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Blood Serum Biochemical Changes and Milk Fatty Acids Profile Due to Using Garlic Plant as Feed Additives for Sheep

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Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

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ABSTRACT

This study was carried out to investigate the effect of using garlic (*Allium sativum*) as feed additives on immune status and milk fatty acids components for ewes. The study consists of two parts: the first part included metabolism trial using 16 adult barki rams divided into four equal groups. The first group (T1) fed control ration (concentrate mixture and alfalfa hay), the 2nd (T2) and 3rd (T3) groups fed control ration with 2% and 3% (on dry matter basic) garlic powder while the 4th group (T4) fed control ration plus 2 ml/head/days of garlic oil, respectively. Results revealed that dry matter, organic matter and crude protein digestibility for T2 and T3 were significantly higher than their values for control group. The second part included feeding trial using 18 pregnant Barki ewes aged 2-3 years and weighed 40.9 kg body weight at late gestation. Ewes were selected and randomly distributed into three similar groups (6 in each) which fed the same rations that were used in the metabolic trial except T3. Results showed improvement of total proteins, albumin and Immunoglobulin (IgG) in blood serum of ewes by garlic additives. Also, feeding ewes on ration contents 2% of garlic powder or 2 ml of garlic oil were insignificantly increased IgG and protein of colostrum and the content of milk from C18:1ω9, C18:2ω6 and C18:2ω3 and C20:0 fatty acids compared with control group. It could be illustrate that ration which supplemented by 2%

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garlic powder and 2 ml /h/d of garlic powder has positive results on dry matter, organic matter and crude protein digestibility, immune globulin in ewes blood and colostrum, the proportions of conjugated linoleic acid, omega 3 and unsaturated fatty acids in ewes milk which consider a good indicator for animal immune status and healthy milk for consumers.

Keywords: Sheep; garlic oil; garlic powder; digestibility; milk fatty acids; immunoglobulin.

1. INTRODUCTION

In the desert rangeland areas of Egypt Barki sheep are reached about 470,000 heads [1] which consider the main resource of income for the Bedouin. These animals are characterized by poor feeding, lack of modern management skills and inadequate adoption of technologies essential to improve their productivity.

According to the FAO, the lack of drugs to treat diseases and infections causes losses of 30 to 35% in the breeding sector of many developing countries, where poor animal health remains the major constraint to breeding. The use of antibiotic as production enhancers has been criticized for the risk of residues into animal derived food products and into the environment. Since the use of antibiotics in animal feeds has been banned, considerable effort has been devoted towards developing alternatives to antibiotics. Among the natural additives, aromatic plants, their extracts and essential oils have been examined due to their advantages over the antibiotics as growth promoters; they are residue free and generally recognized as safe. Furthermore it could help smallholders in desert areas to manage their only income resources from diseases and mortality.

One of the most important herbal plants is garlic *(Allium sativum*) for feeding sheep that could improve rumen fermentation, feed efficiency and animal health [2,3,4]. Therefore the objective of this present study was to evaluate the effects of garlic powder and garlic oil additives on nutrient digestibility, immune status and milk fatty acids profile for lactating ewes.

2. MATERIALS AND METHODS

2.1 Experimental Location

This study was conducted at Maryout Research Station belongs to Desert Research Center (DRC), 35 km south of Alexanderia, Egypt during February to June 2016. The experimental procedures were approved by the Animal and Poultry Production Division of DRC committee and as followed by the Veterinary and Animal Care Department.

2.2 Metabolism Trial

This study was divided into two parts. The first part included metabolism using 16 adult barki rams, aged 3-4 year and average weighed 40.47 kg ± 0.99, divided into four equal groups which fed on concentrate mixture (CM) and alfalfa hay (as control ration) at 40:60% of dry matter bases. The first group fed (T1) fed control ration without any supplementation, the 2nd (T2) and 3rd (T3) groups fed control ration with 2% and 3% (on dry matter basic) garlic powder while the 4th group (T4) fed control ration plus 2 ml/head/days of garlic oil, respectively. Garlic bulb yielded about 0.10% garlic oil on a wet weight basis. The GC–MS analyses of garlic oil revealed that trisulfide di-2-propenyl (29.8%), diallyl disulfide (25.4%) and trisulfide methyl 2-propenyl (11.3%) are the main components of the oil. The chemical composition of feed ingredient and control ration is shown in Table 1. Feed offered was calculated to cover the maintenance requirements for adult rams [5]. Garlic powder was mixed with CM for daily offered. Each rams was taken garlic oil dosage individually. Daily feed offered and refused if any were recorded to estimate the actual feed intake (the real amount) for each group. Animals were fed in groups for 30 days, and then fed individually in metabolism cages for 15 days (8 days as adaptation period followed by 7 days as collection period) in shaded pens to avoid water loss during collecting feces. Total feces were recorded daily. Representative samples of feces about 10% of the total fresh feces weight were taken daily and few drops of sulfuric acid were added to avoid losing nitrogen. At the end of collection period feces samples of each animal was mixed and ground for sampling. Soft water was available free choice during the experimental period. Urine was allowed to drain into bottles containing 5 ml of Sulfuric acid and a slight amount of thymol granules was added to prevent losing nitrogen and fermentations. The volume of urine was recorded daily and a sample of urine represents 10% of total urine for each animal was taken for proximate analysis.

Table 1. Chemical composition of garlic powder, concentrate mixture (CM), alfalfa hay and control ration (% on DM basis)

Item	Composition % on DM					
	DM	OM	CP	CF	NDF	TDN
Garlic powder	92.6	94.5	16.3	0	0	--
CM	91.1	91.1	16.1	16.5	33.1	67.9
Alfalfa hay	91.33	87.5	15.5	31.9	48.3	56.5
Control ration	91.4	89.6	16.4	21.2	36.7	62.8

DM = dry matter, OM = organic matter, CP = crude protein, CF = crude fiber, NDF = neutral detergent

Samples of feed offered, refused, feces and urine were taken and stored during the collection period for analysis. Proximate analyses of concentrate mixture, alfalfa hay, urine and feces samples were determined according to AOAC [6].

2.3 Feeding Trial

From the results of the metabolism trial, a feeding trial was conducted by 18 pregnant Barki sheep (weighting an average 40.86 kg) at the last month of gestation and extend for the end of lactation period (approximately 100 days). Ewes were allocated to three feeding regimes in a complete randomize (6 animals each). The three ewes groups fed the same rations which were used in the metabolic trial except T3. The treatments included: R1 (control ration) consisted of clover hay + concentrate feed mixture (CFM); R2 is control ration + garlic powder (as 2% of DMI) and R3 is control ration + 2 ml/head/day of garlic oil. All experimental ewes were fed concentrate mixture design to live body weight and alfalfa hay according to physiological status [5]. Voluntary feed intake was measured at 30, 60, 90 days by weighing the offered and the refusal feeds from the previous day. Fresh water was available free choice.

2.3.1 Sampling and analysis of colostrum and milk

After parturition, colostrum samples were taken from all ewes in each group and kept for analysis. Colostrum samples were taken and kept in plastic bottles under -20ºC for chemical analysis. Chemical compositions of colostrum, in terms of fat, protein, lactose and total solids were determined by using milk scan (MilkoScan, Bentley, Belguim). Milk production was recorded biweekly starting from the second week of lambing till the 12^{th} week of lactation using the hand-milking procedure after separation of lambs from their dams. Fatty acids in milk were extracted and methylated according to method 996.06 of AOAC [7] using HPLC system.

2.3.2 Sampling and analysis of blood

During mid-lactation period blood samples were taken from all animals group. Blood samples were withdrawn. Then, centrifuged and separated blood serum was stored into a clean dried glass vial at -20ºC for analysis. Biochemical analyses (total proteins, albumin, and globulin) were measured in serum using kits provided by Diamond Company. Immunoglobulin (IgG) was measured by ELISA kits after serum dilution and according to Abbott Laboratories instruction (Abbotta Park, IL 60064) USA.

2.4 Statistical Analysis

Data collected was subjected to Analysis of Variance (ANOVA) using statistical analysis software (SAS) at 5% (*P*=.05) level of significance [8].

3. RESULTS AND DISCUSSION

3.1 Metabolism Trial

3.1.1 Feed intake and apparent digestibility

Data of dry matter intake (Table 2) showed that there were no differences among groups because nutrient requirements for maintenance were given (restricted feeding) according to body weights [5]. Organic matter (OM), crud fiber (CF) and crude protein (CP) intakes followed the same trend as DM intake. These results were in agreement with those obtained by Rasoul et al. [9] they indicated there were no significant differences of adding different dosages from garlic powder to experimental rations in dry matter intake on pre-partum dairy goats.

According to nutrient digestibility, it is noticed that Addition of 2% of garlic powder in T2 improved apparent digestibility of DM, OM, CF and CP compared to control ration with significant differences. This may be due to that garlic powder could modify the microbial population profile, reducing of *Prevotella spp* activity, which

is mainly responsible for protein degradation and amino acids deamination resulting improvement in protein digestion and metabolism [2]. These findings are in agreement with those obtained by Rongzhen, et al. [10] when they stated that the apparent digestibility of DM and CP increased by garlic powder supplementation. Despite of the addition 3% garlic powder in T3 has similar positive effect of T2 for DM and OM digestibility, T3 has negative effect on CP and CF digestibility compared to control ration. This result reflected that T2 is more adequate to be used for feeding animals under the present experimental condition.

Otherwise, data in Table 2 showed suppressing effects of garlic oil on crude fiber digestibility. It appeared that gram positive bacteria are more sensitive to be inhibited by plant essential oils compounds which include most of the cellulolytic bacteria capable of hydrolysing fiber (e.g. *Ruminococcus albus, Ruminococcus flavefaciens and Butyrivibrio fibrisolvens*) as reported by Panghal et al. [11]. As result of the large number of chemical compounds present in garlic oil, the antibacterial activity cannot be easily attributed to a specific mechanism. Several mechanisms have been suggested to explain the antibacterial action, including degradation of the cell wall, damage to the cytoplasmatic membrane, leakage of cell contents and coagulation of cytoplasm [12,13]. On an opposite trend, Busquet et al. Yang, et al. [3,14] found that the supplementation of garlic oil did not affect all nutrients digestibility.

Generally, the effects of feeding garlic or garlic extracts on nutrient digestibility were not consistent in the literature. For example, Chaves, et al. [15] reported that dietary garlic oil supplementation had no effects on DMI and ADG of lambs, whereas Hasan, et al. [16] found that the ADG of grazing goats increased 10.3% when goats were fed with a water solution of garlic.

It could be illustrate from Table 2 that ration which supplemented by 2% garlic powder has positive results to all nutrient digestibility compared to T1 and T3. Adding 2 ml garlic oil to the ration (T4) has the same positive effect on all nutrient digestibilities except on crude fiber digestibility and their effect on female performance need to be studied. Therefore author choose ration T2 and T3 to be continued for the feeding trial.

3.2 Feeding Trial

3.2.1 Live body weight changes of ewes

Results of ewes live body weight changes revealed that there were no significant differences among the experimental groups (Table 3). At the beginning of the experiment (late pregnancy period), body weight of ewes within each group was nearly similar. Just before kidding, all experimental ewes gained in their body weights resulting of increasing level of feeding and parturition effect. Just after kidding, all ewes' loss their weights, ewes fed control ration (R1) recorded the highest weight loss compared with their mates which fed R2 and R3 (expressed as % of weights before kidding). In this respect, the weight loss of ewes is correlated with the summation of the weight of offspring, placenta and fetal fluids.

During lactation phase, It was noticeable that treated with garlic forms recorded the less lost weights compared to their mates in control group. When animals are in negative energy balance (lactation period), the additional energy available due to the essential oil from medicinal supplementation is used to improve performance and reduce body reserve losses [17].

Generally, the data of ewes weights during different physiological stages indicated that no significant effects on live weight. This might be due to the feeds were proposed to cover the requirements of milk production for each all animal. The same results were obtained by Shams [18] when they indicated there were no significant effects of adding different dosages from ginger powder to experimental rations on live weight without adverse effect on dairy cows during different periods.

3.2.2 Blood biochemical parameters

Data in Table 4 revealed that total proteins, albumin and Immunoglobulin (IgG) differed ($P = .05$) as affected by garlic additives. Serum albumin significant increased either for R2 or R3 compared to control ration (R1). These results may be owing to the improvements of ruminal microbial protein synthesis which increased blood albumin [4]. Total proteins ($P = .05$) increased by adding garlic powder (R2) or their oil (R3) compared to control ration which may be due to that the additives enhanced the secretion of saliva, the efficiency of digestion enzymes, the

digestion and metabolism and slow the time of feed passage which increased the absorption of protein in small intestine [19,20].

Concerning Immunoglobulin (IgG) values, the results demonstrated that garlic powder (R2) and garlic oil (R3) had higher (*P* = .05) IgG values than control group R1, this increment in IgG might be attributed to the presence of active components in garlic which brought about boom cellular antioxidant defenses activity, act as anti-lammation and induced improved immune response of the body and subsequently globulin was increased [21,22].

Table 2. Feed intake and apparent digestibility of rams fed experimental rations

Items	T1	T2	T3	T4	±SE
Number of animal	4	4	4	4	
Live body weight, Kg	35.5	34.0	35.2	34.6	0.902
Intakes, g					
DM concentrate	420.9	404.8	408.5	410.9	8.746
Roughage	320.8	310.0	297.9	312.5	7.45
Total	741.7	714.8	706.4	723.4	15.86
OM	648.8	625.4	618.0	632.8	13.86
CF	161.5	156.4	153.6	158.4	3.499
CP	104.3	100.5	99.7	101.8	7.701
Digestibility,%					
DM	55.9^{b}	59.6^{a}	59.4^{a}	59.1^{a}	0.546
OM	53.7^{b}	56.8^{a}	55.8^{a}	55.3^{ab}	0.414
CF	52.5^{b}	60.9^{a}	55.8^{b}	47.7^{c}	1.556
CP	65.1^{b}	70.6^{a}	69.2^{ab}	66.6^{ab}	0.908

^{a,b,c} means at the same row with different superscript are significantly (P = .05) different.. T1: control ration, T2: control ration plus 2% of garlic powder, T3: control ration plus 3% of garlic powder, T4: control ration plus 2ml/h/d of garlic oil

Table 3. Dry matter intake and body weight changes of ewes fed the experimental rations during last gestation and lactation period

Items	R1	R2	R3	±SE
Number of animal	6	6	6	
During late pregnancy period				
Initial body weight	40.3	41.2	41.2	1.48
Average DMIg	1568	1567	1498	0.86
Just before kidding	47.4	46.8	48.3	1.02
Just after kidding	40.8	39.9	41.9	1.09
Body weight loss as %*of weight before kidding	13.8	12.1	13.3	0.20
During lactation period				
Average DMIg	1310	1398	1530	26.3
Average Weight	34.9	36.8	38.5	1.47

** % = Body weight loss after kidding / body weight before kidding; R1: control ration, R2: control ration plus 2% of garlic powder, R3: control ration plus 2 ml/h/d of garlic oil*

Table 4. Serum protein and immune status of ewes fed the experimental rations

Items	R1	R2	R3	±SE
IgG (IU/L)	155.6^{b}	163.6^{b}	203.4^{a}	7.25
Protein%	12.5^{b}	15.4^{a}	14.3^{ab}	1.204
Fat%	10.4	10.6	9.9	1.082
Lactose%	3.5	2.5	2.0	0.216
TS%	29.4	31.9	33.0	0.677

*a,b means at the same row with different superscript are significantly (P = .05) different , R1: control ration, R2: control ration plus 2% of garlic powder, R3: control ration plus 2 ml/h/d of garlic oil, *IgG: Immunoglobulin*

3.2.3 Productive traits of ewes

3.2.3.1 Colostrum composition

Table 5 shown that addition of garlic to ewes' rations reflected a significant differences (P =.05) in protein and IgG of colostrum. These results might be due to the improvements in protein digestion and metabolism (Table 2) and blood protein synthesis (Table 4). Nevertheless, the obtained percentages of fat, protein and lactose were within the range reported by Banchero et al. [23] with values being (8.2 to 10.6) for fat (%), (7.7 to 15.7) for protein (%) and (1.7 to 3.6) for lactose (%).

3.2.3.2 Milk yield and fatty acids profile

The obtained data (Table 6) showed that using garlic additives either powder or oil for feeding lactating ewes had non-significant effects on average milk yield and saturated fatty acids in milk. Benchaar et al. [23] found no variations in milk yield and milk fatty acids when supplementing the ration of dairy cows with a mixture of essential oils compounds.

Also, data showed that feeding ewe's tested ration which contained garlic powder (R2) and garlic oil (R3) led to decrease milk fatty acids; C10:0, C12:0, C14:0 and C16:0 compared with control ration (R1). This reduction might be because of the potential inhibitory effect of the dietary polyunsaturated fatty acids (PUFA) or its metabolites on the nova fatty acids synthesis in the mammary gland or a dilution effect [24,25].

Feeding ewes on R2 or R3 ration were insignificantly increased the content of milk from C18 fatty acids. These results agree with findings of Banchero et al. [23]. The increase of C18:1 for ewes milk fed experimental ration may be resulted from partial bio-hydrogenation of C18:2 and C18:3 fatty acids and the desaturation of C18:0 in the mammary gland [26]. Also, adding garlic powder (R2) and garlic oil (R3) in ewes ration induced a higher content of C20:0 than control group. These results were in agreement with Kholif et al. [4] who recorded the increasing of C20:0 fatty acid, unsaturated fatty acids and conjugated linoleic acid (CLA) in lactating goats fed ration supplemented with garlic, cinnamon or ginger oils.

Table 5. Chemical composition of colostrum for ewes fed tested rations during lactation period

Items	R1	R2	R3	SE
Total Protein (g/dl)	6.91^{b}	7.26^{ab}	7.61^{a}	0.246
Albumin(g/dl)	4.01^{b}	4.35^{a}	4.91^{a}	0.042
Globulin(g/dl)	2.90	2.81	2.70	0.021
A/G ratio	1.38	1.55	1.82	0.44
*IgG(IU/L)	1.23^{c}	3.08^{b}	4.74^{a}	1.38

*a,b,c means at the same row with different superscript are significantly (P = .05) different , R1: control ration, R2: control ration plus 2% of garlic powder, R3: control ration plus 2 ml/h/d of garlic oil, *IgG: Immunoglobulin*

Table 6. Milk production and fatty acid profile for ewes fed tested rations during lactation period

Items	R1	R2	R3	SE
Average milk yield, gm	223.1	301.2	157.5	6.25
Saturated fatty acids				
C8:0 (Caprlyic acid)	0.61	0.56	0.67	0.0567
C10:0 (Capric acid)	4.49	4.1	3.6	0.345
C12:0 (Lauric acid)	3.25	2.3	2.1	0.249
C14:0 (Myristi acid)	8.34	7.4	6.9	0.495
C16:0 (Palmtic acid)	26.8	22.9	23.61	1.226
C18:0 (Stearic acid)	15.6^{ab}	20.5^{a}	12.56^{b}	1.365
Unsaturated fatty acids				
C18:1ω9 (Oleic acid)	26.7^{b}	29.18^{a}	29.56^{a}	2.744
C18:2ω6 (Linoleic acid)	4.05^{b}	5.79^{ab}	7.40^{a}	0.607
C18:3ω3 (Linolenic acid)	0.33^{b}	0.45^{b}	0.89^{a}	0.519
C20:0 (Arechidic acid)	0.34^{b}	0.48^{a}	0.49^{a}	0.144

a,b means at the same row with different superscript are significantly (P = .05) different , R1: control ration, R2: control ration plus 2% of garlic powder, R3: control ration plus 2 ml/h/d of garlic oil

In the present result, C18:1ω9, C18:2ω6 and C18:2ω3 were insignificantly increased compared with control group. Similar trend was observed by Boutoial, et al. [27] when using rosemary leaves to lactating goats and was reported by Kholif, et al. [4] when using essential oils (Garlic, Cinnamon or Ginger Oils) in lactating goats rations.

In general, supplementation of garlic powder or oil changed the fatty acids profile of the milk fat that the proportions of CLA and omega 3 fatty acids were increased, proportions of unsaturated fatty acids were increased and saturated fatty acids were decreased.

4. CONCLUSION

It could be conclude that supplementation of garlic powder or oil improved dry matter, organic matter and crude protein digestibility, increased immune globulin in ewes blood and colostrum, changed the fatty acids profile of the milk fat that the proportions of CLA and omega 3 fatty acids were increased, proportions of unsaturated fatty acids were increased and saturated fatty acids were decreased which consider a good indicator for healthy milk for consumers.

ETHICAL APPROVAL

The experimental procedures were approved by the Animal and Poultry Production Division of DRC committee and as followed by the Veterinary and Animal Care Department.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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