EVALUATION OF MEDICINAL PLANTS (*ASTRAGALUS ERIOCEPHALUS* AND *QUERCUS INFECTORIA*) AS FEED ADDITIVES IN AWASSI EWES' RATION

2. SOME BLOOD BIOCHEMICAL, HORMOAL LEVELS AND MILK HYIGEN.

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ABSTRACT

The aim of this study was to investigate the effect of addition two medical plants or their mixture on hematological, biochemical parameters, hormonal levels and milk microbiology of lactating Awassi ewes. Twenty-eight ewes at mid lactation were divided into four groups and fed rations containing 1% Gall oak, 2% Astragalus, 0.5 Gall oak + 1% Astragalus, and Control. At biweekly intervals, blood samples were withdrawn. A bulk milk samples was collected for microbiological test under sterilized conditions. Results revealed that medicinal plants significantly influenced Erythrocyte Sedimentation Rate being lowest in the Astragalus group. Ewes feed Mixture group had significantly higher blood glucose concentration. Cholesterol level was significantly lower in the blood of ewes fed Gall oak diet and was highest in ewes fed Astragalus diet. Animals fed diet supplemented with Gall oak had a significantly higher concentration of T3 hormone. Total viable bacterial and mold count was decreased in the milk of Astragalus and Gall oak group, respectively. It can be concluded that *Astragalus eriocephalus* and *Quercus infectoria* improved immunity, metabolism and milk hygiene of lactating Awassi ewes.

Keywords: herbs, hematology, blood biochemical parameters, hormones, milk hygiene, ewes

مجلة العلوم الزراعية العراقية -2019 :50(2):526-533 برواري وأخرون

تقيم النباتات الطبية (*Quercus infectoria* and *Astragalus eriocephalus*) كاضافة علفية في عليقة النعاج العواسية 2 –بعض صفات الكيمياوية للدم ومستوى الهورمات وصحة الحليب

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المستخلص

هدف الدراسة,استعمال النباتات (العفص والكثيراء) بشكل فردي اوخليط منهما كاضافة في عليقة نعاج العواسية الحلوبة للكشف عن تاثيرها في الصفات الدموية ومستوى الهورمونات (T3, T4, بروﻻكتين, كورتيزول) واحتواء الحليب على الاحياء المجهرية. تم استعمال ثمانية وعشرون نعجة حلوبة في منتصف مرحلة ادرار الحليب وقسمت الى اربع مجاميع حيث غذيت نعاج المجاميع الاربعة على علائق حاوية على 1% العفص, 2% الكثيراء, 0.5 العفص+1% الكثيراء ومجموعة المقارنة. تم سحب الدم كل اسبوعين مرة. وجمع عينات الحليب من كل مجموعة لدراسة الاحياء المجهرية في الحليب. كانت للنباتات المضافة تاثيراً معنيويا في معدل ترسيب كريات الدم الحمراء اذ كانت ادناها في المجموعة المغذاة على الكثيراء. كان مستوى الكلوكوز اعلى معنوياً في المجموعة المغذاة على خليط النباتين. كان مستوى الكوليسترول ادنى معنويا في دم النعاج المغذاة على عليقة العفص واعلاه في دم النعاج المغذاة على نبات الكثيراء. كما كان مستوى هورمون T3 اعلى معنوياً في مجموعة النعاج المغذاة على عليقة العفص. انخفض العدد الكلي للبكتيريا والفطريات في حليب النعاج المعاملة. يمكن الاستنتاج بان اضافة النبات العفص والكثيراء يعمل على تحسن المناعة و الايض وصحة الحليب في النعاج العواسية الحلوبة.

الكلمات المفتاحية: اعشاب, الدم, احياء المجهرية,هورمونات, الاغنام

***Received:5/7/2018, Accepted:15/11/2018**

INTRODUCTION

Various attempts have been manipulated to maximize the productivity of the animals in different species, including antibiotics, hormones, chemical growth promoters, enzymes, and minerals. Most of them were not safe for human consumption because of their residual in the product and many others were either expensive and/or may affect physiological state, immunity, and health of animals. Therefore, in recent years, using herbal additives from natural origin in human and animal feeding has been encouraged (21). Moreover, Wanapat et al. (28) observed that secondary metabolites in plant extracts are considered the best candidate to achieve these objectives of productivity. In addition, herbs and their secondary metabolites have ethnoveterinary medicinal properties like immunomodulators, growth promoters, antioxidants and antimicrobials. This could be the route to overpass the development of pathogens resistance to drugs or antibiotics (11). Also, they stimulate the intermediary nutrient metabolism, endocrine system and nutrient requirement of the animals (30). Therefore, feed medicinal plants additives may replace the traditional growth promoters due to their beneficial effects on animal performance (5), through enhancing their digestibility, production, reproduction, and health (9). The important index of an animal physiological state is hematological blood profile. Mirzaei et al. (21) indicated that as a response of using polyherbal mixture the metabolic processes of animals improved, and liver activity or animal's health was affected. Also, Galbat et al. (9) clarified that using polyherbal ration had a positive effect on general health, as indicated from blood parameters of lactating goats. Moreover, many studies cited by Galbat et al. (9) showed that herbal or medicinal plants have either negative or positive action on blood biochemical parameters of different ruminants. Also, as a part of their functions, they can be used as antimicrobial and this is because of the secondary metabolites, e.g. saponins, terpenoids, phenylpropanoids, tannins, and essential oils (20). Moreover, they enhance the bile acid synthesis in the liver and consequently lead to bile excretion which causes the lipids absorption. Galbat et al. (9) indicated that, seeds from herbal plants enhancing the effect of hormonal alert by increasing prolactin and therefore release the somatotropin hormones which consequently increase the level of glucose through udder tissues activation and this leads to improvement the lactating animal productivity. Safety in dairy animals, mammary health and milk hygiene can be monitored by using variables such as total bacterial count from bulk tank milk (10). Therefore, Ibrhim (13) observed that healthy property of milk from dairy animals fed essential oils was improved and suggesting that its consumption benefits human health. In addition, immediately after milking the types and number of micro-organisms in bulk tank milk are influenced by many factors including, feed, animal, equipment cleanliness, season and animal health (27). Therefore, this study was aimed to determine the effects of using *Astragalus eriocephalus* and *Quercus infectoria* as single and as a mixture use in Awassi ewe's rations on hematological, blood serum biochemical parameters, hormonal levels, and milk microbiology.

MATERIALS AND METHODS

Twenty-eight Awassi ewes (48.87±1.17 kg) body weight were divided into four groups, at mid-lactation within lambing date ranging from 20, November to 10, December. Each group was fed on the following rations: Control group was fed basal diet without any supplement, Astragalus group was fed basal diet supplemented with 2% (20 g/kg) of *Astragalus eriocephalus* , Gall oak group was fed diet supplemented with 1% (10 g/kg) of *Quercus infectoria* and Mixture group was fed diet supplemented with 1% (10 g/kg) *Astragalus eriocephalus* and 0.5% (5 g/kg) *Quercus infectoria*. Full details of herbal preparation, GC-MS analysis, apparent digestibility, management, and feeding are described in our previous paper part 1 (19).

Blood sampling and parameters:

Hematology

Samples of blood were withdrawn from the jugular vein from each animal at biweekly intervals. The collected blood was immediately preserved in 10 mL Eppendorf tubes containing 100 µL of 0.5 M ethylene-diamine tetraacetic acid (EDTA). Preserved

blood samples were brought to Laboratory at Department of Animal production, College of Agriculture, Duhok University. Hemogram inspections; Pack Cell Volume (PCV), Hemoglobin (Hb), Erythrocyte Sedimentation Rate (ESR), and WBC deferential count were determined directly from fresh blood by conventional assays. Microhematocrit centrifuge was used to measure PCV according to Kerr (15) and from results of PCV; Hb was measured by related formula. Westergrentubes were used to determine ESR by standing the tubes for 24 hours vertically (18). Giemsa dye was used to stain blood films and counting neutrophils and lymphocytes as described by Coles (3).

Blood biochemical

During the trial period, at biweekly intervals other blood samples were collected from the jugular vein of each ewe and stored in vacuum glass tubes containing no anticoagulant. Then, the samples were left for 20 minutes at room temperature. Next, the samples were centrifuged for 10 minutes at 3,000 rpm and were kept at -25°C, for measuring blood biochemical and hormones level. By using commercial kits (BIOLAPO SA, France), the biochemical parameters (Glucose, Total protein, Albumin, Total globulin, Triglyceride, and Cholesterol) were measured by the UV Spectrophotometer.

Hormones measurement

Cortisol, Prolactin, triiodothyronine (T3) and thyroxine (T4) hormones were measured by ELISA using Monobind Inc Kits. 25ml of standards and serum samples were added to assigned wells. 25ml of working reagent (50ml for cortisol) was added. Microplates were swirled gently for 20-30 seconds to mix and they were covered (for cortisol, 50ml of cortisol Bioten Reagent was added and swirled again before incubation). After incubation for 60 minutes, the contents of microplates were discarded and dried with absorbent paper. Microplates were washed with 350ml (300ml for cortisol) of wash buffer three times manually using squeeze bottle. 100ml of working substrate was added, avoiding shaking. Then, the microplates were incubated for 15 minutes. Next, 50 ml of stop solution was added to all wells with the gentle mix for 15-20 seconds. The absorbance was taken by (ELISA Tool) within 30 minutes at 450 nm. Their concentrations were counted by GraphPad Prism 7 Software.

Microbiology sampling

At the final sampling, 50 ml of milk was taken as bulk tank sample from each group for the microbiological test (Bacteria and Mould count) under sterilized conditions. Total Viable Count (TVC) and mold count were recorded according to Yousef and Carlstrom (31). For TVC, nutrient agar plates were used by spread platting under sterilized conditions. The plates were incubated for 24-48 hours at 35-37^{o}C. The TVC was recorded by using colony counter. For mold inspection, potato dextrose agar plates were used by spread platting under sterilized conditions. The plates were incubated at 20-25 ºC for 3-5 days. Mold count inspected by using colony counter.

Statistical analysis

Data were analyzed as a completely randomized design by ANOVA using the General Linear Model (GLM) procedures of SAS (25). The model included the fixed effect of treatment. Treatment differences were calculated using Duncan's option of the same software.

RESULTS AND DISCUSSION

Hematological parameters

The effect of medicinal plants on hematological parameters is presented in Table 2. ESR, Neutrophile, lymphocyte and L/N ratio was significantly influenced by supplementation of medicinal plants, whereas no significant effect of treatments on PCV and Hb was noticed. The blood from ewes fed Control diet had the higher value (8.36 %) of ESR and the lower value (6.0 %) was recorded in ewes from Gall oak group followed by Mixture group (6.8 %) and Astragalus group (7.04 %) and the differences between them were significant. A significant rise (P<0.05) was observed in neutrophils of Astragalus group compared to Mixture group, while the lymphocytes and L/N ratio increased significantly in Mixture group as compared to Astragalus group (Table 2). The values of PCV, Hb, ESR and L/N ratio obtained in the present work were within the range of lactating Iraqi Awassi ewes observed by Badawi and AL-Hadithy (1) in sheep and Weiss and Wardrop (29) in lactating ewes.

This means that the supplementation of the experimental plants had no negative influence on hematological profile of ewes during lactation, with tendency to improve the immunity of the lactating ewes of Gall oak group as points, the declining ESR values in the blood. Moreover, Bombik et al. (2) reported that the level of immune cells increased in calf ration supplemented with herbal as a source of natural immunostimulants. Animal physiology may be affected by unconventional sources of feeds, and this was proved through the impact of different feeds on the livestock hematological profile (6). Decreasing (P<0.05) the ESR value in the Gall oak group as compared to Control group may be due to the presence of active compounds, particularly, flavonoids and terpenoids components (19). Furthermore, these molecules are considered the dominant active compounds with anti-inflammatory action (5). The increase in lymphocytes and L/N ratio and the decrease in neutrophils in the Gall oak and Mixture groups may be attributed to the presence of active compound (Carvacrol) in the *Quercus infectoria* (19). Havsteen (12) claimed that an increase in the number of lymphocytes stimulate the immune system through the effect of flavonoids compounds in the propolis. It was noticed that the addition of medicinal plants chamomile and Nigella sativa seeds to lactating ewes' rations significantly increased total WBC's and lymphocytes counts and none significantly affecting Hb (5). A similar trend of lymphocytes and Hb results were also reported in the present study. Similarly, Galbat et al. (9) observed that adding mixture of medicinal plant to the diet of dairy goats have no effect on Hb and PCV, in contrast to the present finding (Table 2) he indicated that neutrophil and lymphocyte were similar. To our knowledge there are no previous reports on the mechanism of how the active compounds of studied plants act to influence the hematological profile of lactating ewes.

Table 2. Effect of medicinal plants on some hematological parameters of lactating Awassi ewes

Hematological Parameters	Treatment[1]				SEM[2]	P-Value
	Control	Astragalus group	Gall oak group	Mixture group		
PCV %	34.53 [a]	34.59 [a]	34.71 [a]	32.96 [a]	0.41	0.38
Hb g/dl	11.51[a]	11.61[a]	11.57[a]	10.99[a]	0.14	0.34
ESR mm/24hr	8.36 [a]	7.04 [ab]	6.0 [b]	6.80 [ab]	0.35	<0.05
Neutrophile %	40.56 [ab]	44.96 [a]	39.89 [ab]	38.28 [b]	0.93	<0.05
Lymphocyte %	59.44 [ab]	55.04 [b]	60.11 [ab]	61.71 [a]	0.93	<0.05
L/N ratio	1.65 [ab]	1.31 [b]	1.64 [ab]	1.79 [a]	0.07	<0.05

[a,b]**Means in the same row with different superscripts differ significantly for treatment effect**

[1]**Ewes were fed a basal diet (Control) or basal diet supplemented with either 2% *Astragalus eriocephalus* (Astragalus group), 1% *Quercus infectoria* (Gall oak group), or 1% *Astragalus eriocephalus* and 0.5% *Quercus infectoria* (Mixture group).**

[2]**Standard error of least squares means**

Blood biochemical's

With the exception of glucose and cholesterol, all other blood biochemical traits (total protein, albumin, total globulin and triglyceride) were similar among all groups (Table 3). Blood of ewes from Mixture group had significantly higher glucose concentration (53.04 mg/dL) than Astragalus group (47.11 mg/dL), Gall oak group (44.22 mg/dL) and Control group (43.22 mg/dL). Therefore, glucose as an energy indicator in the serum was improved in the treated groups, and this may be due to the nutrient digestibility improvement and/or, higher utilization of dry matter in the treated groups. As a result of this, sufficient energy is provided to enhance milk yield (19). Moreover, Increasing T3 hormone, in the hyperthyroid state, stimulates gluconeogenesis and regulating glycogenolysis which lead to increase glucose level (22). Also, results of cholesterol showed that Astralagus group (73.72 mg/dL) was highest while control and mixture group (68.68 and 64.40 mg/dL, respectively) were intermediate and gall oak group (59.69 mg/dL) was the least (Table 3). This may be due to the cholesterol oxidation in the liver to bile acids (22) and the excretion of cholesterol into the intestine by stimulating effect of unsaturated fatty acid (16), or may be due to an increase in

the T3 hormone which regulates the cholesterol metabolism through regulating LDL-R gene transcription (22). These results illustrated the healthy effect of Oak acorn supplementation to lactating ewe's rations to reduce cholesterol levels. Blood cholesterol concentrations found in the current work were similar to those of EL-Ghousein (5) who noticed that addition of Chamomile flowers and Nigella sativa seeds to lactating Awassi ewe's ration significantly affected blood cholesterol level. Similarly, Ibrhim (13) recorded that Zinger and Garlic supplementation to the diets decreased cholesterol level (P<0.05). Furthermore, the addition of medicinal plants (Cinnamon, zinger or garlic) to goat's ration decreased cholesterol levels (9). Total protein, albumin, total globulin, and triglyceride concentrations were numerically higher in the blood of ewes fed rations with medicinal plant supplementation than Control group. Moreover, blood relevant metabolites concentrations were similar with the finding of EL-Ghousein (5) who noticed that blood albumin and triglyceride were not significantly affected by the addition of Chamomile flowers and Nigella sativa seeds in lactating Awassi ewes. Also, Galbat et al. (9) reported that serum albumin and globulin showed no significant differences between Control and supplemented groups with poly-herbal medicinal plant mixture. Similarly, supplementation of Zinger and Garlic to the diets had no effect on total protein (13).

Table 3. Effect of Medicinal plants on blood biochemical's of lactating Awassi ewes

Traits	Treatment [1]				ESM [2]	P-Value
	Control	Astragalus group	Gall oak group	Mixture group		
Glucose mg/dL	43.22 [b]	47.11 [b]	44.22 [b]	53.04 [a]	1.42	<0.05
Total Protein g/dL	3.76 [a]	3.97 [a]	3.77 [a]	3.99 [a]	0.10	0.73
Albumin g/dL	2.83 [a]	2.86 [a]	2.90 [a]	2.87 [a]	0.11	0.99
Total globulin g/dL	1.10 [a]	1.36 [a]	1.25 [a]	1.24 [a]	0.07	0.62
Triglyceride mg/dL	21.17 [a]	24.03 [a]	24.30 [a]	22.94 [a]	1.85	0.93
Cholesterol mg/dL	68.68 [ab]	73.72 [a]	59.69 [b]	64.40 [ab]	1.84	<0.05

[a,b]**Means in the same row with different superscripts differ significantly for treatment effect**

[1]**Ewes were fed a basal diet (Control) or basal diet supplemented with either 2% *Astragalus eriocephalus* (Astragalus group), 1% *Quercus infectoria* (Gall oak group), or 1% *Astragalus eriocephalus* and 0.5% *Quercus infectoria* (Mixture group).**

[2]**Standard error of least squares means**

Hormonal levels

Ewes fed diet supplemented with *Quercus infectoria* had significantly (P<0.05) higher concentration of T3 hormone (1.56 ng/ml) than other groups Astragalus, Mixture, and Control (1.51 ng/ml, 1.37 ng/ml and 1.35 ng/ml, respectively) (Table 4). Similarly thyroxin and prolactin hormones level were higher (P >0.05) in the blood of ewes from Gall oak group and followed by Mixture, Control, and Astragalus groups. Cortisol hormone concentration was similar among all groups. To the authors' knowledge, there is no information available on the effect of medicinal plants on hormones in lactating ewes. However, Colodel et al. (4) reported that the concentration of serum T3 and T4 hormones in different physiological states of normal sheep including lactation is in line with our observations. Moreover, the serum cortisol levels obtained in the present study agree with the reference range (2.24 ± 0.36 µg/dL) obtained by Kaneko et al. (14). While, the range of prolactin hormone was (5-20 ng/ml) during late lactation of ewes (17), which is higher to those reported in the present work. The lower concentration of prolactin could be due to the sampling, lambing season and lower milk yield of the ewes used in the current study. In addition, Peterson et al. (23) noticed that ewes lambed in autumn had a lower concentration of prolactin and associated to lower milk yield than those lambed in spring. In our previous part of the article, it was illustrated that milk yield was significantly higher in ewes fed supplemented diet with Gall oak compared to Control group (19). The significant increase in T3 hormone may be attributed to the high demand of precursors for milk biosynthesis, which stimulates the increased metabolism to provide more glucose under the influence of increasing T3 hormone.

Table 4. Effect of Medicinal plants on hormonal response of lactating Awassi ewes

Hormone	Treatment [1]				SEM [2]	P-Value
	Control	Astragalus group	Gall oak group	Mixture group		
Prolactin ng/ml	2.76 [a]	2.67 [a]	2.81[a]	2.73 [a]	0.04	0.56
Triiodothyronine (T3) ng/ml	1.35 [b]	1.51 [ab]	1.56 [a]	1.37 [b]	0.03	<0.01
Thyroxin (T4) µg/dL	7.20 [a]	7.03 [a]	7.55 [a]	7.51[a]	0.12	0.35
Cortisol µg/dL	2.04 [a]	2.15 [a]	1.99 [a]	1.95 [a]	0.12	0.94

[a,b] **Means in the same row with different superscripts differ significantly for treatment effect**

[1]**Ewes were fed a basal diet (Control) or basal diet supplemented with either 2% *Astragalus eriocephalus* (Astragalus group), 1% *Quercus infectoria* (Gall oak group), or 1% *Astragalus eriocephalus* and 0.5% *Quercus infectoria* (Mixture group).**

[2]**Standard error of least squares means**

Milk microbiology

The average value of TVC was 49.5* 10^3 CFU/ml, 5.5* 10^3 CFU/ml, 8.5* 10^3 CFU/ml and 15* 10^3 CFU/ml for T1, T2, T3 and T4, respectively (Table 5). It was observed that total viable bacterial count per ml of bulk tank raw milk collected from Control group was higher than the milk samples of other treatment groups and lowest was in the milk of ewes fed Astragalus group diets. According to the act of specific hygiene limits by European Parliament (EC) No. 853/2004 and by FDA (7), the CPM value of raw milk should not exceed 1.5 x 10^6 CFU/ml and 30 x 10^3 CFU/ml, respectively. Although TVC was reduced in milk of Astragalus group which is due to active compound (P-Cymene) in *Astragalus eriocephalus* plant (19), the highest TVC observed in Control group, while the highest value among treated groups was Mixture group which result from the efficacy of plant mixture as well as their volatile compounds synergistic effect (8). Additionally, this author claimed that to some extent volatile compounds, separately, inhibit bacterial growth more efficiently than their mixture (Thymol and Carvacrol). The average mold count was 10.5* 10^3 CFU/ml, 5.5* 10^3 CFU/ml, 1* 10^3 CFU/ml and 5.5* 10^3 CFU/ml for Control, Astragalus, Gall oak and Mixture groups, respectively (Table 5). Raw milk from bulk tank sample of the Control group had the higher content of molds and milk from ewes fed rations supplemented with Gall oak (Gall oak group) had the lowest values. Additionally, mold mean number in raw milk is 1x10^3 CFU/ml (26), which is higher than that observed in our results.

Table 5. Effect of Medicinal plants on bacterial and mould counts of Awassi ewe's milk

Treatment[1]	TVC (CFU/ml)[2]	Mould count (CFU/ml)
Control	49.5 x 10^3	10.5 x 10^3
Astragalus group	5.5 x 10^3	5.5 x 10^3
Gall oak group	8.5 x 10^3	1 x 10^3
Mixture group	15 x 10^3	5.5 x 10^3

[1]**Ewes were fed a basal diet (Control) or basal diet supplemented with either 2% *Astragalus eriocephalus* (Astragalus group), 1% *Quercus infectoria* (Gall oak group), or 1% *Astragalus eriocephalus* and 0.5% *Quercus infectoria* (Mixture group).** [2]**TVC: Total viable count, CFU: colony form unit**

In conclusion, lactating ewes fed diet supplemented with studied medicinal plants positively affected blood hematology (ESR, lymphocites and L/N ratio), blood biochemical (Glucose and cholesterol), hormonal levels (T3) and milk microbiology (total bacterial and mold counts). In specific, Gall oak was more effective in improving the studied parameters compared to other used rations. Also, using these plants has no deleterious effects on animal health. Further works are needed to obtain the respective level and mechanisms that elicit these positive effects.

ACKNOWLEDGMENT

The authors wish to thank Professor Dr. Jalal E. Alkass, Animal Production Department, College of Agriculture, University of Duhok for his valuable help in reading the manuscript.

REFERENCES

1. Badawi N.M., and H.A-H. AL-Hadithy, 2014. The hematological parameters in clinically healthy Iraqi Awassi sheep. World's Vet. J., 4(1): 01-05

2. Bombik T., E. Bombik, A. Frankowska, B. Trawinska, and L. Saba, 2012. Effect of herbal extractions on some hematological parameters

of calves during rearing. Bull Vet. Inst. Pulawy, 56: 655-658.
3. Coles E.H., 1986. Veterinary Clinical Pathology. 4th ed. W.B. Saunders (Editor), Philadelphia. pp: 11-41, 114-121
4. Colodel M.M., E. Martins, V.M.V. Martins, and A.P. Marques Júnior, 2010. Serum concentration of thyroid hormones in crioula lanada serrana ewes in gestation and lactation. Arch. Zootec., 59 (228): 509-517.
5. EL-Ghousein S.S., 2010. Effect of some medicinal plants as feed additives on lactating Awassi ewe performance, milk composition, lamb growth and relevant blood items. Egyptian J. of Animal Production, 47 (1): 37-49
6. Emenalom O.O., and A.B.I. Udedibie, 1998. Effect of dietary raw, cooked, toasted mucuna pruriens seeds (velvet bean) on the performance of finisher broilers. Nigerian J. of Anim. Prod., 25: 115-119
7. Food and Drug Admin U.S., Dept. of Health and Human Services. Code of Federal Regulations, Part 1, Title 21, Sections 131, 133, and 135. April 2006 Revision. http://www.gpoaccess.gov/cfr/index.html.
8. Fratini F., S. Casella, M. Leonardi, F. Pisseri, V.V. Ebani, and L. Pistelli, 2014. Antibacterial activity of essential oils, their blends and mixtures of their main constituents against some strains supporting livestock mastitis. Fitoterapia, 96:1-7, 10.1016/j.fitote.2014.04.003
9. Galbat S.A., A. El-Shemy, A.M. Madpoli, M.A.L. Omayma, E.I. Maghraby, and El-Mossalami 2014. Effects of some medicinal plants mixture on milk performance and blood components of egyptian dairy goats. Middle East J. Appl. Sci.., 4(4): 942-948, 2014.
10. Gonzalo C., J.A. Carriedo, M.C. Garcia-Jimeno, M. Pérez-Bilbao, and L.F. De La Fuente, 2010. Factors influencing variation of bulk milk antibiotic residue occurrence, somatic cell count, and total bacterial count in dairy sheep flocks. J. Dairy Sci., 93:1587–95, 10.3168/jds.2009-2838
11. Gopi M., K. Karthik, H.V. Manjunathachar, P. Tamilmahan, M. Kesavan, M. Dashprakash, B.L. Balaraju, and M.R. Purushothaman, 2013. Essential oils as a feed additive in poultry nutrition. Advance Animal Veterinary Science, 2 (1), http://dx.doi.org/10.14737/journal.aavs/2014.2.1.1.7
12. Havsteen B.H., 2002. The biochemistry and medical significance of the flavonoids. Pharmacol Ther., 96 (2-3):67-202
13. Ibrhim I.E., 2015. Effects of dietary herb supplements for ewes on milk contents and some biochemical parameter. Kufa J. Vet. Med. Sci., 6 (2), 170-176
14. Kaneko J.J., J.W. Harvey, and M.L. Bruss, 2008. Clinical Biochemistry of Domestic Animals. 6th ed., Elsevier/Academic Press, Amsterdam
15. Kerr G.M., 2002. Veterinary laboratory medicine; clinical biochemistry and hematology. 2nd ed, Blackwell Science Ltd., 285-286
16. Khodary R., M.H. El-Ezzawy, and I.R. Hamdy, 1996. Effect of Nigella sativa on egg production, hatchability percentage and some biochemical values in laying hens with references to fertility in cockerels. Proc. 7th Sci. Cong., Fac. Vet. Med., Assuit Univ., 17-19 Nov., Egypt
17. Lamming G.E., S. Moseley, and J.R. Mcneilly , 1974. Prolactin release in the sheep. J. Reprod. Fert., 40: 151-168
18. Maghsoodi R., A. Geranser, E. Jahanzad, and L. Ghojezadeh, 2005. A comparative study on the effect of sodium citrate and EDTA in erythrocyte sedimentation rate. Iranian J. Pediatrics, 15(2): 126-131
19. Merkhan K.Y., Mustafa K.N., R.H. Isa, and E.T.S. Buti, M.S.Q. Barwary, and C.A. Mohammed, (Unpublished). Evaluation of medicinal plants (Quercus infectoria and *Astragalus eriocephalus*) as feed additives in Awassi ewe's ration. 1- Digestibility, milk yield and composition
20. Mirzaei F., and K.R. Hari Venkatesh, 2012. Efficacy of phyto medicines as supplement in feeding practices on ruminant's performance: a review. Global J. Res. Med. Plants and Indigen. Med., (9): 391–403.
21. Mirzaei F., S. Prasad, and S.S. Sofla, 2012. Influence of medicinal plants mixture on productive performance cross bred dairy goats. Curr. Res. Dairy Sci., 4(1):6-16
22. Mullur R., Y. Liu, and G.A. Brent, 2014. Thyroid Hormone Regulation of Metabolism. Physiol Rev. Apr., 94(2): 355–382

23. Peterson S.W., D.D.S. Mackenzie, and S.N. Mccuthceon, 1990. Milk production and plasma prolactin levels in spring- and autumnlambing ewes. Proceedings of the New Zealand Society of Animal Production, Vol50: 483-485

24. Regulation (EC) No 853/2004 of The European Parliament and of The Council of 29 April 2004 laying down specific hygiene rules for on the hygiene of foodstuffs. Official Journal of the European Union, L 139/55

25. SAS Institute (2007) Statistical Analysis System. STAT/ User's Guide, Release 9.2, SAS Institute, Cary: NC. USA.

26. Tasci F., 2011. Microbiological and chemical properties of raw milk consumed in Burdur. J. Anim. Vet. Adv., 10 (5): 635-641, 10.3923/javaa.2011.635.641

27. Torkar K.G., and S.G. Teger, 2008. The microbiological quality of raw milk after introducing the two day's milk collecting system. Acta Agriculturae Slovenica, 92 (1): 61–74

28. Wanapat M., A. Cherdthong, P. Pakdee, and S. Wanapat, 2008. Manipulation of rumen ecology by dietary lemongrass (Cymbopogon citratus Stapf.) powder supplementation. J. Anim. Sci., 86: 3497-3503, 10.2527/jas.2008-0885

29. Weiss D.J., and K.J. Wardrop, 2010. Schalms veterinary hematology. 6th ed. Wiley-Blachwell-USA. 168-170, 593-595, 1162-1163

30. Wenk C., 2003. Herbs and botanicals as feed additives in monogastric animals. Asian-Australas J. Anim. Sci., 16: 282-289, https://doi.org/10.5713/ajas.2003.282

31. Yousef A.E., and C. Carlstrom, 2003. Food Microbiology: A Laboratory Manual. John Wiley & Sons, Inc., Hoboken, New Jersey.