M. Makmur (2024). Optimization Indigofera zollingeriana and gambier (*Uncaria gambir*) supplementation on feed consumption, digestibility, methane production and lactation performance of Etawa crossbreed goats. *Int. J. Vet. Sci.* 13(5): 537-544. <https://doi.org/10.47278/journal.ijvs/2024.129>

- Babiker, E.E., F.A.L. Juhaimia, K. Ghafoora and K.A. Abdoun (2017). Comparative study on feeding value of Moringa leaves as a partial replacement for alfalfa hay in ewes and goats. *Livest. Sci.* 195:21-26.
<https://doi.org/10.1016/j.livsci.2016.11.010>
- Bashar, M. K., K.S. Huque, N.R. Sarker and N. Sultana (2020). Quality assessment and feeding impact of moringa feed on intake, digestibility, enteric CH₄ emission, rumen fermentation, and milk yield. *J. Adv. Vet. Anim. Res.* 7 (3): 521–529.
<https://doi.org/10.5455/javar.2020.g449>
- Bashir, F., S. Sharif, F. Manzoor, S. Naz and F. Rashid. (2024). Protective Effects of Moringa oleifera Leaf Extract against Silver Nanoparticles and Arsenic-Induced Hepatotoxicity in Rats. *Pakistan Vet. J.* 44(2):377-383.
<https://doi.org/10.29261/pakvetj/2024.154>
- Beauchemin, K.A., E.M. Ungerfeld, A.L. Abdalla, C. Alvarez, C. Arndt, P. Becquet, C. Benchaar, A. Berndt, R.M. Mauricio, T.A. McAllister and W. Oyhantçabal (2022). Invited review: Current enteric methane mitigation options. *J. Dairy Sci.* 105(12):9297-9326.
<https://doi.org/10.3168/jds.2022-22091>
- Beauchemin, K.A. and J.G. Buchanan-Smith (1989). Effects of dietary neutral detergent fiber concentration and supplementary long hay on chewing activities and milk production of dairy cows. *J. Dairy Sci.* 72(9): 2288-2300.
[https://doi.org/10.3168/jds.S0022-0302\(89\)79360-7](https://doi.org/10.3168/jds.S0022-0302(89)79360-7)
- Bhagat, S., S. Singh (2022). Nanominerals in nutrition: Recent developments, present burning issues and future perspectives. *Food Res. Int.* 160:111703.
<https://doi.org/10.1016/j.foodres.2022.111703>
- Bo, C., W. Jian, Z. Hui-Min, L. Hu, A. Bi-Ya-si, G. Nan, L. Jian-Feng and F. Tao (2023). Biology organic fertilizer affects the quality and yield of flax under different irrigation regimes. *Pakistan J. Agric. Sci.* 60(3):429-436.
<https://doi.org/10.21162/PAKJAS/23.132>
- Boudalia, S., S. Smeti, M. Dawit, E.K. Senbeta, Y. Gueroui, V. Dotas, A. Bousbia and G.K. Symeon (2024). Alternative approaches to feeding small ruminants and their potential benefits. *Anim.* 14(6):904.
<https://doi.org/10.3390/ani14060904>
- Brenda, V.D., M. Zain and F. Agustin (2024). Strategy to reduce methane to increase feed efficiency in ruminants through adding essential oils as feed additives. *Int. J. Vet. Sci.* 13(2):195-201.
<https://doi.org/10.47278/journal.ijvs/2023.087>
- Bryant, J.P., K. Joly, F.S. Chapin, D.L. DeAngelis and K. Kielland (2014). Can antibrowsing defense regulate the spread of woody vegetation in arctic tundra? *Ecography.* 37(3):204–11.
<https://doi.org/10.1111/j.1600-0587.2013.00436.x>
- Chishti, M.F.A., M.A.U. Rahman, K. Jatta, S. Khan, M. Riaz, Q. Bilal, U. Anwar, S. Ahmad, H.M. Bajwa and F. Rasul. (2022). Effect of forage to concentrate ratio on growth performance and feeding behavior of Thalli lambs. *Trop Anim. Health Prod.* 54(4):236.
<https://doi.org/10.1007/s11250-022-03226->
- Chishti, M.F.A., M.A.U. Rahman, M.S.U. Rehman and M.Q. Bilal (2024). Chemical composition and in-situ digestion kinetics of nutritionally enriched straw from genetically improved wheat varieties. *Pakistan J. Agric. Sci.* 61(2):671-684.
<https://doi.org/10.21162/PAKJAS/24.153>
- Dey, A., S.S. Paul, P. Pandey and R. Rathore (2014). Potential of Moringa oleifera leaves in modulating in vitro methanogenesis and fermentation of wheat straw in buffalo. *Ind. J. Anim. Sci.* 84(5):533–538.
- Dong, L., T. Zhang and Q. Diao (2019). Effect of dietary supplementation of Moringa oleifera on the production performance and fecal methanogenic community of lactating dairy cows. *Anim.* 9 (5): 262. <https://doi.org/10.3390/ani9050262>
- Ebeid, H. M., L. Mengwei, A.E. Kholif, F.U. Hassan, P. Lijuan and L. Xin (2020). Moringa oleifera oil modulates rumen microflora to mediate in vitro fermentation kinetics and methanogenesis in total mix rations. *Curr. Micro.* 77(7): 1271–1282. <https://doi.org/10.1007/s00284-020-01935-2>
- Daba, M. (2016). Miracle tree: A review on multi-purposes of Moringa oleifera and its implication for climate change mitigation. *J. Earth Sci. Clim. Change,* 7(4):1-5. Gerber, P.J., H. Steinfeld, B. Henderson, A. Mottet, C. Opio, J. Dijkman, A. Falcucci, and G. Tempio (2013). Tackling climate change through livestock- A global assessment of emissions and mitigation opportunities; Food and Agriculture Organization of the United Nations (FAO): Rome, Italy.
<http://www.fao.org/docrep/018/i3437e/i3437e00.htm>
- Haque, M.N. (2018). Dietary manipulation: a sustainable way to mitigate methane emissions from ruminants. *J Anim. Sci. Technol.* 60(1):15.
<https://doi.org/10.1186/s40781-018-0175-7>
- Hironaka, R., G.W. Mathison, B.K. Kerrigan and I. Vlach (1996). The effect of pelleting of alfalfa hay on methane production and digestibility by steers. *Sci. Total Environ.* 180(3): 221-227.
[https://doi.org/10.1016/0048-9697\(95\)04948-7](https://doi.org/10.1016/0048-9697(95)04948-7)

- Huifen, D., L. Hong, H. Renke, Z. Baizhong, Y. Kangle and S. Ao (2023). Study on mixed silage of forage ramie and pennisetum purpureum (schum.) in different proportions. *Pakistan J. Agric. Sci.* 60(3):593-597. <https://doi.org/10.21162/PAKJAS/23.623>
- Ishaq, S.L., M.M. Lachman, B.A. Wenner, A. Baeza, M. Butler, E. Gates, S. Olivo, J.B. Geddes, P. Hatfield and C.J. Yeoman (2019). Pelleted-hay alfalfa feed increases sheep wether weight gain and rumen bacterial richness over loose-hay alfalfa feed. *PloS one*, 14(6):1-20. <https://doi.org/10.1371/journal.pone.0215797>
- Jiang, F.G., X.Y. Lin, Z.G. Yan, Z. Hu, G.M. Liu, Y.D. Sun, X.W. Liu and Z.H. Wang (2017). Effect of dietary roughage level on chewing activity, ruminal pH, and saliva secretion in lactating Holstein cows. *J. Dairy Sci.* 100(4):2660-2671. <https://doi.org/10.3168/jds.2016-11559>
- Jiajun H.E., W.U. Zhenzhou, Y.U. Liu, L. Li, Z. Guoqiang, W. Dangdang, X. Xiangxue (2024). Effects of Dietary Supplementation with *Clostridium butyricum* on Rumen Fermentation, Rumen Microbiota and Feces in Beef Cattle. *Kafkas Univ. Vet. Fak. Derg.* 30 (3): 325-333. <https://doi.org/10.9775/kvfd.2023.31114>
- Johnson, K., R. Kincaid, H. Westberg, C. Gaskins, B. Lamb and J. Cronrath (2002). The effect of oilseeds in diets of lactating cows on milk production and methane emissions. *J. Dairy Sci.* 85(6):1509-1515. [https://doi.org/10.3168/jds.S0022-0302\(02\)74220-3](https://doi.org/10.3168/jds.S0022-0302(02)74220-3)
- Kekana, T. W., U. Marume, C.M. Muya and F.V. Nherera-Chokud (2019). Lactation performance and blood metabolites in lactating dairy cows microsupplemented with *Moringa oleifera* leaf meal. *S. Afr. J. Anim. Sci.* 49(4): 709-716. <https://doi.org/10.4314/sajas.v49i4.12>
- Kendall, C., C. Leonardi, P.C. Hoffman and D.K. Combs (2009). Intake and milk production of cows fed diets that differed in dietary neutral detergent fiber and neutral detergent fiber digestibility. *J. Dairy Sci.* 92(1):313-323. <https://doi.org/10.3168/jds.2008-1482>
- Kholif, A.E., M.M.Y. Elghandour, A.Z. Salem, A. Barbabosa, O. Márquez and N. Odongo (2017). The effects of three total mixed rations with different concentrate to maize silage ratios and different levels of microalgae *Chlorella vulgaris* on in vitro total gas, methane and carbon dioxide production. *J. Agri. Sci.* 155(3):494–507. <https://doi.org/10.1017/S0021859616000812>
- Kholif, A.E., G.A. Gouda, T.A. Morsy, A.Z.M. Salem, S. Lopez and A.M. Kholif (2015). *M. oleifera* leaf meal as a protein source in lactating goat's diets: feed intake, digestibility, ruminal fermentation, milk yield and composition, and its fatty acids profile. *Small Rumin. Res.* 129:129–37. <https://doi.org/10.1016/j.smallrumres.2015.05.007>
- Kholif, A.E., G.A. Gouda, M.L. Galyean, U.Y. Anele and T.A. Morsy (2019). Extract of *Moringa oleifera* leaves increases milk production and enhances milk fatty acid profile of Nubian goats. *Agrofor. Sys.* 93(5):1877–1886. <https://doi.org/10.1007/s10457-018-0292-9>
- Kholif, A.E., G.A. Gouda, O.A. Olafadehan and M.M. Abdo (2018). Effects of replacement of *Moringa oleifera* for berseem clover in the diets of Nubian goats on feed utilization, and milk yield, composition and fatty acid profile. *Animals* 12(5):964–972. <https://doi.org/10.1017/S1751731117002336>
- Knaus, W., K. Luger, W. Zollitsch, H. Gufler, L. Gruber, C. Muraier and F. Lettner (1999). Effects of grass clover-pellets and whole plant maize-pellets on the feed intake and performance of dairy cows. *Anim. Feed Sci. Technol.* 81(3-4): 265-277. [https://doi.org/10.1016/S0377-8401\(99\)00090-5](https://doi.org/10.1016/S0377-8401(99)00090-5)
- Kousar, F., N. Jahan, K.U. Rahman and B. Sultana (2024). Optimization of parameters for the formulation of *moringa oleifera* nanosuspension with enhanced hepatoprotective potential. *Pakistan J. Agric. Sci.* 61(1):116-127. <https://doi.org/10.21162/PAKJAS/24.106>
- Kucukoflaz, M., A.M.Ayyildiz, C. Zaman, E. Aydin and S.Sariözkan (2024). Evaluation of the effect of exchange rate and energy prices on livestock products and feed prices in Türkiye with path analysis. *Kafkas Univ. Vet. Fak. Derg.* 30(4). 533-539. <https://doi.org/10.9775/kvfd.2024.31893>
- Krause, K.M., D.K. Combs and K.A. Beauchemin (2002). Effects of forage particle size and grain fermentability in midlactation cows. I. Milk production and diet digestibility. *J. Dairy Sci.* 85(8):1936-1946. [https://doi.org/10.3168/jds.S0022-0302\(02\)74270-7](https://doi.org/10.3168/jds.S0022-0302(02)74270-7)
- Ku-Vera, J. C., R. Jiménez-Ocampo, S.S. Valencia-Salazar, M.D. Montoya-Flores, I.C. Molina-Botero and J. Arango (2020). Role of secondary plant metabolites on enteric methane mitigation in ruminants. *Front. Vet. Sci.* 7:1-14. <https://doi.org/10.3389/fvets.2020.00584>
- Leitanthem, V.K., P. Chaudhary, M. Bhakat, M. Mohini and G. Mondal (2022). Impact of *Moringa oleifera* on rumen fermentation and methane emission under in vitro condition. *AMB*

- Express, 12(1): 141.
<https://doi.org/10.1186/s13568-022-01480-0>
- Lileikis, T., R. Nainienė, S. Bliznikas and V. Uchockis (2023). Dietary ruminant enteric methane mitigation strategies: current findings, potential risks and applicability. *Anim.* 13(16): 2586.
<https://doi.org/10.3390/ani13162586>
- Loza, C., T. Reinsch, R. Loges, F. Taube, J.I. Gere, C. Klub, M. Hasler, C.S. Malisch (2021). Methane emission and milk production from Jersey cows grazing perennial ryegrass-white clover and multispecies forage mixtures. *Agriculture* 11(2):175.
<https://doi.org/10.3390/agriculture11020175>
- Maña, M.A.T., R.A. Niepes, M.A.C. Florida and R.A. Paculba (2023). Growth performance of goats (*Capra hircus* L.) on forage legumes mixed with guinea grass (*Megathyrsus maximus*). *J. Glob. Innov. Agric. Sci.* 11(3):305-309.
<https://doi.org/10.22194/JGIAS/23.1169>
- Melasari, T., B. Guntoro, M.A.U. Muzayyanah, A. Setiyanto and N.H. Qui (2024). The role of the camara nusantara livestock ship in volatility and disparity of beef and cattle prices in Indonesia. *J. Glob. Innov. Agric. Sci.* 13(2):447-455.
<https://doi.org/10.22194/JGIAS/25.1615>
- Mertens, D. R. (1987). Predicting intake and digestibility using mathematical methods of ruminal function. *J. Anim. Sci.* 64(5):1548–1558.
<https://doi.org/10.2527/jas1987.6451548x>
- Morgavi, D.P., M. Eugene, C. Martin and M. Doreau. (2011). Reducing methane emissions in ruminants: is it an achievable goal. (Mediterranean Options: Series A. Mediterranean Seminars, n. 99). CIHEAM/CSIC/Universidad de León/FAO, Zaragoza, Spain. National Research Council (NRC) (2001). Nutrient Requirements of Dairy Cattle. 7th Edition, National Academic Press, Washington DC, USA.
- Norrapoke, T. and T. Pongjongmit (2025). Effect of high-quality pellet feed level on voluntary feed intake, nutrient digestibility and rumen fermentation in beef cattle. *Scientific Reports*, 15:15343. <https://doi.org/10.1038/s41598-025-96455-z>
- Novianti, J., B.P. Purwanto, D.A. Astuti and A. Atabany (2021). Milk production and metabolite profile of Saanen Goat fed with Moringa oleifera, Sauropus androgynous L. Merr and Coleus amboinicus Lour leaves. *Livest. Res. Rural Dev.* 33(9).
- Oba, M., and M. S. Allen (1999). Evaluation of the importance of NDF digestibility: Effects on dry matter intake and milk yield of dairy cows. *J. Dairy Sci.* 82(3):589–596.
[https://doi.org/10.3168/jds.S0022-0302\(99\)75271-9](https://doi.org/10.3168/jds.S0022-0302(99)75271-9)
- Castelan-Ortega, O.A., P.E. Pedraza Beltrán, G.S. Hernández Pineda, M. Benaouda, M. González Ronquillo, L. Molina, J.C. Ku Vera, H.D. Montelongo Pérez and M.F. Vázquez Carrillo (2020). Construction and operation of a respiration chamber of the head-box type for methane measurement from cattle. *Anim.* 10(2):227. <https://doi.org/10.3390/ani10020227>
- Patra, A.K. and K. Yu (2015). Effects of adaptation of in vitro rumen culture to garlic oil, nitrate, and saponin and their combinations on methanogenesis, fermentation, and abundances and diversity of microbial populations. *Front. Microbiol.* 6: 1434.
<https://doi.org/10.3389/fmicb.2015.01434>
- Pan, J., Y. Wang, Z. Wang, J. Cai, S. Yu, J. Wang, X. Liu, Y. Wu, W. Ou and L. Liao (2023). Effect of different bacterial agents on fermentation quality and microbial community of pigeon pea silage. *Pakistan J. Agric. Sci.* 60(2):193-201.
<https://doi.org/10.21162/PAKJAS/23.225>
- Saleemi, M.K., A. Raza, A. Khatoon, M. Zubair, Y. Xu, B. Murtaza, X. Li, M. Jamil, M. Imran, F. Muhammad and K. Zubair (2023). Toxic Effects of Aflatoxin B1 on hematobiochemical and histopathological parameters of Juvenile White Leghorn male birds and their amelioration with vitamin e and Moringa oleifera. *Pakistan Vet. J.* 43(3):405-411.
<https://doi.org/10.29261/pakvetj/2023.053>
- Saleena, L.A.K., K.L. Nyam and L.P. Pui (2024). Probiotic edible film added with aqueous clove extract milk powder. *Kafkas Univ. Vet. Fak. Derg.* 30(3):375-382.
<https://doi.org/10.9775/kvfd.2024.30895>
- Sarkar, S., M. Mohini, V.M. Nampoothiri, G. Mondal, S. Pandita and M.S.E. Mahesh (2016). Effect of supplementation of Moringa oleifera leaves on in vitro methane emissions and rumen fermentation on roughage-based ration. *Proc. XVI Biennial Animal Nutrition Conference on Innovative Approaches for Animal Feeding and Nutritional Research*, Karnal, India.
- Sarwar, M., J.L. Firkins and M.L. Eastridge (1992). Effects of varying forage and concentrate carbohydrates on nutrient digestibilities and milk production by dairy cows. *J. Dairy Sci.* 75(6):1533-1542.
[https://doi.org/10.3168/jds.S0022-0302\(92\)77910-7](https://doi.org/10.3168/jds.S0022-0302(92)77910-7)
- Shaani, Y., D. Eliyahu, I. Mizrahi, E. Yosef, Y. Ben-Meir, M. Nikbachat (2016). Effect of feeding ensiled mixture of pomegranate pulp and drier feeds on digestibility and milk performance in

- dairy cows. *J. Dairy Res.* 83(1):35–41. <https://doi.org/10.1017/S0022029915000618>
- Shankhpal, S., C. Waghela, P. Sherasia, V. Sridhar, A.K. Srivastava and D. Singh (2019). Effect of feeding moringa (*Moringa oleifera*) as green fodder on feed intake, milk yield, microbial protein synthesis and blood profile in crossbred cows. *Ind. J. Anim. Nutr.* 36 (3):228–234. <https://doi.org/10.5958/2231-6744.2019.00038.0>
- Soliva, C.R., M. Kreuzer, N. Foidl, G. Foidl, A. Machmuller and H.D. Hess (2005). Feeding value of whole and extracted *Moringa oleifera* leaves for ruminants and their effects on ruminal fermentation in vitro. *Anim. Feed Sci Technol* 118(1-2):47–62. <https://doi.org/10.1016/j.anifeedsci.2004.10.005>
- Storm, I.M., A.L.F. Hellwing, N.I. Nielsen and J. Madsen (2012). Methods for measuring and estimating methane emission from ruminants. *Anim.* 2(2)160-183. <https://doi.org/10.3390/ani2020160>
- Tedeschi, L.O., A.L. Abdalla, C. Alvarez, S.W. Anuga, J. Arango, K.A. Beauchemin, P. Becquet, A. Berndt, R. Burns, C. De Camillis and J. Chara (2022). Quantification of methane emitted by ruminants: a review of methods. *J. Anim. Sci.* 100(7)1-22. <https://doi.org/10.1093/jas/skac197>
- Truong, N.B. and N.V. Thu. (2023). Effect of crossbreeding and dietary neutral detergent fiber (NDF) levels on feed intake, nutrient digestibility, rumen parameters and nitrogen retention of beef cattle. *J. Glob. Innov. Agric. Sci.* 11(1):579-586. <https://doi.org/10.22194/JGIAS/23.1094>
- Uden, P. (1988). The effect of grinding and pelleting hay on digestibility, fermentation rate, digesta passage and rumen and faecal particle size in cows. *Anim. Feed Sci. Technol.* 19(1-2):145-157. [https://doi.org/10.1016/0377-8401\(88\)90063-6](https://doi.org/10.1016/0377-8401(88)90063-6)
- United Nations, Department of Economic and Social Affairs, Population Division (2017). World Population Prospects: The 2017 Revision, Key Findings and Advance Tables. Working Paper No. ESA/P/WP/248. United Nations, New York, 2017.
- Van Soest, P. J. (1994). *Nutritional Ecology of the Ruminant*. 2nd ed. Cornell Univ. Press, Ithaca, New York.
- Van Soest, P.V., J.B. Robertson and B.A. Lewis (1991). Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74(10):3583-3597. [https://doi.org/10.3168/jds.S0022-0302\(91\)78551-2](https://doi.org/10.3168/jds.S0022-0302(91)78551-2)
- Waldo, D. R. (1986). Symposium: Forage utilization by the lactating cow. Effect of forage quality on intake and forage-concentrate interactions. *J. Dairy Sci.* 69(2):617–632.
- Weiss, W. P. and D. J. Wyatt (2002). Effects of feeding diets based on silage from corn hybrids that differed in concentration and in vitro digestibility of neutral detergent fiber to dairy cows. *J. Dairy Sci.* 85(12):3462–3469. [https://doi.org/10.3168/jds.S0022-0302\(02\)74434-2](https://doi.org/10.3168/jds.S0022-0302(02)74434-2)
- Woodford, J. A., N. A. Jorgensen, and G. P. Barrington (1986). Impact of dietary fiber and physical form on performance of lactating dairy cows. *J. Dairy Sci.* 69(4):1035-1047. [https://doi.org/10.3168/jds.S0022-0302\(86\)80499-4](https://doi.org/10.3168/jds.S0022-0302(86)80499-4)
- Yue, S., X. Li, J. Qian, J. Du, X. Liu, H. Xu, H. Liu and X. Chen (2023). Impact of enzymatic hydrolyzed protein feeding on rumen microbial population, blood metabolites and performance parameters of lactating dairy cows. *Pakistan Vet. J.* 43(4):804-808. <https://doi.org/10.29261/pakvetj/2023.081>
- Zebeli, Q., J. R. Aschenbach, M. Tafaj, J. Boguhn, B. N. Ametaj and W. Drochner (2012). Invited review: Role of physically effective fiber and estimation of dietary fiber adequacy in high-producing dairy cattle. *J. Dairy Sci.* 95(3):1041–1056. <https://doi.org/10.3168/jds.2011-4421>
- Zhang, T., B. Si, K. Deng, Y. Tu, C. Zhou and Q. Diao (2018). Effects of feeding a *Moringa oleifera* rachis and twig preparation to dairy cows on their milk production and fatty acid composition, and plasma antioxidants. *J. Sci. Food Agric.* 98(2):661-666. <https://doi.org/10.1002/jsfa.8511>
- Zhou, M., Y. Zeng, W. Zhou, H. Zhu, Y. Xing, X. Dong and D. Chen. (2024). Supplementation of *Laminaria japonica* powder influence ruminal microbiota without affecting ruminal fermentation in bulls. *Pakistan Vet. J.* 44(4)1-8. <https://doi.org/10.29261/pakvetj/2024.265>