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## Research Article

# *Moringa oleifera* and *Echinacea purpurea* as Supplements for Rhamani Lactating Ewe's Diets and Their Effect on Rumen Characteristics, Nutrients Digestibility, Blood Parameters, Milk Production, Composition and its Fatty Acid Profile

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## Abstract

**Objective:** This study was designed to evaluate impact of adding *M. oleifera* and *E. purpurea* dried leaves to diets of Rhamani lactating ewes on their rumen fermentation characteristics, nutrients digestibility, blood parameters, milk yield, composition and its fatty acid profile. **Materials and Methods:** Fifteen Rhamani lactating ewes after 1 week of parturition were assigned randomly into three groups of 5 animals each using complete random design. The entire experimental period was 84 days. Ewes were fed dry matter according to 4% of their body weight. The first group was fed the basal diet which consisted of 30% CFM and 70% berseem (control diet). The second group was fed the control diet supplemented with *Moringa oleifera* (MO) dried leaves at 15 g kg<sup>-1</sup> DM (T<sub>1</sub>), while the third group was fed the control diet supplemented with *Echinacea purpurea* (EP) dried leaves at 15 g kg<sup>-1</sup> DM (T<sub>2</sub>). **Results:** The ewes fed MO supplemented diet (T<sub>1</sub>) showed significant increase in most of ruminal parameters (except ruminal pH) and nutrients digestibility coefficients followed by EP supplemented ewe's diet (T<sub>2</sub>), while MO and EP supplementation decreased ruminal protozoal count significantly. There were no significant differences among all groups in blood albumin, globulin, ALT, AST and total lipids concentrations, but the ewes fed MO supplemented diets had higher (p<0.05) plasma protein and glucose values than those of control. The supplemented diets with MO and EP increased ewe's milk productivity by 12.75 and 4.4%, respectively compared with the control diet. Milk component's yield were higher (p<0.05) for MO supplemented ewes group than the other groups (control and T<sub>2</sub>). The EP treated ewes recorded the lowest (p<0.05) milk somatic cells count. The supplemented diets with MO and EP increased milk total unsaturated fatty acids by 14 and 11%, respectively compared with the control diet. **Conclusion:** The supplemented diets with MO and EP enhanced the performance of Rhamani lactating ewes with no harmful effects on their health.

**Key words:** *Moringa oleifera*, *Echinacea purpurea*, Rhamani ewes, milk production, milk fatty acids

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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

One of the most important constraints in Egyptian farm animal's production systems is the presence of limitations in both quantity and quality of feed ingredients<sup>1,2</sup>. Usually, the Egyptian farmers feed their lactating goats and ewes with low-quality berseem hay and agriculture by products, which are low in protein content, high in crude fiber and have short supply of vitamin and mineral contents, which leads to low digestibility and reduced voluntary feed intake<sup>3</sup>. These restrictions lead to low milk quantity which, in turn, gives low net incomes for farmers<sup>4,5</sup>. Therefore, supplementary feeding with high nutritive feeds could be a pre-requisite for successful dairy small ruminant's production<sup>6,7</sup>. On the other hands, the consumers in Egypt do not only look for fresh and tasty milk, but also safe and healthy. The over treat of farm animals with the synthetic antibiotics led to rapid rise number of the pathogenic bacteria which became more resistant to the majority of these antibiotics, this makes infection harder to control<sup>8,9</sup>. But, with gradual withdrawal of antibiotics from animal production system in Egypt, more emphasis has been given to use medicinal plants as natural animal's immune system promoters. In this concern, *Moringa oleifera* and *Echinacea purpurea* have been studied for their nutritional, pharmacological and immunological effects<sup>10-14</sup>.

*Moringa oleifera* Lamarck is a South Asian tree grows near The Himalaya Mountains but has spread overall the world<sup>15</sup>. Its leaves are rich in carotene and ascorbic acid with a good profile of amino acids, vitamins A, B and C, Ca, Fe and P<sup>16</sup>. Also, Thurber and Fahey<sup>17</sup> reported that *Moringa oleifera* leaves have beneficial effects include anti-inflammatory action, inhibition of platelets aggregation, antioxidant, antimicrobial and antitumor activities. In addition, Iqbal and Bhangar<sup>18</sup> reported that *M. oleifera* leaves contained polyphenolic compounds (e.g., kaempferol, rhamnetin, isoquercitrin and kaempferitrin). These flavonoids have important role in scavenging of the free radicals. In relation to antinutritional factors, *Moringa oleifera* leaves have a low quantity of tannins (12 g kg<sup>-1</sup> dry matter), phytates (21 g kg<sup>-1</sup>) and absence of trypsin and amylase inhibitors, lectins, cyanogenic glucosides and glucosinolates but contains significant amount of saponins and alkaloids (80 g kg<sup>-1</sup>) but this amount considered nontoxic to ruminants<sup>19</sup>. Similarly, *Echinacea purpurea* consider as a popular medicinal herb which support the body's immune system. The pharmacological activity of it depends on combined effect of alkamides (inhibit enzymes involved in the production of inflammatory mediators), caffeic acid derivatives (stimulate the activity of the immune cells to exhibit antiviral and

antioxidant activity and inhibit hyaluronidase enzyme which involved in infection and inflammation) and polysaccharides which play an important role in stimulation ability of the immune cells to exhibit anti-inflammatory activity<sup>10</sup>.

No data are available about the Egyptian production of *M. oleifera* and *E. purpurea* leaves; however, their relatively low prices encourage their use as animal feed supplements. Furthermore, their potential use as a small ruminants diet supplementation have not been fully documented, therefore, this study was designed to evaluate impact of adding *M. oleifera* and *E. purpurea* dried leaves to diets of Rhamani lactating ewes on their rumen fermentation characteristics, nutrients digestibility, blood parameters, milk yield, composition and fatty acid profile.

## MATERIALS AND METHODS

This work was carried out at Agricultural Experimental Station, Sheep and Goat Research Unit, Faculty of Agriculture, Cairo University, Giza, Egypt. The entire experimental period was extended from November 4, 2014 to January 26, 2015. The chemical and microbiological analyses were carried out at the Laboratories of Dairy Sciences Department, National Research Center, Dokki, Giza, Egypt and Animal Nutrition Department, Desert Research Center, Cairo, Egypt.

**Feeding and management:** Fifteen Rhamani lactating ewes (about 2 years old and weighing on average 45.8±0.3 kg) after 1 week of parturition were assigned randomly into three groups of 5 animals each using complete random design. The entire experimental period was 84 days (12 weeks). Ewes were fed dry matter according to 4% of their body weight changed continuously according to animal weight changes. The first group was fed the control diet which consisted of 30% concentrate feed mixture (CFM) and 70% berseem (Egyptian clover). The second group was fed the control diet supplemented with *Moringa oleifera* dried leaves powder at 15 g kg<sup>-1</sup> DM (T<sub>1</sub>), while the third group was fed the control diet supplemented with *Echinacea purpurea* dried leaves powder at 15 g kg<sup>-1</sup> DM (T<sub>2</sub>). The concentrate feed mixture (CFM) and berseem were offered once daily at 7.00 and 8.00 am, respectively. Fresh water was available to the animals all the time. The feed ingredients and the chemical composition of basal diet are shown in Table 1.

**Apparent digestibility:** A grab sample method was applied at which acid insoluble ash (AIA) was used as an internal marker for determination of nutrient digestion coefficients

Table 1: Ingredients and chemical composition of the basal diet fed to Rhamani lactating ewes (percentage per kilogram dry matter)

Ingredients	DM (% kg <sup>-1</sup> )
Berseem (Egyptian clover)	70.00
Yellow corn	18.00
Wheat bran	6.00
Sunflower meal	3.00
Soybean meal	1.50
Mineral and vitamin mix*	1.50
<b>Chemical composition</b>	
Organic matter	89.36
Crude protein	19.15
Ether extract	5.98
Crude fiber	15.22
Nitrogen free extract	49.01
Ash	10.64

\*Mineral and vitamin mix contained 42 ppm Co, 3500 ppm Cu, 20,000 ppm Fe, 12,000 ppm Mn, 12,000 ppm Zn, 1200 ppm I, 3800 IU g<sup>-1</sup> of vitamin A, 1200 IU g<sup>-1</sup> of vitamin D and 3 IU g<sup>-1</sup> of vitamin E

according to Ferret *et al.*<sup>20</sup>. At the end of each month of the experimental period, fecal grab samples were collected in cloth bag connected to the animal back at 11 am for two successive days from two animals of each group. The collected feces were dried at 60°C for 48 h and then ground for chemical analysis. The digestibility coefficient of nutrient was calculated according to the following formula<sup>20</sup>:

$$\text{Digestion co-efficient} = 100 - \left[ 100 \times \frac{\text{Indicator in feed (\%)}}{\text{Indicator in feces (\%)}} \times \frac{\text{Nutrient in feces (\%)}}{\text{Nutrient in feed (\%)}} \right]$$

**Feed and fecal analysis:** Feedstuffs and fecal samples were analysed according to the AOAC<sup>21</sup> methods to determine Dry Matter (DM), Crude Protein (CP), Ether Extract (EE), Crude Fiber (CF) and ash contents. Organic Matter (OM) and Nitrogen Free Extract (NFE) contents were calculated by difference.

**Sampling and analysis of rumen liquor:** At the last day of each month of experimental period, rumen liquor samples were collected by stomach tube from two animals each group at 12 pm. Samples were strained through two layers of gauze cloth to remove feed particles and immediately used for determination of ruminal pH using digital pH meter. Rumen liquor samples were stored in glass bottles with drops of toluene and thin layer of paraffin oil and stored in a deep freeze (-20°C) until analysis. Ammonia nitrogen concentration (NH<sub>3</sub>-N), total nitrogen (TN) and non-protein nitrogen (NPN) were determined by the modified semi-micro-kjeldahl digestion method according to AOAC<sup>21</sup> while, true protein nitrogen (TP) was calculated by subtracting the non-protein nitrogen content from total nitrogen content. The total volatile fatty acids (TVFA's) were determined according to method of Warner<sup>22</sup>. The ruminal microbial protein was

estimated as described by Makkar *et al.*<sup>23</sup>. For classification and determination of ruminal ciliate protozoal count, the filtered rumen liquor were fixed and stained with 4 times volume of methyl-green formalin saline solution as described by Ogimoto and Imai<sup>24</sup>, then stoked in dark place until examination. After gentle mixing of fixed rumen liquor sample, one drop was poured on hemocytometer slide, covered with a cover slip and examined under a light microscope for identification of protozoal genera and species according to the description published by Dehority<sup>25</sup>.

**Sampling and analysis of blood plasma:** Blood samples were taken from jugular vein of two animals each group through the last 3 days of each month of the experimental period at about 4 h after feeding of CFM and directly collected in glass tubes containing EDTA as anticoagulant agent. Blood plasma samples were separated by centrifuge at 4000 rpm for 20 min and kept frozen for later analysis. Plasma total protein was determined according to method of Armstrong and Carr<sup>26</sup>, albumin<sup>27</sup>, globulin was calculated by subtracting the albumin from total protein, urea<sup>28</sup>, glucose<sup>29</sup>, total lipids<sup>30</sup>, plasma aspartate aminotransferase (AST) and alanin aminotransferase (ALT)<sup>31</sup>.

**Sampling and analysis of milk:** The ewes were milked twice a day at 7.00 am and 4.00 pm during the last 3 days of each month of experimental period. Milk samples were immediately collected from each animal after morning and evening milking and milk yield was recorded. The sample of each animal represented a mixed sample of constant percentage of the morning and evening yield. Milk samples were analysed for total solids, fat, protein and lactose by bentley 150 infrared milk analyzer (Bentley Instruments, Chaska, MN, USA) according to AOAC<sup>21</sup> procedures. Solids-not-fat (SNF) was calculated by subtracting fat from total solids percentage. Fatty acids profile of milk fat was determined as methylated according to Park *et al.*<sup>32</sup> and separated by gas liquid chromatography. Fat corrected milk (4% fat) was calculated by using the following equation according to Gaines<sup>33</sup>:

$$\text{FCM} = 0.4 \text{ M} + 15 \text{ F}$$

where, M is milk yield (g) and F is fat yield (g).

**Statistical analysis:** Data obtained from this study were statistically analysed by IBM SPSS Statistics for Windows<sup>34</sup> using the following general model procedure:

$$Y_{ij} = \mu + T_i + e_{ij}$$

where,  $Y_{ij}$  is the parameter under analysis of the  $ij$  bottles of rumen liquor trails or ewes of digestibility and lactation trails,  $\mu$  is the overall mean,  $T_i$  is the effect due to treatment on the parameter under analysis and  $e_{ij}$  is the experimental error for  $ij$  on the observation, the Duncan's multiple range tests was used to test the significance among means<sup>35</sup>.

## RESULTS

**Rumen characteristics:** Ruminal characteristics values of all ewe's groups are illustrated in Table 2. The ruminal pH values showed significant decrease by ewes fed *Moringa oleifera* (MO) supplemented diet ( $T_1$ ), while no significant differences were found between the ewe's group fed the *Echinacea purpurea* (EP) supplemented diet ( $T_2$ ) and the other groups. All pH values were above 6.0 which indicated a better digestion of fibrolytic materials<sup>36</sup>. In contrast, the ruminal total volatile fatty acids (TVFA's), non-protein nitrogen (NPN), total nitrogen, ammonia nitrogen ( $NH_3-N$ ), true protein and microbial protein concentrations showed significant ( $p > 0.05$ ) increase for ewes fed MO supplemented diet ( $T_1$ ) followed by EP supplemented ewes ( $T_2$ ), while the lowest values were recorded for the control ewes. No significant differences were detected between EP supplemented group ( $T_2$ ) and the other groups in ruminal ammonia nitrogen and microbial protein concentrations. Concerning with MO and EP effect on rumen protozoa; 5 species with 13 subspecies of ruminal protozoa were identified in the rumen liquor of ewe's groups (Table 3). Ruminal protozoal subspecies density (count  $\times 10^4$  cell  $mL^{-1}$  rumen liquor) showed the lowest ( $p < 0.05$ ) value by ewes treated with EP ( $T_2$ ) followed by MO treated ewes ( $T_1$ ), while the heights protozoal count were detected in the rumen liquor of the control ewes. In addition, no significant difference among all ewes groups in *E. simplex*, *D. elongatum*, *I. prostoma*, *I. intestinalis* and *D. rummantium* count.

**Nutrients digestibility:** Data of Table 4 showed significant ( $p < 0.05$ ) increase of apparent digestibility of DM, OM, CP and NFE for ewes fed MO supplemented diets ( $T_1$ ) compared with those fed the control or EP supplemented diets, while EE and CF digestibility were not affected by the treatments. Although, there were insignificant ( $p > 0.05$ ) differences between EP treated group and the control group in all nutrients digestibility, but EP treated group had slightly increase in most nutrients digestibility compared with the control. Furthermore, the nutritive values of the tested diets as TDN and DCP take the same trend of the nutrients digestibility.

Table 2: Effect of treatments on ewe's ruminal characteristics

Items	Treatments			Mean $\pm$ SE
	Control	MO	EP	
pH	6.87 <sup>a</sup>	6.49 <sup>b</sup>	6.62 <sup>ab</sup>	0.06
Total volatile fatty acids mg/100 mL	11.24 <sup>c</sup>	13.03 <sup>a</sup>	12.00 <sup>b</sup>	0.18
Ammonia-Nitrogen mg/100 mL	45.43 <sup>b</sup>	55.01 <sup>a</sup>	48.87 <sup>ab</sup>	1.48
Non protein nitrogen mg/100 mL	54.14 <sup>b</sup>	66.13 <sup>a</sup>	58.15 <sup>b</sup>	1.47
Total nitrogen mg/100 mL	147.97 <sup>c</sup>	187.93 <sup>a</sup>	164.28 <sup>b</sup>	2.94
True protein mg/100 mL	93.82 <sup>c</sup>	121.79 <sup>a</sup>	106.13 <sup>b</sup>	2.31
Microbial protein mg/100 mL	47.31 <sup>b</sup>	72.79 <sup>a</sup>	63.38 <sup>ab</sup>	3.08

Means with different letters with each row are significantly different ( $p < 0.05$ )

Table 3: Effect of treatments on ewe's ruminal ciliate protozoa count ( $\times 10^4$  cell  $mL^{-1}$  rumen liquor)

Items	Treatments			Mean $\pm$ SE
	Control	MO	EP	
<b>Entodinium spp.</b>				
<i>E. caudatum</i>	294.67 <sup>a</sup>	291.67 <sup>ab</sup>	188.67 <sup>b</sup>	9.91
<i>E. simplex</i>	208.00	192.67	179.00	10.78
<i>E. minimum</i>	309.00 <sup>a</sup>	312.33 <sup>a</sup>	198.33 <sup>b</sup>	19.28
<i>E. bursa</i>	166.67 <sup>a</sup>	132.67 <sup>b</sup>	104.67 <sup>b</sup>	7.00
<i>E. furca</i>	79.00 <sup>a</sup>	25.33 <sup>b</sup>	13.33 <sup>b</sup>	6.33
<b>Epidinium spp.</b>				
<i>E. ecaudatum</i>	113.67 <sup>a</sup>	32.33 <sup>b</sup>	35.00 <sup>b</sup>	8.37
<b>Polyolastrom spp.</b>				
<i>P. multivesiculatum</i>	69.00 <sup>a</sup>	61.67 <sup>ab</sup>	39.00 <sup>b</sup>	5.06
<b>Diplodinium spp.</b>				
<i>D. psitaceum</i>	113.00 <sup>a</sup>	42.33 <sup>b</sup>	89.33 <sup>a</sup>	10.28
<i>D. dentatum</i>	39.67 <sup>a</sup>	27.67 <sup>b</sup>	31.00 <sup>ab</sup>	2.09
<i>D. elongatum</i>	20.67	23.67	15.67	1.86
<b>Isotrichia spp.</b>				
<i>I. prostoma</i>	44.33	35.67	34.00	2.16
<i>I. intestinalis</i>	33.00	28.33	33.00	1.54
<b>Dasytrachia spp.</b>				
<i>D. rummantium</i>	48.67	50.33	43.66	6.63
Total count	1539.35 <sup>a</sup>	1256.67 <sup>b</sup>	1004.66 <sup>c</sup>	21.11

Means with different letters with each row are significantly different ( $p < 0.05$ )

Table 4: Apparent nutrients digestibility as affected by the treatments

Items	Treatments			Mean $\pm$ SE
	Control	MO	EP	
<b>Apparent nutrients digestibility (%)</b>				
Dry matter	69.01 <sup>b</sup>	74.99 <sup>a</sup>	70.24 <sup>b</sup>	1.17
Organic matter	74.56 <sup>b</sup>	77.86 <sup>a</sup>	75.12 <sup>b</sup>	0.65
Crude protein	71.07 <sup>b</sup>	77.64 <sup>a</sup>	71.83 <sup>b</sup>	1.35
Ether extract	76.98	76.98	77.37	0.13
Crude fiber	68.51	69.19	68.18	0.27
Nitrogen free extract	77.51 <sup>b</sup>	80.74 <sup>a</sup>	78.28 <sup>b</sup>	0.11
<b>Nutritive value (%)</b>				
Total digestible nutrients (TDN)	72.37 <sup>b</sup>	75.38 <sup>a</sup>	72.90 <sup>b</sup>	0.06
Digestible crude protein (DCP)	13.61 <sup>b</sup>	14.87 <sup>a</sup>	13.75 <sup>b</sup>	0.12

Means with different letters with each row are significantly different ( $p < 0.05$ )

**Blood parameters:** The ewes fed MO supplemented diets had higher ( $p < 0.05$ ) plasma protein and glucose values than those of control, while the control group recorded the highest ( $p < 0.05$ ) blood urea concentration (Table 5). Although, there were no significant ( $p > 0.05$ ) differences between treated

Table 5: Blood plasma metabolites as affected by the treatments

Items	Treatments			Mean±SE
	Control	MO	EP	
Total protein (g dL <sup>-1</sup> )	6.22 <sup>b</sup>	6.74 <sup>a</sup>	6.66 <sup>a</sup>	0.10
Albumin (g dL <sup>-1</sup> )	4.18	4.33	4.09	0.04
Globulin (g dL <sup>-1</sup> )	2.34	2.41	2.57	0.10
Urea (mg dL <sup>-1</sup> )	34.07 <sup>a</sup>	29.50 <sup>b</sup>	31.60 <sup>ab</sup>	0.78
Glucose (mg dL <sup>-1</sup> )	59.90 <sup>b</sup>	66.90 <sup>a</sup>	60.46 <sup>ab</sup>	2.19
Total lipids (mg dL <sup>-1</sup> )	394.65	419.03	410.69	6.03
AST (U mL <sup>-1</sup> )	37.20	32.60	32.00	1.81
ALT (U mL <sup>-1</sup> )	17.00	16.60	19.60	0.80

Means with different letters with each row are significantly different ( $p < 0.05$ )

Table 6: Effect of treatments on ewe's milk yield, composition and somatic cell count

Items	Control	MO	EP	Mean±SE
<b>Yield (g day<sup>-1</sup>)</b>				
Milk yield	484.70 <sup>b</sup>	546.50 <sup>a</sup>	505.90 <sup>ab</sup>	9.27
Fat corrected milk yield (4%)	487.41 <sup>b</sup>	578.47 <sup>a</sup>	515.77 <sup>ab</sup>	10.17
Total solids yield	62.82 <sup>b</sup>	76.30 <sup>a</sup>	67.02 <sup>b</sup>	1.37
Fat yield	19.57 <sup>b</sup>	23.99 <sup>a</sup>	20.89 <sup>b</sup>	0.43
Solids not fat yield	43.25 <sup>b</sup>	52.31 <sup>a</sup>	46.13 <sup>b</sup>	0.93
Protein yield	19.98 <sup>b</sup>	24.48 <sup>a</sup>	21.26 <sup>b</sup>	0.44
Lactose yield	19.88 <sup>b</sup>	23.85 <sup>a</sup>	21.18 <sup>b</sup>	0.42
Ash yield	3.39 <sup>b</sup>	4.00 <sup>a</sup>	3.67 <sup>b</sup>	0.07
Somatic cell count ( $\times 10^3$ cm <sup>-3</sup> )	1324.40 <sup>a</sup>	580.60 <sup>b</sup>	227.00 <sup>c</sup>	64.24
<b>Milk composition (%)</b>				
Total solids	12.96 <sup>b</sup>	13.96 <sup>a</sup>	13.25 <sup>b</sup>	0.13
Fat	4.04 <sup>b</sup>	4.39 <sup>a</sup>	4.13 <sup>ab</sup>	0.07
Solids not fat	8.92 <sup>c</sup>	9.57 <sup>a</sup>	9.12 <sup>b</sup>	0.09
Protein	4.12 <sup>b</sup>	4.48 <sup>a</sup>	4.20 <sup>ab</sup>	0.06
Lactose	4.10	4.36	4.19	0.05
Ash	0.70	0.73	0.73	0.05

Means with different letters with each row are significantly different ( $p < 0.05$ )

Table 7: Milk fatty acids profile as affected by the treatments

Items	Control	MO	EP	Mean±SE
C8	2.63 <sup>a</sup>	2.57 <sup>a</sup>	2.32 <sup>b</sup>	0.05
C10	8.27 <sup>a</sup>	3.25 <sup>b</sup>	3.37 <sup>b</sup>	0.83
C11.0	1.06	0.95	0.98	0.03
C12	4.54 <sup>b</sup>	7.62 <sup>a</sup>	3.46 <sup>c</sup>	0.03
C14.0	12.09 <sup>a</sup>	10.38 <sup>b</sup>	10.14 <sup>b</sup>	0.31
C14.1	0.78 <sup>b</sup>	0.97 <sup>a</sup>	0.94 <sup>a</sup>	0.03
C15.0	0.98 <sup>a</sup>	0.92 <sup>b</sup>	0.84 <sup>b</sup>	0.02
C16.0	27.17 <sup>b</sup>	26.00 <sup>c</sup>	28.78 <sup>a</sup>	0.40
C16.1	1.45 <sup>c</sup>	1.87 <sup>b</sup>	2.51 <sup>a</sup>	0.15
C17.0	1.16 <sup>b</sup>	1.80 <sup>a</sup>	0.80 <sup>c</sup>	0.14
C18.0	11.40 <sup>b</sup>	14.38 <sup>a</sup>	14.93 <sup>a</sup>	0.55
C18.1N9T	25.33 <sup>b</sup>	27.71 <sup>a</sup>	27.14 <sup>a</sup>	0.36
C18.1N9C	1.66 <sup>b</sup>	2.54 <sup>a</sup>	1.85 <sup>a</sup>	0.13
C18:2 cis-9, trans-11	0.15	0.17	0.16	0.01
C18:2 trans-10, cis-12	0.36 <sup>c</sup>	0.70 <sup>a</sup>	0.53 <sup>b</sup>	0.05
C18.3N3	0.33	0.31	0.40	0.01
C18.3N6	0.64	0.62	0.66	0.01
Total unsaturated fatty acids	30.70 <sup>c</sup>	34.89 <sup>a</sup>	34.20 <sup>b</sup>	0.65
Total saturated fatty acids	69.30 <sup>a</sup>	67.87 <sup>b</sup>	65.61 <sup>c</sup>	0.54
Mono-unsaturated fatty acids	29.21 <sup>c</sup>	33.09 <sup>a</sup>	32.44 <sup>b</sup>	0.60
Poly-unsaturated fatty acids	1.49 <sup>b</sup>	1.80 <sup>a</sup>	1.76 <sup>a</sup>	0.05
Total CLA	0.52 <sup>c</sup>	0.86 <sup>a</sup>	0.69 <sup>b</sup>	0.05
N6/n3 ratio	1.95	1.99	1.65	0.06

Means with different letters with each row are significantly different ( $p < 0.05$ )

groups (MO and EP) in all tested blood parameters, but the EP supplemented group had the highest blood globulin concentration. Moreover, There were no significant differences ( $p > 0.05$ ) among all groups in blood albumin, globulin, ALT, AST and total lipids concentrations. In the current study, ewe's diets supplementation with MO or EP through entire experimental period did not badly affect the liver or kidney functions.

**Milk yield, composition and its somatic cell count:** Milk yield and 4% Fat Corrected Milk (FCM) yield were higher ( $p < 0.05$ ) for MO treated ewes compared to ewes of the control, while the differences among ewes fed EP treated diets ( $T_2$ ) and the other ewes (control and  $T_1$ ) were not significant (Table 6). Feeding diets supplemented with MO and EP increased ewe's milk productivity by 12.75 and 4.4%, respectively compared with the control diet, while fat corrected milk productivity increased by 18.7 and 5.81%, respectively. As in results of milk yield, milk component's yield were higher ( $p < 0.05$ ) for ewes group fed  $T_1$  diet than the other groups (control and  $T_2$ ), while no significant differences among control and  $T_2$  in all milk component's yields. Concerning with the milk composition, milk fat, protein, Solids Not Fat (SNF) and Total Solids (TS) percentages were higher ( $p < 0.05$ ) for MO supplemented group compared to the control one, while the supplementation with EP did not affect percentages of milk components except in case of SNF%. The supplemented group with EP showed significant increase in SNF% when compared with the control but still significantly lower than MO supplemented group. No significant differences among treated groups ( $T_1$  and  $T_2$ ) in fat and protein percentages, while no significant differences among all groups in percentages of lactose and ash. Furthermore, the Somatic Cells Count (SCC) showed significant increase ( $p < 0.05$ ) in milk of control ewes followed by MO treated ewes while the EP treated ewes recorded the lowest ( $p < 0.05$ ) milk somatic cells count. In other words, EP and MO supplementation led to decrease somatic cell count in milk to be 17.14 and 43.84% of its count in control milk, respectively.

**Milk fatty acids profile:** The impact of treated ewes with MO and EP on milk methylated fatty acids profile is shown in Table 7. The conjugated linoleic acid (CLA), mono and total unsaturated fatty acids were significantly higher in milk of MO treated ewes followed by EP treated ewe's milk then the control's milk, while the control's milk recorded the highest ( $p < 0.05$ ) total saturated fatty acids concentration. It is worth mention that, supplemented diets with MO and EP increased



total unsaturated fatty acids (about 14 and 11%, respectively) and decreased total saturated fatty acids (2.1 and 5.3%, respectively) of milk compared with the control diet. The poly unsaturated fatty acids were higher ( $p < 0.05$ ) in milk of treated groups ( $T_1$  and  $T_2$ ) than of the control one, while omega3 and omega6 (n6/n3) ratio were not affected by the treatments.

## DISCUSSION

The study of ruminal characteristics is fundamental for more understanding of ruminal microbial activity, digestion and metabolism as affected by the treatments. In current study, the reduction of ruminal pH values and increase ruminal concentrations of TVFA's and protein fraction concentrations after MO and EP treatment could be regarded to antioxidant and antimicrobial effects of MO and EP which may provide a suitable environment for the growth of beneficial microflora in the rumen and let for more diet nutrients fermentation and subsequently TVFA's production<sup>12,17</sup>. In this concern, Soliva *et al.*<sup>37</sup> reported that *Moringa* leaves promotes rumen microbial protein synthesis due to its substantial contents of readily fermentable N and energy. Also, MO leave's  $\alpha$ -linolenic acid, tannins and saponins have suppressor effect on the growth and activity of the methane producing bacteria and this may save energy which otherwise lost as methane and support more TVFA's production. Our finding agree with those of Hadhoud<sup>38</sup> who found that ruminal pH and ammonia concentration were not affected by *Echinacea purpurea* supplementation to Damascus lactating goats at level of  $8 \text{ g kg}^{-1}$  DM, while TVFA's concentration were higher ( $p < 0.05$ ) for supplemented goats than control's goats. Similarly, Sarwatt *et al.*<sup>39</sup> reported that the small amounts of *Moringa* leaves improved the rumen environment.

Concerning with effect of MO and EP supplementation on ruminal protozoa; Newbold and Chabernain<sup>40</sup> reported that unsaturated C18 fatty acids are toxic to rumen ciliate protozoa. Olaofe *et al.*<sup>41</sup> stated that *Moringa oleifera* leaves are rich in unsaturated C18 fatty acids and these acids represent 41.5% of *M. oleifera* leaves total fat. It has been reported that the fresh biomass of *Echinacea purpurea* also rich in unsaturated C18 fatty acids and these fatty acids represent 80.8% of *E. purpurea* total fat<sup>42</sup>; this may illustrate the reason for protozoa count reduction in the rumen of MO and EP supplemented groups.

The positive effect of MO on nutrients digestibility can be attributed to its positive effect on the microbial activity in the rumen as it has high content of slow degradable protein and essential amino acids which can enhance dietary N utilization

by the treated animals. Moreover, the obvious high ruminal production of TVFA's and microbial protein yield and apparent dietary protein digestibility (Table 2, 4) may indicate that MO leaves improved the synchrony between dietary energy and protein in the rumen of the treated ewes. Hadhoud<sup>38</sup> stated that supplemented goat's diets with *Echinacea purpurea* at level of 4 or  $8 \text{ g kg}^{-1}$  DM significantly enhance the entire nutrients digestibility coefficients. In addition, Khalel *et al.*<sup>11</sup> reported that the digestible protein in the intestine (DPI) for *Moringa* leaves was higher than various conventional protein supplements like seed meal and this may enhance nutrients digestibility. Diet's TDN and DCP increased as a result of the higher nutrients digestibility associated with *Moringa* leaves supplementation.

Increase plasma total protein values in the treated groups (MO and EP) may be due to the improvement of rumen environment after MO and EP supplementation which led to enhance dietary protein digestion and microbial protein synthesis (Table 2, 4). The significant lower of plasma urea concentration associated MO supplementation can be attributed to occur improvements in the metabolic process of treated ewes including highly dietary nitrogen utilization than ewes of the control. However, the significant increase of plasma glucose concentration in the blood of MO treated animals may be reflect increasing of NFE digestibility (Table 4) or may support the assumption of Khalel *et al.*<sup>11</sup> that, feeding *Moringa* may help in bypassing some soluble carbohydrates to be absorbed as glucose. It can be notes that, ewes supplemented with *E. purpurea* had the higher globulin values and this may support the results of Teleb *et al.*<sup>10</sup> who reported that *E. purpurea* stimulate animal's own defense system. Hadhoud<sup>38</sup> reported that supplemented goat's diets with *Echinacea purpurea* at level of 4 or  $8 \text{ g kg}^{-1}$  DM significantly increase blood serum total protein of the treated goats, but had no effect on blood serum albumin, globulin, urea, glucose, ALT, AST, cholesterol and triglycerides concentrations

The enhancement of milk and fat corrected milk yields by treated ewes with MO leaves could be attributed to positive effect of MO on fermentation process in the rumen which reflects increased microbial protein synthesis and TVFA's concentration (Table 2). Singh *et al.*<sup>43</sup> stated that higher concentrations of TVFA's in galactagogue treated groups have been regarded as an indicative of better energy supply for milk production. In addition, Aerts *et al.*<sup>44</sup> assumed that phenolics and tannins of MO leaves negatively affects the methanogenesis in the rumen and this may cause saving amount of waste energy in the form of  $\text{CH}_4$ , resulting in improved milk production efficiency by the treated animals.

Moreover, higher milk fat, protein, Solids Not Fat (SNF) and Total Solids (TS) percentages and yields and lactose yield following MO supplementation might be due to higher rumen fermentation (Table 2), high CP, DM, OM and NFE digestibilities (Table 4) or due to increase milk yield in the treated ewes. In this concern, Rocha and Mendieta<sup>42</sup> fed dairy cows with different levels of *Moringa* leaves and they found that MO supplementation at a level of 0.3% b.wt., increased cow's milk yield by 13% compared with the control cows, which was grazing only. The earlier data of Reklewska *et al.*<sup>45</sup> who found that the addition of *Echinacea* extracts to diets of white polish goats did not significantly affect milk yield. However, Hadhoud<sup>38</sup> stated that supplemented goat's diets with *Echinacea purpurea* at level of 4 g kg<sup>-1</sup> DMI significantly increase milk protein, lactose, ash, solids not fat (SNF) and total solids (TS) percentages and yields. Another studies by Kholif *et al.*<sup>13,14</sup> found a significant increase in daily milk yield and improved milk composition in goats fed *Moringa* fresh or silage as a protein supplement.

In the current study, the Somatic Cells Count (SCC) reduction following addition of medicinal plants especially *E. purpurea* can be attributed to increase lactoferrin secretion, which is anti-bacterial, anti-viral and immuno-stimulating compound. The obtained results were in line with those obtained by Hadhoud<sup>38</sup> who reported that adding *Echinacea purpurea* to dairy goat's diets at level of 4 or 8 g kg<sup>-1</sup>. Significantly decrease SCC in the produced milk. In contrast, Dymnicka *et al.*<sup>46</sup> didn't observe any significant changes in milk SCC in cows given 300 g head<sup>-1</sup> of dried whole *Echinacea purpurea* plant over 3 weeks. However, *Echinacea purpurea* effectiveness may vary according to plant growth conditions, as well as to its harvesting and conservation methods. Also, dry matter intake, feed utilization efficiency, type of basal diets, animal breed and physiological status may cause the observed differences in the different studies.

The significant alteration of fatty acid profile after MO and EP supplementation could be attributed to high concentrations of fatty acids generally and unsaturated fatty acids especially in *M. oleifera* and *E. purpurea* leaves. In this concern, Olaofe *et al.*<sup>41</sup> reported that total unsaturated fatty acids (TUFA) of *M. oleifera* leaves represent 42% of its total fat, while TUFA in fresh biomass of *E. purpurea* represent 82% of its total fat<sup>47</sup>. As ruminants can not synthesize unsaturated fatty acids; so milk fatty acids concentration in milk depends mainly on their absorbed amounts from the small intestine which came from the digested feed<sup>13,14</sup>, consequently, the alteration of milk fatty acids profile observed in the current study could be attributed to *M. oleifera* and *E. purpurea* direct effect.

## CONCLUSION

In the light of the obtained results it can be concluded that the supplemented diets with *Moringa oleifera* (MO) dried leaves at 15 g kg<sup>-1</sup> DM showed superior positive effect on nutrients digestibility, milk production, milk composition and milk fatty acids profile of Rhamani lactating ewes than those of control and *Echinacea purpurea* supplemented diets. While, *Echinacea purpurea* showed superior positive effect on the ewe's udder health as its supplementation led to decrease ewe's milk somatic cell count to be about 17% of the control. *Moringa oleifera* and *Echinacea purpurea* supplementation had no harmful effects on the general health of the treated ewes.

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