

EFFECT OF MORINGA OLEIFERA SEED OIL AS NATURAL FEED SUPPLEMENT ON THE PRODUCTIVE PERFORMANCE OF LACTATING EWES

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SUMMARY

Moringa seed oil (MSO) was used as a feed additive to evaluate its potential to manipulate rumen fermentation and productive performance in lactating Ossimi ewes. Cold extracted MSO was supplemented at four different levels (1, 2, 3, and 4%) in diet using *in vitro* batch culture system for optimizing best supplementation level for sheep. Results of *in vitro* study revealed non-significant ($P>0.05$) effect of MSO on true dry matter degradability (TDMD) up to 3% inclusion level, however, decrease in TDMD was observed by 4% MSO supplementation as compared to control and other treatment groups. Accumulated gas production was significantly ($P<0.05$) increased by MSO supplementation while non-significant decrease in ammonia concentration was observed. Fermentation pattern and TDMD revealed 1% MSO as an appropriate level for supplementation, which was further evaluated by *in vivo* trial. Fourteen lactating Ossimi ewes (about 3 years old with an average body weight of 51 ± 0.5 kg after 5 days of parturition) were randomly assigned into two experimental groups (seven each). One group was fed a basal diet without any supplementation and served as control. Other group was fed basal diet supplemented with 1% MSO on dry matter basis. Animals were fed these diets for a period of 45 days. Nutrient digestibility, milk production and composition were determined. Results revealed that supplementation of MSO significantly ($P<0.05$) increased milk yield and fat corrected milk. Similarly, it is also significantly ($P<0.05$) increased yield of milk components (protein, lactose, and SNF) as compared to the control group. However, milk composition (%) was not significantly ($P>0.05$) affected by treatment. Our study revealed that MSO could be used as a natural fat supplement to meet energy requirements of lactating sheep. Moreover, antioxidants and other bioactive compounds, present in MSO can effectively modulate rumen fermentation which makes it a potential alternative of chemical feed additives (especially antibiotics) to improve feed digestibility and utilization for increasing animal productivity.

Keywords: *Moringa oleifera*, rumen, fermentation, milk yield and milk composition.

INTRODUCTION

Dietary fat is usually supplemented in animal feeds to address gross energy requirements and avoid negative energy balance especially in intensive production system (Weisbjerg *et al.*, 2013, Palmquist and Jenkins, 2017). Normally, different oils are supplemented to manipulate fermentation kinetics, and microbial populations in rumen to improve performance while reducing methane production (Huws *et al.*, 2015 and Enjalbert *et al.*, 2017), enhancing meat and milk fatty acids profiles (Steinfeld *et al.*, 2006; Shingfield *et al.*, 2013 and Gawad *et al.*, 2015a), increased efficiency of feed utilization (Myer *et al.*, 2015 and Yoshimura *et al.*, 2018), and enhancing health and welfare (Nagaraja and Titgemeyer, 2007). Different sources of dietary fat are used (vegetable oils, marine oils) in different forms (protected or unprotected forms) for supplementation (Ashes *et al.*, 1992; Gawad *et al.*, 2015b; Palmquist and Jinkens, 2017 and Barfouroushi *et al.*, 2018). One of the emerging sources of vegetable oils is *Moringa oleifera* seed oil. In many countries in tropical and sub-tropical regions, *Moringa oleifera* (*M. oleifera*) is a quite resilient plant with ability to thrive under diverse agro-climatic conditions and widely used as a rich

source of nutritious food and phytochemicals (Falowoa *et al.*, 2018). Different parts of *Moringa oelifera* have been utilized in livestock feeding as feed additive or feed ingredients, owing to its rich nutrients like carbohydrates, fat, proteins and minerals. *Moringa oleifera* seed oil is one of the most promising feed supplements due to its rich fatty acids contents comprising both saturated and unsaturated fatty acids. Moreover, *M. oleifera* seed oil contains many other bioactive compounds including polyphenolic, tannins, and saponins other than fatty acids. These phytochemicals are mainly responsible for its biological activity to manipulate rumen fermentation and also it's potential as an alternative to replace synthetic feed additives (such as antibiotics) for high yielding dairy animals (Nouman *et al.*, 2014, Soltan *et al.*, 2017). Main objective of this study was to evaluate effect of Moringa seed oil on rumen fermentation and productive performance of lactating ewes.

MATERIALS AND METHODS

This study was conducted within a cooperation work among Morinaga Production Unit, National Research Center; Dairy production Lab., Dairy Sciences Department, National Research Center and Atomic Energy Authority, Inshas, Cairo, Egypt. Morinaga trees were cultivated and Moringa seed oil was extracted by Moringa production unit. Moringa seed oil was analyzed and evaluated *in vitro* on dairy animal production lab. *In vivo* evaluation using lactating ewes was carried out at the experiments station, Atomic Energy Authority, Inshas, Cairo, Egypt.

Determination of fatty acid profile of Moringa seed oil:

Fatty acids in Moringa oil were determined by gas chromatography (GC) system according to the method of Tsaknis *et al.* (1999). Analysis was performed on a Varian 3600 gas chromatograph (Varian, Palo Alto, CA, U.S.A.) equipped with a Supelcowax 10 (Supelco, INC., Supelco Park, Bellefonte, PA) fused silica capillary column 30m_0.32mm i.d., 0.25 mm film thickness. The temperature profile used was 60°C for 10 min and then increased @ 2°C/min up to 220°C. Injector and FID temperatures were set at 160 and 280°C, respectively, sample volume was 0.2 mL, the carrier gas was N₂ at a flow rate of 30mL/min, chart speed was set at 0.5 cm/ min and the attenuation at 10_10_32. The internal standard used was nonadecanoic acid. Samples were prepared and measured separately for each replicate in triplicate.

In-vitro rumen fermentation:

Batch culture system was used to evaluate the effect of different levels of MSO on *in-vitro* rumen fermentation parameters. Rumen fluid was collected from 3 cannulated sheep (mean weight 53±0.7 kg). The fistulated sheep were fed experimental ration containing clover hay and concentrate (50:50 on DM basis). Rumen fluid was collected before morning feeding and squeezed through four layers of cheesecloth into a Schott Duran® bottle (L) with an O₂-free headspace and immediately transported to laboratory at 39°C where it was used as a source of inoculum. Same experimental diet which fed to sheep was also as a substrate for *in vitro* batch culture (Table 1). Moringa seed oil was supplemented in substrate at four different levels on dry matter basis: control (substrate without supplementation), T2 (substrate + 1% MSO), T3 (substrate + 2% MSO), T4 (substrate + 3% MSO), and T5 (substrate + 4% MSO). Four incubation vessels were for each treatment and control group as well as four vessels as blanks (without substrate). About 400 mg of milled substrate was added to each incubation vessel (100 mL capacity) containing 40 mL incubation buffer (292 mg K₂HPO₄, 240 mg KH₂PO₄, 480 mg (NH₄)₂SO₄, 480 mg NaCl, 100 mg MgSO₄·7H₂O, 64 mg CaCl₂·2H₂O, 4 mg Na₂CO₃ and 600 mg cysteine hydrochloride per 1 liter of ddH₂O). All vessels were incubated at 39 °C for 48 h.

Samples collecting and examination:

After 48 h of incubation, overall gas production was measured immediately according to Makkar and McSweeney (2005) after removing vessels from incubator. Immediately after opening, pH was measured (pH-meter). Different aliquots from each sample were taken and stored at -20°C until further processing. Concentration of VFAs was measured using GC system as described previously (Tangerman and Nangengast, 1996). Quantitative analysis of ammonia (NH₃) was carried out by a modified Nessler's method (Szczechowiak *et al.*, 2016). Substrate residues after 48 h of incubation were dried at 70°C and analyzed for TDMD according to AOAC (1995).

Table (1): Feed ingredients and chemical composition of CFM used in batch culture system.

Item	Control	MO 1%	MO 2%	MO 3%	MO 4%
Feed ingredients, %					
Corn	35.5	35.5	35.5	35.5	35.5
Beet pulp	15	15	15	15	15
Cotton meal	23	23	23	23	23
Soya meal	4	4	4	4	4
Wheat bran	20	20	20	20	20
Moringa oil, %	0	1	2	3	4
Salt	1	1	1	1	1
Dicalcium phosphate	1	1	1	1	1
Minerals	0.5	0.5	0.5	0.5	0.5
Total	100	101	102	103	104
Chemical composition, %					
DM	92.13	92.21	92.29	92.36	92.43
OM	92.07	91.08	90.11	89.16	88.23
CP	15.77	15.6	15.43	15.27	15.11
NDF	46.59	46.09	45.6	45.12	44.65
ADF	25.4	25.13	24.86	24.6	24.34
EE	3.97	5.01	6.02	7	7.97
Ash	7.93	7.85	7.77	7.68	7.6

In-vivo evaluation:

Based on results of *in vitro* experiment, 1% level of MSO was selected in-*vivo* evaluation. For this purpose, 14 lactating Ossimi ewes (about 3 years old with average body weight of 51 ± 0.5 kg) after 5 days of parturition were randomly divided into two groups (seven each) using complete random design. The first group was fed control ration (0% MSO) while the second group was fed control ration supplemented by 1% MSO (on DM basis). Rations (1:1 R: C ratio) were fed according to 4% of their body weight twice daily (at 8.00 and 15.00) to all ewes for an experimental period of 45 days. Animals were provided with free access to water. Feed ingredients and chemical composition of experimental rations are given in Table 2.

Table (2): Feed ingredients and chemical composition of the experimental rations.

Item	Control	MSO
Ingredients, g/kg DM		
Berseem	500	500
Yellow corn	177.5	177.5
Dry olive pulp	75	75
Cotton seed meal	115	115
Moringa oil	-	10
Soybean meal	20	20
Wheat bran	100	100
Salt	5	5
Di-calcium phosphate	5	5
Mineral mixture	1.5	1.5
Vit. AD3	0.5	0.5
Bicarbonate sodium	0.5	0.5
Chemical composition, g/kg DM		
Organic matter	933.4	934.0
Crude protein	169.8	171.2
Ether extract	29.9	41.0
NDF	339.0	349.8
ADF	509.2	511.3
Ash	66.6	66.0

Samples collection and analysis:

Total tract apparent digestibility of OM, CP, EE, NDF and ADF were determined by total fecal collection. Total feces excreted from three ewes of each group were manually collected, during three consecutive collection periods of eight hours, on days 43 to 45 of experiment. Fecal composite samples were oven dried at 55°C for 48h and then at 100°C until no further reduction in weight was observed. Representative feces samples were ground using Wiley mill to pass a 1 mm sieve, and thereafter subjected to chemical analysis for CP, EE, NDF, ADF and ash contents according to AOAC (1995). The acid insoluble ash (AIA) method (Van Keulen and Young, 1977) was used as an internal marker for determination of nutrient digestibility as reported previously (Sales and Janssens, 2003). Lactating ewes were milked twice a day at 09.00 and 17.00 hours biweekly for collection of milk samples and recording of milk yield throughout the experiment. Milk samples were analyzed for total solids, fat, total protein and lactose by Bentley 150 infrared milk analyzer (Bentley Instruments, Chaska, MN, USA). Solids-non-fat (SNF) was calculated by subtracting fat from total solids (percent). Fat corrected milk (4% fat) was calculated by using the following equation: $FCM = 0.4 M + 15 F$

Where; M= milk yield (g), F= fat yield (g).

Statistical analysis

Data from in vitro experiment were subjected to analysis of variance using GLM procedure of SAS (2015) using the following model; $Y_{ij} = \mu + T_i + e_{ij}$. Where; Y_{ij} is performance trait of i^{th} ewe with j^{th} group, μ is the overall mean, T_i is the effect of treatment, e_{ij} is the experimental error. While Data of production performance were statistically analyzed for Two-Way Repeated Measures ANOVA using GLM procedure of SAS (2015) using following model; $Y_{ijk} = \mu + R_i + T_j + (RT)_{ij} + e_{ijk}$, Where; Y_{ijk} is k^{th} observation ($k = 1 \dots 14$) for group i in time j ; μ is the overall mean; R_i is the effect of diet i ($i = 1 \dots 2$); T_j is the effect of time j ($j = 15, 30, 45$); RT_{ij} is the interaction; e_{ijk} is the experimental error. Duncan's multiple range tests were used to compare the treatment means (Duncan, 1955).

RESULTS AND DISCUSSION**Moringa oil fatty acids content:**

Results revealed that MSO contains higher contents of oleic acid and other oleic acid derivatives ($C_{18:1n9, n7}$) amounting to 73.78% of total fatty acids (Table 3). Moreover, MSO also showed considerable contents of palmitic acid (8.11%), linoleic acid (2.31%), and behenic acid (5.72%). Fatty acids profile of MSO observed in this study is quiet similar to previous studies conducted on Moringa trees under different conditions and methods of extraction (Sonntag 1982; Tsaknis *et al.*, 1999; Lalas and Tsaknis, 2002, and Soltan *et al.*, 2017). Moreover, Moringa oil has comparable oleic acid contents with olive oil and avocado oil (Banerji *et al.*, 2003). This rich profile of Moringa oil especially oleic acid contents make it an attractive option as an edible oil to replace oils based diets rich in trans-unsaturated and saturated fatty acids associated with increased risk of cardiovascular diseases caused by high blood cholesterol levels (Lalas and Tsaknis, 2002).

Table (3): Moringa oleifera oil fatty acids fractions.

Fatty acids	% (of total MSO fatty acid fraction)
C 16:0	6.44
C 16:1n ₇	1.67
C 18:0	4.73
C 18:1n ₉	67.89
C 18:1n ₇	5.89
C 18:2n ₆	2.31
C 20:0	3.07
C 20:1n ₉	2.27
C22:0	5.72
Total	100%

Effect of MSO supplementation on DM and CP degradability and in vitro fermentation parameters:

Effect of MSO supplementation on TDMD degradability and rumen fermentation parameters are given in Table (4).

Table (4): Rumen fermentation parameters affected by MSO supplementation in vitro.

Item	Treatment					SEM	P value			Contrast
	Control	MSO 1%	MSO 2%	MSO 3%	MSO 4%		TRT	Linear	Quad.	
IVTDMD	56.62 ^a	56.33 ^a	56.38 ^a	57.94 ^a	49.39 ^b	0.898	0.0454	0.0420	0.0396	0.4172
TGP, %	29.67 ^d	41.00 ^c	43.50 ^{cb}	47.57 ^{cb}	51.25 ^a	1.695	<.0001	<.0001	0.0120	<.0001
TVFAs	6.12 ^b	8.84 ^a	8.58 ^a	7.69 ^{ab}	6.87 ^{ab}	0.323	0.0287	0.8718	0.0041	0.0118
pH	5.09 ^a	5.04 ^b	5.04 ^b	5.04 ^b	5.05 ^b	0.005	0.0081	0.0269	0.0090	0.0005
NH3	8.00	6.75	6.58	7.19	6.39	0.232	0.1991	0.1105	0.3894	0.0286

The results indicated that MSO supplementation exhibited no significant ($P>0.05$) effect on IVTDMD up to 3% level, however, IVTDMD was significantly ($P<0.05$) decreased with 4% MSO level. These findings are consistent with earlier reports revealing non-significant effect on DM degradability in ruminants in response to supplementation of linseed oil (Gawad *et al.*, 2015a), soybean (Toral *et al.*, 2009), and fish oil (Keady and Mayne, 1999), despite the shift in fermentation pattern in the rumen was observed. These reports suggested that, although, cellulolytic bacteria usually might be negatively, affected by lipid supplementation (Doraueu and Chilliared, 1997), other population of bacteria can replace their niches. Contrarily, some studies have also reported negative effects of linseed oil on DM degradability sheep mainly due significant reduction in protozoa population (Gawad *et al.*, 2015b).

Results of *in vitro* fermentation revealed that MSO not only disturbed the fermentation pattern but also enhanced it and leading to gradual increase in gas and VFA production. The values of pH, however, were significantly ($P<0.05$) decreased by treatments than control, but it still very close to control. Maximum value of gas production was achieved with 4% MSO supplementation, however, highest value of VFA was observed with 2% MSO. Variable effects of plant extracts or plant essential oil on total VFA production have been already reported as lower, higher, and even no effect (Christaki *et al.*, 2012). These variations in results may be partially explained by experimental conditions of these studies including type of diets, plant species and/or their active substances used and source (animal species, site of sampling and pH values) of rumen fluid (Tajodini *et al.*, 2014). Non-significant decrease in ammonia concentration was observed in response to oil supplementation. *Moringa oleifera* oil owing to its rich bioactive compounds has shown to reduce rumen ammonia production while increasing by-pass protein in ruminants (Gassenschmidt *et al.*, 1995; Newbold *et al.*, 1999, and Belewu *et al.*, 2014). This biological significant activity of Moringa oil ultimately leads to efficient utilization of feed by ruminants leading to better performance per unit of feed consumed. Results of our study are in agreement with previous studies regarding enhanced feed degradability by supplementation of Moringa seed or seed extract (Hoffmann *et al.*, 2003; Kutlu *et al.*, 2007). *In vitro* incubation of aqueous extract of Moringa seeds with pure carbohydrates (at a concentration of 1 mg/ml) reduced the degradation of the total true protein (Hoffmann *et al.*, 2003). Furthermore, Plant extracts has shown its ability to control ammonia synthesis and nitrogen binding activity which is responsible for potential decrease in ammonia production during fermentation (Kutlu *et al.*, 2007). These findings revealed potential of Moringa seed extract or oil to be a good alternative of synthetic feed additives (antibiotics) being utilized in ruminant to decrease protein degradation and deamination in the rumen in order to bypass it for post-rumen digestion for better feed efficiency and performance (Kutlu *et al.*, 2007; Soltan *et al.*, 2017, and Ebeid *et al.*, 2019).

Effect of MSO supplementation on productive performance of lactating ewes:

Digestibility coefficients:

Effects of MSO supplementation on feed digestibility in lactating ewes are presented in Table 5. Results revealed that MSO significantly ($P<0.05$) improved digestibility coefficients of organic matter,

fat, NDF, and ADF. Crude protein was not significantly ($P>0.05$) affected by MSO supplementation. This improvement in feed digestibility is mainly due to its bioactive contents from saponins, tannins, and other antioxidant substances. These compounds also possess appetizing properties, which promote feed intake, secretion of endogenous enzymes and digestive juices ultimately leading to better nutrient digestibility (Kutlu *et al.*, 2007 and Soltan *et al.*, 2017). Plant extracts especially saponins, have also strong antiprotozoal activity. Elimination of protozoa from rumen reduces degradation of microbial protein which ultimately enhances post ruminal protein supply to host. Additionally, MSO has also shown to decrease methanogenesis by inhibiting methanogens ultimately leading to reduce methane production (Benchaar *et al.*, 2007 and Tajodini *et al.*, 2014).

Table (5): Effect of MSO supplementation on feed digestibility.

Item	Control	MSO	SEM	P value
OM	79.95 ^b	86.30 ^a	2.769	0.041
CP	76.01	77.42	2.304	0.552
EE	77.29 ^b	85.33 ^a	3.981	0.057
NDF	60.29 ^b	72.22 ^a	3.354	0.004
ADF	55.34 ^b	61.64 ^a	3.613	0.001

This ability of MSO can greatly enhance feed efficiency by preventing energy loss from methanogenesis that represent about 2-15% loss of dietary energy in the rumen. Moreover, it is well established that oils rich in unsaturated fatty acids pose a negative effect on cellulolytic bacteria (Maczulak *et al.*, 1981; Doham *et al.*, 2001 and Gawad *et al.*, 2015a,b), ultimately leading to decrease in crude fiber and its digestibility. Contrarily, this not happened in our present study. The digestibility of NDF and ADF was significantly ($P<0.05$) increased. Major reason for these contrasting effects may be attributed to nature of unsaturated fatty acids present in MSO as compared to other plant oils. Toxic effects of oils on rumen microbes have been mainly reported for oils rich in long chain fatty acids like fish oil and linseed oil. However, in case of MSO, major component is oleic acid which is a mono-unsaturated fatty acid. Additionally, other bioactive contents of MSO may also have positive effects on rumen microflora leading to enhanced digestibility of fiber components (Naziroğlu *et al.*, 2002 and Lins *et al.*, 2019).

Effect of MSO on milk yield and composition of lactation ewes:

Effects of supplementation of 1% MSO on milk yield and milk composition of lactating ewes are illustrated in Table (6). The MSO has shown overall positive effects on productive performance of lactating ewes. Milk yield and FCM yield were significantly ($P<0.05$) increased with MOS group as compared to control group. Similarly, yield of milk components (protein, lactose and SNF) were also increased significantly ($P<0.05$) with MSO treatment than control group owing to overall increase in milk yield. However, fat yield did not affect to MSO supplementation in ewe's ration. Moreover, milk composition (%) was not significantly ($P>0.05$) affected by MSO treatment.

Table (6): Effect of Moringa oil supplementation on milk yield and composition.

Items	Treatments		SEM	P value
	Control	MSO		
Milk yield, kg	485.1 ^b	733.6 ^a	27.6	0.002
FCM, kg	511.3 ^b	736.2 ^a	40.0	0.023
Chemical composition, %				
Fat	4.34	4.12	0.39	0.785
Protein	4.41	4.61	0.10	0.376
Lactose	6.61	6.94	0.15	0.311
SNF	11.98	12.53	0.28	0.349
Ash	0.95	0.98	0.02	0.565
Milk yield composition, g/d				
Fat	21.15	29.52	2.26	0.102
Protein	21.87 ^b	33.60 ^a	1.43	0.005
Lactose	32.76 ^b	50.64 ^a	2.12	0.004
SNF	59.34 ^b	91.37 ^a	3.87	0.004

High unsaturated fatty acids contents of MSO are important energy source for lactating ewes as observed in this study. In addition to different fatty acid fractions, MSO also possess many natural bioactive components like saponins and tannins which can effectively modulate rumen fermentation to improve feed degradation and utilization ultimately leading to better animal productivity. Moreover, appetizing activity, secretion of endogenous enzymes and digestive juices mediated by bioactive compounds can also promote feed intake, nutrient digestibility and milk production. Effect of MSO on milk production and composition of lactating animals is not well documented. Aerial parts of Moringa tree have been used as a substitute of food and/or feed supplement for ruminants. For example, Babiker *et al.* (2017) replaced alfalfa with Moringa leaves in the diets of lactating ewes and goats and reported that Moringa leaves positively affected milk yield, composition and growth performance of kids and lambs, in spite of higher protein contents of alfalfa. Furthermore, it has also been reported that inclusion of Moringa leaves as a protein supplement to low quality diets significantly improved DM intake and digestibility while increasing milk production with no effect on milk composition as observed in our present study (Nadir *et al.*, 2006). Moringa seed cake has also been used as a rich protein supplement in sheep to improve milk production (Unpublished data). Many *in vitro* and *in vivo* studies have provided evidence for potential of plant oils (essential oils or other bioactive components) to modulate rumen fermentation to improve energy/protein utilization leading to better animal productivity (Benchaar *et al.*, 2007; Tajodini *et al.*, 2014 and Soltan *et al.*, 2017). Our study indicated that MSO can be used as a fat supplement for lactating animals to avoid negative energy balance during lactation and transition periods. Moreover, MSO with more than 70% unsaturated fatty acid contents, can be used as an effective tool to alter fatty acid profiles of milk and meat by reducing saturated and trans fatty acids while increasing unsaturated fatty acids. Results of supplementation of MSO on milk production observed in this study are in agreement with earlier studies which used fish oil (Whitlock *et al.*, 2006; Heravi *et al.*, 2007; and Barfourooshi *et al.*, 2018), rapeseed and linseed oil (Majewska *et al.*, 2017) as a fat supplements. Other studies have not reported any effect of fish oil on milk production. Many factors can influence milk production response to dietary fat supplements including, forage to concentrate ratio, stage of lactation and diet composition (Heravi *et al.*, 2007).

CONCLUSION

Generally, MSO has shown desirable effects in ruminant nutrition. Owing to its high unsaturated fatty acid contents, it can also be used as an energy source for high producing dairy animals. Moreover, antioxidants and other bioactive compounds, present in MSO can effectively modulate rumen fermentation which make it a potential alternative of chemical feed additives (antibiotics in particular) to improve feed digestibility and utilization for increasing animal productivity. However, further studies involving larger group of animals are warranted to evaluate effect of supplementation of MSO at different stages of lactation with different feeding regimens.

REFERENCES

- AOAC (1995). Official Methods of Analysis. Association of Analytical Chemists. 16th ed. Washington, D.C., USA.
- Ashes, J.R., B.D. Siebert, S.K. Gulati, A.Z. Cuthbertson, and T.W. Scott (1992a). Incorporation of n-3 fatty acids of fish oil into tissue and serum lipids of ruminants. *Lipids*; 27:629.
- 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. *Livestock Sci.* 195: 21–26.
- Banerji R., S.C. Verma, and P. Pushpangadan (2003). Oil potential of moringa Natural Product *Radiance*, 2(2) March-April 2003.
- Barfourooshi, H.J., A. Towhidi, H. Sadeghipanah, M. Zhandi, S. Zeinoaldini, E. Dirandeh, and R.M. Akers (2018). Effect of dietary fish oil on mammary gland development and milk production of Holstein cow. *Annals of Animal Science.*, DOI: 10.2478/Aoas-2018-0028.

- Belewu, M.A., M.A. Ahmed, A.H.A. Badmos, T.O. Esan, K.O. Abdulsalam, M.B. Odebisi and A.K. Arise (2014). Effect of different levels of moringa oleifera oil on performance characteristics of pregnant goat. *Nigerian J. of Agric., Food and Environ.*, 10(2):29-33.
- Benchaar, C., H.V.Petit, R. Berthiaume, D.R. Ouellet, J. Chiquette, and P.Y. Chouinard (2007). Effects of essential oils on digestion, ruminal fermentation, rumen microbial populations, milk production, and milk composition in dairy cows fed alfalfa silage or corn silage. *J. Dairy Sci.*, 90:886–897.
- Christaki, E., E. Bonos, I. Giannenas, and P. Florou-Paneri (2012). Aromatic plants as a source of bioactive compounds. *Agriculture*, 2(3), 228-243.
- Dohme F., A. Machmuller, A. Wasserfallen, and M. Kreuzer (2001). Ruminal methanogenesis as influenced by individual fatty acids supplemented to complete ruminant diets. *Lett. Applied Microb.*, 32: 47–51.
- Doreau, M., and Y. Chilliard (1997). Effects of ruminal or postruminal fish oil supplementation on intake and digestion in dairy cows. *Reprod. Nutr. Dev.*, 37:113.
- Duncan, D.B. (1955). Multiple range and multiple F tests. *Biometrics*, 11: 1-42.
- Ebeid, H.M., A.E. Kholif, M. Chrenkova, and U.Y. Anele (2019). Ruminal fermentation kinetics of *Moringa oleifera* leaf and seed as protein feeds in dairy cow diets: *in sacco* degradability and protein and fiber fractions assessed by the CNCPS method. *Agroforest Syst.*, 1-11.
- Enjalbert, F., S. Combes, A. Zened, and A. Meynadier (2017). Rumen microbiota and dietary fat: A mutual shaping. *J. of Applied Microb.*, 123(4): 782-797.
- Falowo, A.B., Felicitas E. Mukumbo, E.M.C. Idamokoro, C. José, M. Lorenzob, A.J. Afolayanc, and V. Muchenje (2018). Multi-functional application of *Moringa oleifera* Lam. *Inter. Nutr. And Anim. Food Products: A Review. Food Research International* 106, 317–334
- Gassenschmidt, U., K.D. Jany, B. Tauscher, and H. Niebergall (1995). Isolation and characterization of a flocculating protein from *Moringa Oleifera* Lam. *Biochimica Biophysica Acta*, 1243: 477-481.
- Gawad, R.M.A., H.M. Kattab, M. Strabel, S.A. Abo El- Nor, A. Cieslak, P. Zmora, M. Elnashar, H. El-Sayed, and S.M. Kolif (2015a). Effect of different levels from linseed oil and linseed oil beads on rumen fermentation and microbial parameters using gas production system and rumen simulation technique. *Asian J. of Anim. and Veter. Advan.* 10(3): 97-118.
- Gawad, R.M.A., M. Strabel, S.A. Abo El-Nor, H.M. Kattab, A. Cieślak, S.M. Kolif, and M. Elnashar (2015b). Encapsulation method to protect unsaturated fatty acids from rumen biohydrogenation *in vitro*. *J. of Innovations in Pharmaceuticals and Biological Sciences*, 2(3): 240-251.
- Heravi, A.R.M., R.O. Gilbert, T.R. Overton, D.E. Bauman, and W.R. Butler (2007). Effects of feeding fish meal and n-3 fatty acids on milk yield and metabolic responses in early lactating dairy cows. *J. Dairy Sci.*, 90:136–144.
- Hofmann, E.M., S. Muetzel, and K. Becker (2003). Effects of *Moringa oleifera* seed extract on rumen fermentation *in vitro*. *Arch. Anim. Nutr.*, 57: 65-81.
- Huws, S.A., E.J. Kim, S.J.S. Cameron, S.E. Gridwood, L. Davies, J. Tweed, H. Vallin, and N.D. Scollan (2015). Characterization of the rumen lipidome and microbiome of steers fed a diet supplemented with Flax and Echium oil. *Microbial Biotechnol.*, 8; 331–341.
- Keady, T.W.J., and C.S. Mayne (1999). The effects of level of fish oil inclusion in the diet on rumen digestion and fermentation parameters in cattle offered grass silage based diets. *Anim. Feed Sci. Technol.* 81: 57–68.
- Kutlu, A.T.L.Ç.H. (2007). Plant extracts; a new rumen moderator in ruminant diets. *Tekirdağ Ziraat Fakültesi Dergisi*, 4(1):71-79.
- Lalas, S., and J. Tsaknis (2002). Characterization of *Moringa oleifera* seed oil variety “Periyakulam 1”. *J. of food composition and analysis*, 15(1):65-77.
- Lins, T.D.A., S. Terry, R. Silva, L. Pereira, L. Jancewicz, M. He, Y. Wang, T. McAllister, and A. Chaves (2019). Effects of the inclusion of *Moringa oleifera* seed on rumen fermentation and methane

- production in a beef cattle diet using the rumen simulation technique (Rusitec). *Animal* 13 (2):283-291.
- Maczulac, A.E., B.A. Dehority, and D.L. Palmquist (1981). Effects of long-chain fatty acids on growth of rumen bacteria. *Appl. Environ. Microbiol.* 42: 856–862.
- Majewska, M.P., R. Miltko, G. Belżeczki, J. Skomial, and B. Kowalik (2017). Supplementation of rapeseed and linseed oils to sheep rations: effects on ruminal fermentation characteristics and protozoal populations. *Czech J. of Anim. Sci.*, 62(12):527-538.
- Makkar, H.P.S., and C.S. Mcsweeney (2005). Methods in gut microbial ecology for ruminants. IAEA, 3-19.
- Myer, P.R., T.P. Smith, J.E. Wells, L.A. Kuehn, and H.C. Freetly (2015). Rumen microbiome from steers differing in feed efficiency. *PloS one*, 10(6), p.e0129174.
- Nadir, R.S., S. Eva, and L. Inger (2006). Effect of feeding different levels of foliage of *Moringa oleifera* to creole dairy cows on intake, digestibility, milk production and composition. *Livest. Sci.*, 101:24–31.
- Nagaraja, T.G., and E.C. Titgemeyer (2007). Ruminal acidosis in beef cattle: the current microbiological and nutritional outlook. *J. Dairy ci.*, 90, E17-E38.
- Naziroğlu, M., T. Güler, and A. Yüce (2002) Effect of vitamin E on ruminal fermentation in vitro. *J Vet Med A* 49 (5):251-255.
- Newbold, R.W., P.R. Jensen, W. Fenical, and J.R. Pawlik (1999). Antimicrobial activity of Caribbean sponge extracts. *Aquat. Microb. Ecol.*, 19: 279–284.
- Nouman, W., S.M.A. Basra, M.T. Siddiqui, A. Yasmeen, T. Gull, and M.A.C. Alcayde (2014). Potential of *Moringa oleifera* L. as livestock fodder crop: a review. *Turkish J. of Agric. and Fores.*, 38(1):1-14.
- Palmquist, D.L., and T.C. Jenkins (2017). A 100-Year Review: Fat feeding of dairy cows. *J. Dairy Sci.*, 100:10061–10077.
- Sales J., and G.P.J. Janssens (2003). Acid insoluble ash as a marker in digestibility studies: a review. *J. Anim. Feed. Sci.*,12:383-401
- SAS. (2015). SAS User's Guide: Statistics. Version 9.4, SAS Institute Inc., Cary, NC., USA.
- Shingfield, K.J., M. Bonnet, and N.D. Scollan (2013). Recent developments in altering the fatty acid composition of ruminant-derived foods. *Animal*, 7(s1), 132-162.
- Soltan, A., A.S. Morsy, N.M. Hashem, and S.M. Sallam (2017). Utilization of *Moringa Oleifera* in ruminant nutrition (Review article). 3rd. International Conference "Sustainable Development Of Livestock's Production Systems" (Sdlps2017)" From 7-9 November, 2017., Alexandra University, Egypt.
- Sonntag, N.O.V. (1982). Analytical methods. Bailey's industrial oil and fat products, 2: 407-527.
- Steinfeld, H., P. Gerber, T.D. Wassenaar, V. Castel, M. Rosales, M. Rosales, and C. de Haan (2006). Livestock's long shadow: environmental issues and options. Food & Agriculture Organization.
- Szczechowiak, J., M. Szumacher-Strabel, M. El-Sherbiny, E. Pers-Kamczyc, P. Pawlak, and A. Cieslak, (2016). Rumen fermentation, methane concentration and fatty acid proportion in the rumen and milk of dairy cows fed condensed tannin and/or fish-soybean oils blend. *Anim. Feed Sci. Techn.*, 216:93-107.
- Tajodini, M., F. Samadi, S.R. Hashemi, S. Hassani, and M. Shams-Shargh (2014). Effect of different levels of Artichoke (*Cynara Scolymus* L.) powder on the performance and immune response of broiler chickens. *Int. J. Agric. Sci.*, 4: 66-73.
- Tangerman, A., and F.M. Nagengast (1996). A gas chromatographic analysis of fecal short-chain fatty acids, using the direct injection method. *Analytical biochemistry*, 236(1):1-8.

- Toral, P.G., A. Belenguer, P. Frutos, and G. Hervás (2009). Effect of the supplementation of a high-concentrate diet with sunflower and fish oils on ruminal fermentation in sheep. *Small Rumin. Res.*, 81: 119–125.
- Tsaknis, J., V. Spiliotis, S. Lalas, V. Gergis, and V. Dourtoglou (1999). Quality changes of *Moringa oleifera*, variety Mbololo of Kenya, seed oil during frying. *Grasas y Aceites*, 50(1):37-48.
- Van Keulen, J.V., and B.A. Young (1977). Evaluation of acid insoluble ash as a natural marker in ruminant digestibility studies. *J. Anim. Sci.*, 44:282.
- Weisbjerg, M.R., M.K. Larsen, L. Hymøller, M. Thorhauge, U. Kidmose, J.H. Nielsen, and J.B. Andersen (2013). Milk production and composition in Danish Holstein, Danish Red, and Danish Jersey cows supplemented with saturated or unsaturated fat. *Livest. Sci.*, 155(1):60-70.
- Whitlock, L.A., D.J. Schingoethe, A.A. Abu-Ghazaleh, A.R. Hippen, and K.F. Kalscheur (2006). Milk Production and composition from cows fed small amounts of fish oil with extruded soybean. *J. Dairy Sci.*, 89:3972-3980.
- Yoshimura, E.H., N.W. Santos, E. Machado, B.C. Agostinho, L.M. Pereira, S.C. de Aguiar, R. Franzolin, E. Gasparino, G.T. dos Santos, and L.M. Zeoula (2018). Effects of dairy cow diets supplied with flaxseed oil and propolis extract, with or without vitamin E, on the ruminal microbiota, biohydrogenation, and digestion. *Anim. Feed Sci. Techn.*, 241:163-172.

تأثير زيت بذرة المورينجا كإضافات غذائية طبيعية على الأداء الإنتاجي للنعاج الحلابة

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تهدف هذه الدراسة إلى إستخدام زيت بذرة المورينجا كإضافات غذائية لتقييم تأثيرها الكامن على تخمرات الكرش والأداء الإنتاجي للنعاج الأوسيمى الحلابة. تم استخدام اربع مستويات مختلفة (1، 2، 3 و 4%) من زيت بذرة المورينجا المستخلص على البارد كإضافة فى عليقة الأنفيترو (التجارب المعملية) لأختبار أفضل مستوى فى أختباره على الحيوانات المزرعية. وقد أظهرت نتائج التجارب المعملية أن معامل هضم المادة الجافة الحقيقية لم يتأثر معنوياً بإضافة الزيت حتى مستوى 3%، ولكن إنخفضت مع مستوى 4% مقارنة بالعليقة الكنترول. أظهرت المعاملات زيادة معنوية فى إنتاج الغاز، بينما إنخفض إنتاج الأمونيا فى هذ المعاملات عن الكنترول. وقد لخصت النتائج المعملية إلى إن أفضل مستوى معملى كان 1% من إضافة زيت المورينجا والذي تم إختباره لأختباره على الحيوانات المزرعية. التجربة المزرعية: تم أختبار 14 نعجة أوسيمى حلابة (متوسط أعمارهم 3 سنوات) و متوسط أوزانهم 51 كجم وقد بدأت التجربة بعد مرور 5 أيام من الولادة وقسمت عشوائياً الى مجموعتين وكل مجموعة بها 7 حيوانات. وكانت المجموعات عبارة عن مجموعة كنترول بدون أى إضافات و مجموعة كنترول مع إضافة 1% من زيت المورينجا على أساس المادة الجافة وأستمرت التجربة لمدة 45 يوم. وقد تم تقدير المادة الغذائية المهضومة و إنتاج اللبن وتركيبه. وأظهرت النتائج أن إضافة 1% من زيت بذرة المورينجا أدى إلى زيادة محصول اللبن ومكوناته من الدهن والبروتين واللاكتوز والجلوكوز واللاكتون فى اللبن زيادة معنوية مقارنة بالكنترول. وتوصلت الدراسة إلى انه يمكن استخدام زيت المورينجا كمصدر زيت طبيعى مضاف الى علائق الأغنام لتغطية الأحتياجات من الطاقة بالإضافة لأحتوائه على مضادات الأكسدة والمركبات الفينولية الأخرى التى تحسن من تخمرات الكرش وكبديل للمواد الكيميائية مثل المضادات الحيوية وبالتالي تحسين عملية الهضم وزيادة الإنتاجية.