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TO WHAT EXTENT DRY *MORINGA OLEIFERA* LEAVES SUPPLEMENTATION COULDINFLUENCE GROWTH PERFORMANCE OF SHEEP AND AFFECT METHANE PRODUCTION.

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SUMMARY

In a feeding experiment lasted 70 days, the nutritional supplementation effect of Dry Moringa *oleifera* leaves (DML) was investigated on growth performance and rumen fermentation indices on 20 local Barki male lambs aged 6 months and weighed 24.4±1.12 kg. Lambs were randomly allocated into four equal groups where they individually fed daily on a uniform diet consisted of: 3% of body weight (BW) concentrate feed mixture (CFM) plus 1.25 kg whole corn silage and chopped rice straw fed ad-libitum. The first group (R1) was fed on un-supplemented CFM (control), while diets of R2, R3 and R4 were supplemented with 100,200 and 400 mg DML/kg BW, respectively. The offered amounts of CFM + DML were bi-weekly adjusted according to body weight change. The results illustrated that; the daily DM intake reduced by 17% with R4 diet (400mg DML) than other groups. The best ($P<0.05$) values of final body weight, ADG and feed conversion efficiency were recorded for lambs fed R3 diet (200mg DML), while corresponding parameters of R2(100mg ML) and control showed comparable values. With increasing DML supplementation to 400mg/kg BW (R4) ADG was remarkably fallen dawn to nearly 50% than other groups. Similar trend was achieved for nutrients digestibility and dietary nitrogen utilization, where the best results were recorded with R3 and the worst with R4. Moreover, blood plasma urea, creatinine, AST and ALT had the highest ($P<0.05$) values with R4 than other groups, while cholesterol was decreased ($P<0.05$) with increasing DML supplementation level. Ruminal NH_3-N concentration was ($P<0.05$) decreased with increasing DML level, while the highest TVFAs was recorded with R3 and the lowest with R4. In-vitro enteric methane production (mg/day) was decreased ($P<0.05$) with increasing DML supplementation level, however, the lowest CH_4 (mg/kg DMI) was recorded with R3 diet. *In-vivo* ruminal microbial protein had the highest yield ($P<0.05$) with R3 and the lowest with R4. It is worth saying that, *dry Moringa oleifera* leaves is useful growth promoter for lambs when supplemented at the maximum of 200mg/kg BW or in average 5 gm/day.

***Key words**: Moringa oleifera leaves, lambs, growth performance, enteric methane production, microbial protein yield.*

INTRODUCTION

Since the year 2006, the vigor use of antibiotic growth promoters (AGPs) had been banned by the European Commission due to the growing resistance of pathogenic microbes to antibiotics and its possible accumulation in animal products (milk and meat) which could badly hurt consumers health. This situation has forced nutritionists to find safe, effective, and low cost economic bioactive substances to replace AGPs in the feeding strategy of commercial livestock.

Nowadays, numerous feed additives are available and efficiently used in the daily feeding practices of beef and dairy farm stations. Unsaturated fatty acids, essential-sulfur-containing amino acids, vitamins, chelated minerals, organic acids, ionophores, probiotics (Helal *et al*., 2021), prebiotics, and plant extracts and their secondary metabolites (El-Naggar *et al*. 2017a), all are being used as feed supplements to enhance feed utilization and promote animal productivity (Soliva *et al*. 2005; El-Naggar *et al*. 2018). In tropical and subtropical regions where good quality forages are scarcely available, so the use of fodder trees, browses and shrubs had been confirmed for ruminant feeding (Benninson and

Paterson, 2003). Their use as supplements to poor quality roughages has been shown to improve intake, increase growth rate and improve reproduction efficiency in ruminants (El-Naggar and Ibrahim 2018).

Moringa oleifera Lam is cultivated in all types of soils whenever irrigated water is available and can tolerate the climatic changes of tropical and subtropical zones (Trigo *et al*. 2021). Almost all parts of Moringa (flowers, leaves, park, fresh pods and seeds) are traditionally consumed fresh or dry for their nutritious and therapeutic effects not only for human but also for monogastric and ruminant animals (El-Naggar 2017b). Moringa dry leaves have been noted to contain 23-31% crude protein with appreciable essential amino acids content, low fiber content (5.9-6.9), and relatively high fat (6-8%), also α-linolenic acid has the highest content where more than half of fatty acids are unsaturated fatty acids, besides those leaves contain effective phytogenic substances, vitamins, and minerals (Moyo *et al*., 2011). Also Moringa leaves could provide Vit.C 7 times than in orange, calcium 17 times more than milk, Vit. A 10 times more than carrots, potassium 15 times more than bananas, protein 9 times more than yoghurt, and iron 25 times more than spinach (Rockwood *et al*. 2013). Moringa leaves are also a rich source of some important phytochemical compounds such as flavonoids, isoflavones, terpenoids, sterols, saponins and tannins, along with glycosylate and isothiocyanate (Abdel-Raheem and Hassan 2021). It's doubtless, that there are considerable variations of chemical and nutritional characteristics of Moringa among different research studies due to different effective factors i.e.; plant genetic make-up, type of planting soil, irrigation water quality, cultivation method, age at harvest, and environmental temperature (Brisibe *et al*. 2009). Moringa leaves (ML) are gradually gaining importance as a protein supplement to upgrade the nutritional quality of some crop residues (Mendieta-Araica *et al*. 2011). The beneficial effects of feeding ML to different livestock were noted by many workers; ML protein was recommended to replace oil seed cake protein up to 50% in the diets of cattle (Mendieta-Araica *et al*. 2011), goats (Babeker and AbdAlbagi 2015, Kholif *et al*. 2016), and rabbits (Bakr 2019). Redekar *et al*. (2019) noted that inclusion of moringa leaves as a protein source up to 20% of the diet enhanced growth performance of growing sheep. The antipathogenic effect of Moringa was studied by Abd El-Moez *et al*. (2014) who stated that Moringa ethanolic extract was more effective than oxytetracycline against *E. coli*, *Salmonella typhimurim*, *Shigella boydii*, and *Staphylococcus aureus.* Moreover, dry moringa leaves were noted to eliminate enteric methane emission in the rumen environment of lactating cattle, goats and growing sheep (SU and Chen 2020). Moringa leaves are a rich source of some important phytochemical compounds which increase immunity of animals and decrease methane production, so that enhance animal performance and mitigate climate change.

The current study aimed to investigate the effects of different supplementation levels of dry Moringa leaves (DML) at 100, 200, and 400 mg/kg BW in diets of Egyptian Barki lambs on feed intake, weight gain, nutrients digestibility, dietary nitrogen utilization, blood biochemical indices, ruminal fermentation and *in vitro* enteric methane production.

MATERIAL AND METHODS

Site of work and Moringa leaves preparation:

This study was implemented jointly between Animal Research Nubaria Station Affiliates Animal Production Research Institute, Ministry of Agriculture, Dokki, Giza, Egypt, and laboratories of Animal Production Department of the National Research Centre, Ministry of Higher Education and Scientific Research, Dokki, Giza, Egypt. The experimental animal farm is located in Nubaria province (150 Km West of Northern Cairo city). Fresh Moringa oleifera leaves (ML) were brought from a private plant farm of newly reclaimed sandy soil. Fresh Moringa leaves were collected from Moringa shrubs, washed, spread in a thin layers over a plastic sheet, and left to dry in an open shaded area with daily shuffling upside down until sun drying (50 to 60% moisture). Moringa leaves were then oven dried at $40C^{O}$ for 96 hours until the moisture reached 11-13%. Dry leaves were collected, finally ground, sieved through 1mm mesh screen and the whole amount was then packed in polyethylene bags until been used.

Experimental lamb's management and feeding program:

Twenty Barki male lambs weighed 24.4±1.12 kg aged 6 months old were used in a feeding experiment lasted 70 days. Lambs were vaccinated against infectious diseases and injected against internal and external parasites with Ivermectin four weeks before starting the experiment. All lambs were housed in open shaded pens fitted with wooden barriers to allow individual feeding.

Experimental lambs were randomly divided by weight into four equal groups of 5 animals each. Animals in the control group (R1) were fed daily on a concentrate feed mixture (CFM) at 3% of body weight + 1.25Kg fresh whole corn silage + *adlibitum* chopped rice straw (RS), while animals of R2, R3, and R4 groups were fed the same amounts of the basal diet with CFM supplemented with 100, 200 and 400 mg of DML/Kg body weight, respectively. Daily offered amounts of un-supplemented or DML supplemented CFM were biweekly adjusted according to body weight changes. The CFM was offered once daily at 8.00 AM and silage at 11,00 AM, while RS was offered at all times. Drinking water in buckets was freely available all day round. Feed residues (of mostly RS) were daily collected, sun-dried, and weekly weighed to calculate the actual feed intake and tested diets were manipulated to provide 66-68% TDN and 14-15% CP and the daily offered amounts of experimental diets were calculated to provide adequate energy and protein needed for maintenance balance and 200 g daily gain requirements (NRC, 2007).

At end of the feeding period, four metabolism and nitrogen balance trials were conducted on three random lambs from each group to determine nutrient digestibility and dietary nitrogen (N) utilization of experimental diets. In parallel, blood samples from the jugular vein were collected in heparinized tubes, centrifuged at 3500 rpm for 15 minutes and separated blood plasma were kept in clean vails at - 4C^{o} until further analysis.

Samples of rumen liquor were individually collected from three fistulated adult rams fed the same rations after 3hrs. of feeding the CFM, collected liquor was directly tested for pH and then strained through four layers of cheese close to determine ammonia nitrogen (NH_3 -N) and total VFAs contents. *In vitro* enteric methane production and *in-vivo* microbial protein synthesis using the three fistulated adult rams were also investigated.

Analytical methods and calculations:

Determination of chemical composition of Moringa leaves were conducted at the Central Lab. of the National Research Centre**,**Moringa leaves chemical composition was determined according to AOAC (2019). Macro minerals (Ca, P, K and Mg) were determined by a flame photometer (model: Jenway PFP7) and micro minerals (Mn, Zn, Fe and Cu) were determined by atomic absorption (model: Spectrometer analysis 400). Amino acids were estimated by amino acid auto-analyzer. Phytochemical compounds of dry Moringa leaves were estimated in 70% ethanolic solution. Total chlorophyll was spectrophotometrically detected at 649 and 665 nm. Total carotenoids content was spectrophotometrically estimated at 450 nm according to AOAC (2019). Total phenolic compounds were calorimetrically estimated using Folin-Ciocalteu’s with galic acid as a standard. Total flavonoids were estimated spectrophotometrically at 420 nm and the concentration was calculated relative to a standard quercetin calibration curve.

Feeds and feces chemical composition of dry matter (DM), crude protein (CP), crude fiber (CF), ether extract (EE), ash and Urinary-N were carried out according to AOAC (2019). Organic matter (OM) and nitrogen free extract (NFE) were calculated by difference. Collected rumen liquor was directly tested for pH using Orian 680 digital pH meter. Rumen ammonia-N (NH_3N) was determined calorimetrically according to Searle (1984). Total VFAs of strained rumen liquor was determined using a gas chromatograph (GC-2010, Shimadzu, Kyoto, Japan) equipped with a Flame Ionization Detector and a capillary column (HP-INNOWAX, 1909N-133, Agilent Technologies, Santa Clara, CA, USA). Blood plasma total protein, albumin, Urea, Creatinine, Cholesterol, Liver transaminases activity for aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined using colorimetric methods (Biodiagnostic, Egypt), while globulin was calculated as the difference between total protein and albumin.

Ruminal microbial protein (MP) synthesis was estimated on three fistulated adult rams fed sequentially the tested diets each for 12 days as an adaptation period and two days for rumen liquor collection at 3, 6, 9, 12 and 24 hrs. of feeding. Total VFAs production rate was determined to predict the microbial protein yield from the equation suggested by Borhami *et al*. (1992) as follows:

$$\text{MP g/day} = \text{mole VFAs produced/day} \times 2 \times 13.48 \times 10.5 \times 6.25/100$$

Where one-mole VFA yields about 2 moles ATP, one mole ATP produces 13.48 g DM microbial cells, N% of dry microbial cells= 10.5 and 6.25/100 as g protein contains 16% nitrogen.

In vitro methane production was carried out by applying the gas production (GP) assay adapted to a semi-automatic system (Bueno *et al*. 2005). Ground feed samples (0.3g) were incubated with 45ml buffered rumen fluid in 120ml serum bottles closed with a rubber stopper and the bottles were kept in a shaker waterbath at 39^{o}C. At end of the incubation period (24 hrs.), the methane (CH_4) fraction of accumulated gas was determined by gas chromatography and its production was estimated according to the following equation:

CH_4= [GP+HS] x Conc., where CH_4 is the volume (ml) of methane, GP is the volume of gas produced (ml), HS is the volume (ml) of the bottle headspace and Conc. is the percentage of methane in the gas samples (Tavendale *et al*. 2005).

Statistical analysis:

Collected data of measured parameters were subjected to one-way analysis of variance using the general linear model of SAS (2007) following the mathematical model Yij = μ + Rij + E ij, where: μ is the overall mean; Rij is the treatment effect; Eij is the experimental error. Duncan's Multiple Range Test (Duncan, 1955) was applied to separate significant means.

RESULTS AND DISCUSSIONS

The proximate chemical composition of experimental feedstuffs is given in Table (1). Moringa dry leaves (ML) used in this study (Table 2) had 27.3**5**% crude protein, Su and Chen (2020) reported from different studies that on a dry matter basis, the CP content of Moringa leaves was ranging 23.0 to 30.3% comparable to the CP content of cotton seed meal.

Table (1): Chemical composition of diets fed to growing sheep.

Item	Moisture %	DM composition, %					
		OM	CP	CF	EE	NFE	Ash
*CFM	11.42	94.31	14.55	7.31	2.53	69.92	5.69
Whole corn silage	70.43	90.56	8.12	23.84	1.73	56.87	9.44
Rice straw	11.43	83.06	3.19	36.32	0.84	42.71	16.94

**CFM: 35% ground yellow corn, 15% wheat bran, 10% barley grains, 10% soya bean meal, 16.5% un-decorticated cotton seed meal, 5% sun flower meal, 5% cane molasses, 2% lime stone, 1.5% sodium chloride.*

Table (2): Summary of the most noticeable substances of dry Moringa leaves used.

Chemical composition, %							
DM	OM	CP	EE	NDF	ADF	ADL	Ash
89.82	87.54	27.35	08.33	12.01	10.22	04.95	12.46

Amino Acids, %					
The highest			The lowest		
Leucine	Phenylalanine	Lysine	Methionine	Tryptophan	Histidine
1.95	1.79	1.64	0.13	0.43	0.79

Macro-elements, %				Micro-elements, mg/kg			
Ca	P	K	Mg	Mn	Zinc	Iron	Cu
3.72	0.38	1.37	0.47	48.51	40.00	558.69	6.34

Phytochemicals, mg/kg			
Total chlorophyll	Total carotenoids	Total phenols	Total flavonoids
9.87	1.36	41.34	22.57

Lipids content of DML was 8.34% which noted to be higher than lipids found in other woody plant forages, and was higher than 6.50% noted by Moyo *et al*. (2011), but within the lipids content mentioned by Su and Chen (2020). Fiber fractions namely; NDF, ADF, ADL contents were 12.01, 10.22, and 4.95%, respectively and were slightly different than those mentioned by Moyo *et al*. (2011), but in general most studies pointed to that DML had a low level of structural carbohydrates which makes it a valuable source of readily fermentable carbohydrate for ruminants feeding and nutritious feed for monogastric animals. Ash content of DML in this study was high (12.46%) which could be regarded to its high contents of macro and micro-elements, while comparable ash content (10.50%) was noted by

Babeker and Abdalbagi (2015) for Moringa leaves cultivated in Sudan, while, lower values (8.21 %) were recorded by Zaza *et al*. (2017) in Egypt.

Moringa oleifera used in this study, contained 16 amino acids where 6 of which were essential namely; Methionine, Leucine, Lysine, Histidine, Tryptophan and Phenylalanine as shown in Table (2), DML had poor content of Methionine (1st limiting amino acid) and this agree with reported by Su and Chen (2020). followed by Tryptophan but high contents of Leucine (1.95 %), Phenylalanine (1.79%) then (1.64%) for Lysine. These essential amino acids particular are the principle molecular units that determine protein quality and its utilization efficiency (Su and Chen 2020).

Calcium (Ca) had the highest value of 3.72% followed by potassium (K) (1.37%) and phosphorus (P) had the least value of 0.38% among macro-elements, dry moringa leaves were rich in Iron being 558.69 mg/kg followed by Manganese (48.51) and 40.00 mg/kg for Zinc, while Copper recorded the lowest value (6.36), micro and macro elements of DML in these results were in accordance with those reported by Moyo *et al*. (2011). Phytochemical contents of ML revealed that it contained 1.36 mg total carotenoid, 41.34 mg total phenols, and 22.57 mg of total flavonoids per kg dry leaves. Moreover, Moringa leaves were noted to contain abundant contents of polyphenols in this study (41.34 mg/kg dry leaves), where flavonoids consisted nearly 50 % of it (22.57 mg/kg). β-carotene is the main precursor for vitamin A and consisting 47% of the total carotenoids in Moringa leaves, beside its natural high content of vitamin A (Su and Chen,2020). Polyphenols especially flavonoids have antioxidant properties that eliminate the harmful effect of oxidative stress that takes place in animals exposed to unfavorable environmental and nutritional conditions (Ceci *et al*. 2022).

In general, the variations among different studies concerning chemical composition and other nutritional substances of dry Moringa leaves could be due to age at harvest, type of soil and its fertility, the proportion of leaf, and agro-ecological zone where trees are grown (Brisibe *et al*. 2009).

Growth performance:

The effect of DML supplementation on feed intake, body weight gain, and feed conversion are presented in Table (3).

Table (3): Feed intake, body weight gain and feed conversion of lambs in experimental groups (means±SE).

Item	Experimental groups			
	0 ML R1 (Control)	100 mg ML/kgBW R2	200 mg ML/kgBW R3	400 mg ML/kgBW R4
Experimental feeding period	70 days			
No. of animals	5	5	5	5
Initial body weight, kg	24.6±1.03	24.4±0.60	24.4±1.09	24.2±2.32
Final body weight, kg	35.7 ^{b} ±1.64	35.9 ^{b} ±0.84	36.4 ^{a} ±1.56	30.2 ^{c} ±2.63
Total gain, kg	11.1	11.5	12.0	6.0
Average daily gain, g	158.6 ^{b} ±4.51	164.3 ^{b} ±3.24	171.4 ^{a} ±5.62	85.7 ^{c} ±4.59
Feed intake (DM basis), g/h/day:				
Concentrate feed mixture	794.4	774.9	772.5	716.1
Corn silage	286.5	318.8	351.6	252.0
Rice straw	125.5	128.2	130.7	100.0
Total DM intake (DMI)	1206.4 ^{a} ±29.5	1221.9 ^{a} ±23.7	1254.8 ^{a} ±44.6	1068.1 ^{b} ±91.8
Feed conversion, g DMI/g gain	7.65 ^{b} ±1.52	7.35 ^{b} ±1.28	7.24 ^{b} ±0.86	12.56 ^{a} ±3.58

a,b,c,d means within rows followed by different superscripts are significantly different at (P<0.05).

The average daily gain of lambs was significantly higher (P<0.05) in R3 (171.4 g) than those of R2 (164.3 g) and control (158.6 g) groups. The lowest (P<0.05) body weight gain was recorded on lambs of R4 (85.7 g) where weight gain was dramatically fallen by 50% than other groups, and this similar with found In some previous studies concerned with ML as an alternative feed protein (Melesse *et al*. 2015, Abdel-Raheem and Hassan 2021) who reported that average daily gain of buffalo calves and goat kids were significantly improved than the control group with a diet contained 15-20% ML but that improvement was reduced with diet contained 50% ML. On the contrary, Zaza *et al*. (2017) did not find any adverse effect of weight gain or feed intake on growing Rahmani sheep fed 10 and 20 gML/kg diet.

The daily DM intake of lambs in R1, R2, and R3 groups were nearly comparable, this result is agreed with reported by Soliva *et al*. (2005) that ML is a good protein source that is a convenient substrate of some meals (soybean and rapeseed) for ruminants, and it can improve the microbial protein synthesis in the rumen without deleterious effect on daily intake, while with of R4 diet (400mg Moringa/ kg BW) decreased ($P<0.05$) by about 17% than other groups. Feed conversion efficiency was slightly better than the control for lambs fed 100 or 200mg ML supplemented diets, while lambs fed 400mg ML diet recorded the worst ($P<0.05$) value being 12.56kg DM/kg BW gain.

The growth curve presented in Figure (1) indicates that the bad effect of the highest DML supplementation level (400mg) was detected within the first two weeks of feeding and continued then after until end of the feeding experiment. Meanwhile, BW change of other DML supplemented groups surpassed control after 8 weeks of feeding.

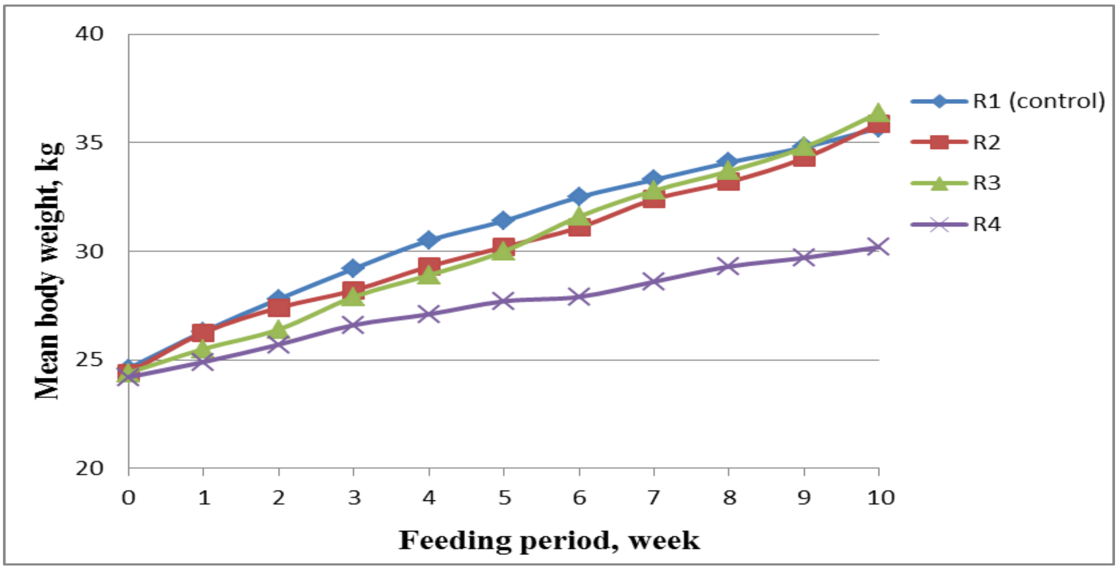

Figure (1): Development of lamb's body weight during experimental feeding period.

Generally, most previous studies illustrated that daily feed intake and body weight gain tended to be reduced with increasing ML supplementation levels. However, the suitable ML feeding level and the reason of positive or negative effects on weight gain or voluntary feed intake were not clearly evidenced in previous studies due to limited numbers of animals, short-term feeding, different animal species, and ages, different managerial systems, age of Moringa trees and way of harvest and drying leaves (Dong *et al*. 2019).

Apparent nutrients digestibility, and dietary N utilization:

Digestibility, nutritive value, and dietary N utilization of experimental diets were presented in Table (4). Diets supplemented with 100 or 200mg ML /kg body weight had higher ($P<0.05$) apparent digestibility for OM, CP, CF, EE, and NFE in comparison to control, and the lowest values were recorded with R4 diet (400 mgML), . The same trend was noticed on dietary nutritive values expressed in terms of TDN and DCP, these results are in agreement with the finding of El-Badawi *et al*. (2014) who found that nutrients digestibility along with TDN and DCP values were increased in rabbits with increasing level of ML up to 0.30% of the total ration, while the values were significantly decreased with 0.45% ML supplementation level.

The digestibility of CP and CF were inversely related to DM intake however, this statement does not match the digestibility results of R4 group where the daily intake was reduced by nearly 17% than other groups. In the contrast, the higher CP and CF digestibility associated with the gradual increase of DML supplementation up to 200 mg/kg BW suggests an increase in the activities of cellulolytic bacteria in the rumen media due to the high level of antioxidant substances existing in Moringa leaves (flavonoids, carotenoids, flavones, kaempferol, quercetin) (Saini *et al*., 2016), these substances were noted to enhance ruminal microbial multiplication and increase microbial protein yield that enhance fiber digestion in animals. Meanwhile, the adverse effect of the high supplementation level of DML (400mg) on nutrients digestibility could be related to the accumulative effect of some anti-microbial substances (glycosylates and isothiocyanate) which are naturally occurring in ML (Sarwatt *et al*., 2004). It was clear that the supplementation of DML in diets of growing sheep up 200 mg/ kg BW could promote nutrients digestibility and enhance dietary utilization efficiency. In other previous studies that introduced ML as a source of protein, Sanchez *et al*. (2006) stated that with a basal diet of low-quality grass digestibility of nutrients was increased with an increasing level of ML supplementation. In similar trend, Gebregiorgis *et al*. (2012) also reported that the apparent nutrients digestibility increased with increasing ML supplementation levels up to 450 g in diets of sheep fed Rhodes grass hay. None of such studies reported any deleterious or toxic effects of feeding level of polyphenolic compounds which are existing in abundant amounts in Moringa leaves and it has a harmful effects on the rumen microbial population (cellulolytic bacteria and protozoa) and could damage liver and kidney tissues (El-Badawi *et al*., 2014; Stohs and Hartman, 2015; Dong *et al*., 2019).

Table (4): Nutrients digestibility (%), nutritive value and dietary N utilization of experimental diets fed by lambs (means±SE).

Item	Experimental groups			
	0 ML R1 (Control)	100 mgML/kgBW R2	200 mgML/kgBW R3	400 mgML/kgBW R4
OM	61.58^{c} ±0.31	63.75^{b} ±0.29	65.05^{a} ±0.18	59.91^{d} ±0.17
CP	61.39^{b} ±0.49	63.42^{a} ±0.25	65.09^{a} ±0.32	57.98^{c} ±0.66
CF	56.46^{c} ±0.42	59.78^{b} ±0.44	62.04^{a} ±0.23	51.10^{d} ±0.57
EE	71.06^{b} ±0.22	71.80^{b} ±0.20	72.75^{a} ±0.18	68.62^{c} ±0.52
NFE	62.75^{c} ±0.28	64.67^{b} ±0.27	65.66^{a} ±0.25	62.31^{c} ±0.32
Nutritive value, %:				
TDN	64.68^{c} ±0.30	67.57^{b} ±0.28	69.54^{a} ±0.19	61.10^{d} ±0.16
DCP	6.30^{bc} ±0.07	6.46^{ab} ±0.02	6.60^{a} ±0.03	6.10^{c} ±0.09
Dietary N Utilization				
N intake, g	17.54^{b} ±0.02	17.70^{ab} ±0.03	17.97^{a} ±0.12	16.75^{c} ±0.02
Fecal N, g	6.77^{b} ±0.07	6.48^{bc} ±0.06	6.27^{c} ±0.09	7.07^{a} ±0.10
Urinary N, g	5.42^{a} ±0.11	5.40^{a} ±0.21	4.75^{c} ±0.10	5.17^{b} ±0.14
N- retention (NR), g	5.35^{c} ±0.05	5.82^{b} ±0.08	6.95^{a} ±0.07	4.51^{d} ±0.12
NR/NI, %	30.51^{c} ±0.34	32.89^{b} ±0.53	38.69^{a} ±0.32	26.95^{d} ±0.91

a,b,c,d means within rows followed by different superscripts are significantly different at (P<0.05).

Results of Nitrogen (N) balance of sheep fed experimental diets showed that all diets had positive N balance, an indication that the protein requirements for maintenance and growth were adequately met by the dietary treatments. N intake recorded the lowest (P<0.05) value with R4 diet due to mostly the lowest DM intake associated the highest supplementation dosage of DML. Akinyemi *et al*. (2010) concluded that the optimum inclusion level at which the best N-balance and N-retention were reached was with 25% ML inclusion of Panicum based diet fed to sheep.

Fecal N was decreased (P<0.05) than control with diets supplemented with DML up to 200 mg/kg BW. However, fecal N was increased (P<0.05) with 400 mg ML supplemental diet than other groups as a direct result of the lowest nutrients digestibility recorded with R4 diet. In the contrast, urinary N loss was decreased than control (P<0.05) particularly with 200 mgML diet, and the alleviation effect of DML on urinary N loss was also noted with feeding R4 diet than the control or 100 mg DML but was higher (P<0.05) than that with 200 mg ML diet. Apparent N retention generated from the level of intake, rate of digestibility, and absorption showed that DML supplemented diets had a positive effect on dietary N utilization and the best result was recorded with 200 mg diet. Meanwhile, such a positive effect was not attained with feeding lambs on 400 mgML diet where N retention was lower (P<0.05) than control. Calculated N retention relative to N intake had a similar trend as that of apparent N utilization. As known the high N intake coupled with high urinary N excretion demonstrated that the dietary protein fed over animal requirements is catabolized and excreted in the urine. But in the current study, the apparent N retention relative to N intake had the opposite direction under the administration of the high supplementation level of DML (400mg) which might suggest that DML has substances that inhibit protein digestion and utilization of these substances; polyphenols, protease inhibitors, and lectins which make up the majority of antinutritional substances in Moringa leaves (Su and Chen, 2020).

Rumen environment:

Rumen fermentation parameters detected after 3 hrs. of feeding are given in Table (5). Rumen pH values in all experimental diets were in the normal range (Ørskov and Ryle, 1990), but it decreased with increasing DML supplementation up to 200 mg ML, suggested that with lower DML supplementations (100 and 200 mg/kg BW) polyphenols might enhance ruminal microbial activity, while with the highest DML supplementation (R4) accumulation of some anti-microbial substances might inhibit ruminal fermentation and increase rumen pH value (Su and Chen, 2020).

Table (5): Rumen fermentation of lambs fed experimental diets (means±SE).

Item	Experimental groups			
	0 ML R1 (Control)	100 mgML/BW R2	200 mgML/KgBW R3	400 mgML /kg BW R4
pH	6.33^{b} ±0.15	6.31^{b} ±0.12	6.26^{c} ±0.15	6.69^{a} ±0.11
NH_3-N (mg/100ml)	14.56^{a} ±0.15	13.76^{b} ±0.14	13.58^{b} ±0.19	13.52^{b} ±0.22
TVFAs (meq/100ml)	10.44^{c} ±0.11	10.89^{b} ±0.18	12.16^{a} ±0.13	9.13^{d} ±0.13

a,b,c,d means within rows followed by different superscripts are significantly different at (P<0.05).

Values of NH_3-N in all groups were well above the minimum concentration required for optimal microbial growth (Satter and Slyter, 1974). Ammonia-N concentration in the rumen fluid was decreased (P<0.05) with increasing DML supplementation level than the control without any significant difference among supplemental groups, Moante *et al*. (2004) reported that ruminal NH_3-N concentration is regulated by protein intake, rate of protein degradability, the transit time of feed in the rumen, and pH of the rumen liquor. The lower NH_3-N concentration of DML diets than control might be regarded to some protein binding substances existing in Moringa leaves like tannins which could reduce dietary protein degradability.. Total VFAs concentration was increased (P<0.05) with increasing DML supplementation level up to 200 mg/kg BW than control, but it was remarkably decreased to 9.13 meq/dl with 400 mgML diet in comparison to 10.44, 10.89 and 12.16 meq/dl for 0, 100, and 200 mg ML diets, respectively.

The latter result of methane production (Table 6) could not be explained without accurate studies on the effect of the level of Moringa leaves on rumen microbiota.

Where the highest yield of microbial protein (MP) (P<0.05) was recorded with R3 (38.92 g/d) and the lowest with R4 (32.28 g/d), while control and 100 mg DML diets were almost comparable. On basis of ruminal pH and TVFAs concentration, it seems logical to state that the high supplementation dosage of DML (over 200 mg) had a bad effect on the rate of rumen fermentation and have negatively effect on rumen microorganisms and cause lower CP degradability and consequently it affects the microbial protein synthesis possibly due to the accumulation of some antinutritional substances in DML (Abdel-Raheem and Hassan, 2021).

Table (6): In vitro methane production and microbial protein yield of lambs fed experimental diets (means ±SE).

Item	Experimental groups			
	0 ML R1 (Control)	100 mgML/kgBW R2	200 mgML/kgBW R3	400 mgML/kgBW R4
gCH_4 /day	26.50^{a} ±0.15	25.60^{b} ±0.15	24.10^{c} ±0.21	24.00^{c} ±0.12
gCH_4 /kg DMI	26.03^{a} ±0.10	24.68^{c} ±0.16	22.73^{d} ±0.11	25.33^{b} ±0.14
Microbial protein, g/d	35.22^{b} ±0.45	35.47^{b} ±0.56	38.92^{a} ±0.44	32.28^{c} ±0.26

a,b,c,d means within rows followed by different superscripts are significantly different at (P<0.05).

Daily methane production (gCH4/day) was decreased with an increasing level of DML supplementation. The same significant (P<0.05) trend was recorded when CH_4 production was calculated relative to DM intake (g/kg) being 24.68 and 22.73 g with R2 and R3, respectively vs.26.03 g for the control diet. Meanwhile, methane production had an inverted direction where it rose to 25.33 g/kg DMI with R4 diet.

Even though, it was noticed that DML supplementation had a role in direct inhibition of methanogenesis by reducing hydrogen supply so it has been shown to reduce enteric CH_4 emissions (Polyorach *et al*., 2014). Also, the use of a high concentrate portion favors the production of propionate and reduces pH, thus inhibiting methanogen and protozoal growth (Boadi *et al*., 2004). In the same context, Soliva *et al*. (2005) concluded that ML could be used as an effective natural intervention to monensin in sheep diets to not only to reduce CH_4 production, but also to enhance ruminal efficiency of dietary nutrient utilization. The authors added that ML was found to be a methanogen inhibitor, thus it was suggested to be an alternative to replace critical antibiotic feed additives to alternate the ruminal fermentation pathways. Similarly, Dey *et al*. (2014) suggested that such effects might relate to the existence of saponins or tannins in ML.

Blood biochemical indices:

Mean values of blood serum biochemical constituents for lambs in experimental groups (Table 7) were almost within the normal reference range of growing sheep as reported by Varanis *et al*. (2021).

Table (7): Blood plasma biochemical indices of lambs fed experimental diets (means±SE).

Item	Experimental groups			
	0 ML R1 (control)	100 mgML/kgBW R2	200 mgML/kgBW R3	400 mgML/kgBW R4
Total protein, g/dl	6.67 ^{b} ±0.11	6.61 ^{b} ±0.05	6.80 ^{a} ±0.10	6.56 ^{b} ±0.08
Albumin, g/dl	2.32 ^{c} ±0.03	2.52 ^{c} ±0.06	2.90 ^{a} ±0.13	2.74 ^{b} ±0.11
Globulin, g/dl	4.35 ^{a} ±0.08	4.09 ^{b} ±0.12	3.90 ^{c} ±0.06	3.82 ^{c} ±0.11
Urea, mg/dl	37.21 ^{b} ±0.35	36.65 ^{c} ±0.71	34.43 ^{d} ±0.45	38.14 ^{a} ±1.06
Creatinine, mg/dl	0.82 ^{c} ±0.01	0.83 ^{c} ±0.02	0.87 ^{b} ±0.02	1.15 ^{a} ±0.04
Cholesterol, mg/dl	65.00 ^{a} ±0.46	63.67 ^{b} ±0.48	61.40 ^{c} ±0.72	61.55 ^{c} ±0.62
ALT, U/L	18.54 ^{b} ±0.05	18.40 ^{b} ±0.48	17.64 ^{c} ±0.41	20.38 ^{a} ±0.70
AST, U/L	36.47 ^{b} ±0.05	35.73 ^{b} ±0.54	34.46 ^{c} ±0.55	38.28 ^{a} ±0.99a

a,b,c,d means within rows followed by different superscripts are significantly different at (P<0.05).

However, significant differences were detected among groups and the highest (P<0.05) total protein and albumin were recorded for R3 (200mg ML) group, these results are in agreement with Babeker and Abdalbagi (2015) who found that blood plasma total protein and albumin were significantly (P<0.05) increased in Sudan Nubian growing goats with feeding Moringa leaves substituted sorghum leaves at 0, 20 and 50%, however values of protein and albumin showed a significant decrease (P<0.05) with increasing ML level from 20 to 50%.

In the contrast, urea, creatinine, alanine Aminotransferase (ALT) and aspartate aminotransferase (AST) had the highest values (P<0.05) with sheep fed R4. In the present study, there was no clear reaction of feeding 100mg DML supplemented diet (R2) than control on most measured blood biochemical constituents. It is noticeable that there was a tendency of urea, AST, and ALT indices to decline than control with increasing DML supplementation level up to 200 mg. Cholesterol concentration in particular was significantly (P<0.05) reduced with increasing DML supplementation levels. The significant decrease in serum cholesterol with ML supplementation was consistent with Abdel-Raheem and Hassan (2021) in buffalo calves.

In generally Biochemical blood components are influenced by the quantity and quality of consumed feeds and the level of anti-nutritional substances naturally existing in the feed including elements of toxicity, and they also can be used to evaluate dietary protein quality and sufficiency to the animal.

CONCLUSIONS

According to the results of this study, best results had been recorded with R3 and the worst with R4 as final body weight, ADG, feed conversion efficiency, microbial protein synthesis and methan emission, while corresponding parameters of R2 (100 mg ML) and control showed comparable values. It is worth saying that, *Moringa oleifera* dry leaves are a useful growth promoter for lambs when supplemented at the maximum of 200mg/kg BW or in average 5 gm/day. and there is a need in futuristic studies to give more attention to exploring the biological effect of Moringa leaves on animal health, and productivity, the proper supplementation level for different farm animals during different physiological stages and its role in mitigating enteric CH_4 emission.

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إلى أي مدى يمكن أن تؤثر مكملات أوراق المورينجا الجافة على أداء نمو الأغنام وتؤثر على إنتاج الميثان.

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استمرت تجربة التغذية 70 يومًا ، لفحص تأثير المكملات الغذائية لأوراق المورينجا الجافة (DML) على أداء النمو ومقاييس سائل الكرش على 20 من ذكور الحملان البرقي المحلية بعمر 6 أشهر ووزنها 24.4 ± 1.12 كجم. تم تقسيم الحملان بشكل عشوائي إلى أربع مجموعات متساوية حيث يتم تغذيتها بشكل فردي يوميًا على نظام غذائي موحد 3 ٪ من وزن الجسم (BW) عبارة عن خليط علف مركز (CFM)بالإضافة إلى 1.25 كجم من علف الذرة الكامل كما يقدم قش الأرز لحد الشبع. تم تغذية المجموعة الأولى (R1) على العليقة المركزة (مجموعة الكنترول) ، بينما تم اضافة نسب مختلفة من مسحوق اوراق المورينجا الجافة 100, 200 و 400 مجم / كجم من وزن الجسم للعليقة 2, 3, 4 ، على التوالي. تم تعديل الكميات المقدمة من المورينجا والعليقة المركزة كل أسبوعين وفقًا لتغير وزن الجسم.

أظهرت النتائج: انخفاض المأكول اليومي من المادة الجافة بنسبة 17 ٪ مع العليقة R4 (400) مجم مورينجا مقارنة بالمجموعات الأخرى. تم تسجيل أفضل قيم (P <0.05) لوزن الجسم النهائي ، ومتوسط النمو اليومي وكفاءة تحويل العلف للحملان التي تم تغذيتها على العليقة (200) R3 مجم، بينما لم يكن هناك فروق معنوية بين R2 (100 مجم) والعليقة الكنترول R1. مع زيادة اضافة المورينجا إلى 400 مجم / كجم من وزن الجسم (R4) ، انخفض متوسط النمو اليومي بشكل ملحوظ إلى ما يقرب من 50 ٪ من المجموعات الأخرى. تم تحقيق نفس النتيجة مع هضم العناصر الغذائية ومعدل الاستفادة النيتروجينية، حيث سجلت أفضل النتائج مع R3 والأسوأ مع R4.علاوة على ذلك ، فإن اليوريا في بلازما الدم والكرياتينين و AST و ALT كانت لها أعلى قيم (P <0.05) مع R4 مقارنة بالمجموعات الأخرى ، بينما انخفض الكوليسترول (P <0.05) مع زيادة مستوي المورينجا كما انخفض تركيزالامونيا نيتروجين في سائبل الكرش (P <0.05) مع زيادة مستوى المورينجا ، بينما سجل أعلى تركيز للاحماض الدهنية الطيارة مع R3 والاقل مع R4 .

انخفض إنتاج الميثان المعوي المقاس معمليا مع زيادة نسب اضافة المورينجا (P <0.05) ، ومع ذلك ، تم تسجيل اقل تركيز للميثان مجم / كجم مادة جافة ماكولة مع النظام الغذائي R3. كان أعلى إنتاج للبروتين الميكروبي في الكرش (P <0.05) مع R3 والأقل مع R4.تجدر الإشارة إلى أن أوراق المورينجا الجافة هي محفز نمو مفيد للحملان عند تناولها بحد أقصى 200 مجم / كجم من وزن الجسم أو في المتوسط 5 جم / يوم.

الكلمات المفتاحية: أوراق المورينجا ، الحملان ، أداء النمو ، إنتاج الميثان المعوي ، إنتاج البروتين الميكروبي.