

IMPACT OF LEMONGRASS (*CYMBOPOGON CITRATUS*) AND ROSELLE (*HIBISCUS SABDARIFFA*) ON BLOOD METABOLITES AND FECAL ANTICOCCIDIAL EFFECT OF SAIDI EWES

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SUMMARY

Twenty-eight pregnant Saidi ewes were assigned to 4 treatments to study the influence of dietary Lemongrass and Roselle on blood metabolites, antioxidant status and anticoccidial effect. The experiment lasted for 75 days (15 before parturition and 60 after parturition). The treatments were: (1) CON group, animals received control diet without additives, consist of concentrate mixture (CM) and wheat straw; (2) LG group, animals fed control diet plus 4g Lemongrass/ kg dry matter (DM) of CM; (3) RO group, animals fed control diet plus 4g Roselle /kg DM of CM and (4) LGRO group, animals fed control diet plus (2g Lemongrass +2g Roselle)/kg DM of CM. Blood and feces samples were taken at the end of the trial to determine some of blood metabolites and parasitic number. Dietary LGRO decreased ($P<0.05$) plasma protein and albumin while the ewes fed LG had the highest ($P<0.05$) concentration of globulin. Plasma creatinine concentration was lower ($P<0.05$) in treatment groups compared to CON group. There was a significant increase in plasma cholesterol concentration of LG and RO groups, while LGRO group had the lowest concentration of plasma total cholesterol ($P<0.05$) compared with other ones. Also, feeding with LG and LGRO had negative effect on the level of alanine aminotransferase and aspartate aminotransferase enzymes. Ewes fed RO had the highest ($P<0.05$) level of LDH enzyme compared with other groups. Dietary RO decreased ($P<0.05$) triiodothyronine (T_3) concentration, while LGRO groups tended to increase ($P<0.05$) T_4 concentration. Dietary LG and RO improved ($P<0.05$) total antioxidant capacity (TAOC) compared to CON group. Heavy infection was detected with *Coccidia* in the fecal samples of control group compared to LG and RO groups, while tended to have less quantity of oocysts output in LGRO group. It could be concluded that the addition of LG or RO in the diets may improve some blood metabolites, antioxidant status and immune response of Saidi ewes.

Keywords: Lemongrass, Roselle, blood metabolites, anticoccidial, lactating ewes

INTRODUCTION

In the last few years, there has been an increasing interest in isolating antioxidants from plant ingredients and using them in animal nutrition with the intention of replacing antibiotics. Lots of researches have been conducted to investigate the natural antioxidant activity of plant extracts. Herbs have been evaluated for their ability to alter ruminal fermentation and improve nutrient utilization in ruminants (Greathead, 2003; Wang *et al.*, 2009). Phenolic compounds are among phytochemicals in plant extracts that may render their effects via inhibiting the oxidation reaction caused by oxidative stress and relief its consequences (Saltmarsh, 2003).

Lemongrass (*Cymbopogon citratus*) is one herb of interest, and it is widely used in tropical and subtropical countries in human foods (Tajidin *et al.*, 2012). The basic structure of Lemongrass plant is "cital" (Schaneberg and Khan, 2002) and 1 to 2 % essential oils based on dry matter (Carlson *et al.*, 2001). Effects of Lemongrass on antibacterial (Valero and Salmeron, 2003), antioxidant (Mirghani *et al.*, 2012), antinociceptive (Viana *et al.*, 2000), and antihyper-NH₃-producing ruminal bacterial (Wanapat *et al.*, 2008) activities have been studied. In addition, Hosoda *et al.* (2006) investigated the effects of the supplementation of Lemongrass leaf in the diet of Holstein steers and they found that the treatment had an effect on immune activity of steer.

Roselle (*Hibiscus sabdariffa*) extract was reported to be used as an antibacterial, antifungal, diuretic, uricosuric, and mild laxative substance (Farnworth and Bunyapraphatsara, 1992). Anthocyanins and protocatechuic acid are among chemical constituents in Roselle that shown to have strong antioxidant (Kim *et al.*, 2003) and antitumor effects (Choi *et al.*, 2008 and Sharma *et al.*, 2014). In addition, the components of Roselle extract exhibit antitumor characteristics, immune modulating and antileukemic effects (Müller *et al.*, 2007). In the same context Tsuda et al. (2000) reported that Roselle calyx had significant quantities of polyphenolic acids (1.7% of dry weight), flavonoids (1.43% of dry weight) and anthocyanins (2.5% of dry weight).

On the other hand, coccidial infection is worldwide in sheep and goats, and coccidiosis can be a significant problem in young ages. The economic losses due to coccidial contamination are variable from low growth performance, reduction in productivity, death, morbidity, and the cost of prevention and treatment (Khodakaram-Tafti and Hashemnia, 2017). A number of plant-derived compounds have been effectively used as natural products to avoid the incidence of coccidiosis (Abudabos *et al.*, 2017)

The information on the influence of herbs plants, such as Lemongrass and Roselle on antioxidant status and blood metabolites are very scarcity, especially on lactating ewes. Therefore, the objective of this experiment was to elucidate the influence of dietary dried Lemongrass and Roselle powder supplementation on some blood metabolites, antioxidant status and immune response of Saidi ewes.

MATERIALS AND METHODS

Animals and treatments:

The experiment is a part of research program of Animal Production Department and it was carried out at Animal Production Research Farm, Faculty of Agriculture, Assiut University, Assiut, Egypt. Lemongrass and Roselle herbs were obtained from local suppliers in Assiut city, Egypt.

Twenty-eight lactating Saidi ewes (at the 3rd or 4th lactating seasons and had an average body weighed 45.2 ± 2 kg) were used. The experiment lasted from 15 days before parturition and 60 days after parturition. Ewes were divided into four dietary groups (7 ewes each).

The treatments were: (1) CON group, animals received control diet without additives, consist of concentrate mixture (CM) and wheat straw (60:40% on dry matter (DM) basis); (2) LG group, animals fed control diet plus 4g Lemongrass/ kg dry matter (DM) of CM; (3) RO group, animals fed control diet plus 4g Roselle /kg DM of CM and (4) LGRO group, animals fed control diet plus (2g Lemongrass +2g Roselle)/kg DM of CM. The offered feeds were assessed to cover the maintenance and production requirements for each animal (NRC, 1985). The tested feed additives were mixed with concentrate mixture. The weight and chemical composition of the ingredients are shown in Table 1. The concentrate mixture was offered for each animal individually once daily at 9.00 am, while wheat straw was offered at 12.00 hr. Ewes were weighed at the beginning of the experiment and over two weeks to adjust the feed requirements. Drinking water was freely available to the animals.

Sampling and chemical analysis

Chemical analysis of feed ingredient:

The ingredients and chemical composition of experimental diets used in this study are presented in Table (1). Feed Samples ingredient were analyzed for dry matter (DM), ash, crude protein (CP), and ether extract (EE) according to methods of AOAC (2012).while Van Soest et al.(2010) method was used to determine the neutral detergent fiber (NDF) and acid detergent fiber (ADF).

Table (1): The ingredients and chemical composition (g/kg) of control of ration.

Ingredient	%
Wheat Straw	400
Maize Grain	300
Soybean Meal	90
Sunflower Meal	72
Wheat Bran	120
Limestone	12
Salt	3
Trace mineral and vitamin premix*	3
Total	1000.00
Chemical composition (g/kg DM)	
DM	894.19
OM	822.69
CP	116.95
EE	28.46
NDF	424.23
ADF	235.71

* The premix comprised (per kg) 20,000,000 IU vitamin A, 200,000 IU vitamin D3, 10,000 mg vitamin E, 10,000 mg Fe, 2500 mg Cu, 20,000 mg Mn, 100 mg Mo, 100 mg Co, 800 mg I, 20,000 mg Zn and 100 mg Se. Dry matter (DM), organic matter (OM), crude protein (CP), EE ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF).

Blood Samples and analyses

At the end of the experiment, 10 ml of blood samples were collected from the jugular vein from each ewe, before morning feeding, in tubes containing potassium ethylene diamine tetra-acetic acid (K-EDTA). The blood samples were directly centrifuged at 3000 rpm for 15 min and the plasma was separated and stored at -20 °C until the chemical analyses of plasma total protein, albumin (globulin concentration was obtained as the difference between the total protein and albumin concentration), total cholesterol, creatinine, lactic dehydrogenase (LDH), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzymes using assay kits supplied by Diamond diagnostic, Egypt. Thyroid hormones (T3 and T4) concentrations were determined using enzyme-linked immunosorbent assay kits supplied by Biotecx, USA. Total antioxidant capacity (TAOC) was determined according to (Koracevic et al., 2001) while Hydrogen peroxide (H₂O₂) was determined according to (Aebi, 1984).

Examination of fecal samples for parasitic existence

Fecal samples were collected from all groups at the beginning before administration of treatments and at the end of the experiment to analysis parasitic existence for Coccidia and the effect of treatments on Coccidial output in fecal samples. A total of 28 fecal samples from the four groups (7 animals/ each) were collected after defecation in a plastic sack (labeled with date and animal number). The samples were examined by sedimentation and floatation techniques according to Garcia and Bruckner (2001). The different diagnostic stages detected by microscopic examination and measured by an eye-piece micrometer. The culture of coccidian oocysts was tested and identification of different coccidian species were done according to (ARSLAN and TÜZER, 1998).

Statistical Analysis

The results were statistically analyzed using the SPSS statistical package 22 (SPSS Institute, Chigaco, IL, USA). The data were evaluated using the General Linear Models (GLM) procedure for analysis of variance and subjected to one-way ANOVA accompanied by Duncan's multiple range tests to detect the differences among the treatments. The data are presented as means ± SE. Probability values less than 0.05 ($P < 0.05$) was considered significant.

RESULTS AND DISCUSSION

Blood metabolites

The effect of dietary lemongrass and Roselle on some blood plasma metabolites of Saidi ewes are presented in Table (2). Blood metabolites from the present study were within the references ranges reported by Boyd *et al.* (1984). The information on the effect of lemongrass and Roselle on blood metabolites are very scarcity, especially on lactating ewes. The results of previous research are mixed between high and low, depending on the type of animals and the type of production and quantities of herbs used. For example, no effect were observed in goats ($p>0.05$) with the inclusion of lemongrass and rosemary on serum total protein, albumin, globulin, urea-N, glutamic- pyruvic transaminase and glutamate-oxaloacetate transaminase concentration (Kholif *et al.*., 2017) While, El-Bordeny *et al.* (2005) found that serum creatinine concentration didn't differ between the different groups of buffalo calves fed Lemongrass.

As shown in Table (2), total protein and albumin concentrations decreased ($P<0.05$) in blood plasma of LGRO group compared to other ones. Also, ewes fed LG had the highest ($P<0.05$) level of globulin, while ewes fed LGRO had the lowest ($P<0.05$) level of globulin than other groups. Albumin/ globulin ratio did not differ significantly among treatments. These results agree with those reported by Khattab *et al.* (2017) who reported that plasma total protein did not affect in lactating Barki goats fed lemongrass and galangal supplemented diets. In contrast, (El-Ashry *et al.*, 2006) found that serum total protein and albumin were increased ($P<0.05$) with adding lemongrass to buffalo calves but serum globulin was decreased. Our results may indicate that LG and RO supplementation groups had no adverse effect on energy metabolism in skeletal muscle (NE El-Bordeny, MA El-Ashry, 2005). It is recognized that the variation in albumin level mirrors the change in liver function and the presence of the fatty acids may affect muscle protein synthesis and protein admission (Englesbe *et al.*, 2010).

There was a significant increase in total cholesterol concentration in LG and RO groups, while LGRO group had the lowest concentration of total cholesterol ($P<0.05$) compared with other ones. Similarly, many authors reported that cholesterol concentration was decreased in animals treated with lemongrass and Roselle (Badreldin *et al.*, 2005; Kholif *et al.*, 2017). Meanwhile, Agbafor and Akubugwo (2007) studied the effect of lemongrass on albino rats and observed that the elevated total cholesterol concentration was significantly ($P<0.05$) depressed in the animals given lemongrass. Lemongrass decreased serum total cholesterol concentration, the mechanism by which lemongrass supplementation decrease total cholesterol has not been wholly explored (Kholif *et al.*, 2017).

Plasma creatinine levels in treatment groups decreased ($P<0.05$) compared to the CON group (Table 2). El-Bordeny *et al.* (2005) found that serum creatinine concentration didn't differ among the different groups of buffalo calves fed Lemongrass. This result cleared that herbs supplementations had no adverse effect on kidney function.

Table (2) showed an increase ($P<0.05$) in the concentrations of AST, ALT and LDH enzymes in lactating ewes fed LG and RO compared with those fed control diet and this increase was still within normal range. Also, there was an increase ($P<0.05$) in the activity of ALT, AST concentrations in LGRO group compared to the other ones. Ewes fed treated diets showed an increase ($P<0.05$) in the levels of plasma LDH enzyme compared with those fed CON diet. Ewes fed RO had the highest ($P<0.05$) level of LDH enzyme compared with other groups. These results indicated that tested additives to lactating ewes may have adverse effect on liver function and this result needs further investigation. In contrast, the supplementation of lemongrass in the diets had no significant effect on the concentrations of transaminase enzymes (AST and ALT), this results reported by Khattab *et al.* (2017).

Dietary RO decreased ($P< 0.05$) the triiodothyronine (T_3) concentrations, while LGRO groups tended to increase T_4 concentration (Table 2). The effect of fed LG and RO in the diets of animals on the activity of thyroid hormones is very limited. Thyroid hormone activity is considered crucial to sustaining the productive performance in domestic animals (growth, milk or hair fiber production). Variations in blood thyroid hormone concentrations are an indirect measure of the changes in thyroid gland action and circulating thyroid hormones can be considered as indicators of the metabolic and nutritional position of the animals (Todini, 2007). The highest levels of thyroid hormones (T_3 and T_4), as shown in LG (for T_3) and LGRO (for T_4) diets (Table 2) may increase the metabolic rate and improve the nutritional status in treated animals which reflected on animal health, welfare and production.

Table (2): Blood plasma metabolites of lactating ewes fed Lemongrass and Roselle supplemented diets.

Item	Treatment *			
	CON	LG	RO	LGRO
Blood metabolites				
Total protein (g/dl)	6.04 ^a ±0.25	5.48 ^{ab} ±0.18	5.44 ^{ab} ±0.28	4.96 ^b ±0.25
Albumin (Al) (g/dl)	3.10 ^a ±0.15	2.42 ^{ab} ±0.18	2.78 ^{ab} ±0.11	2.48 ^b ±0.19
Globulin (Gl) (g/dl)	2.94 ^{ab} ±0.22	3.06 ^a ±0.18	2.64 ^{ab} ±0.15	2.46 ^b ±0.14
Al/Gl ratio	1.08 ±0.11	0.81 ±0.09	1.07 ±0.08	1.03 ±0.11
Cholesterol(mg/dl)	52.40 ^b ±2.31	66.60 ^a ±3.12	61.60 ^a ±0.81	42.80 ^c ±1.20
Creatinine (mg/dl)	1.14 ^a ±0.15	0.68 ^b ±0.02	0.68 ^b ±0.05	0.51 ^b ±0.10
Enzymes activities				
ALT(U/l)	12.2 ^c ±0.48	18.20 ^b ±1.39	15.20 ^{bc} ±1.52	35.00 ^a ±1.26
AST(U/l)	60.60 ^c ±1.99	86.80 ^b ±5.50	69.80 ^c ±3.51	128.00 ^a ±2.68
LDH(U/l)	616.84 ^c ±24.4	778.74 ^b ±35.1	913.12 ^a ±21.6	791.32 ^b ±38.3
The activity of thyroid hormones				
T3 (ng/mL)	1.97 ^a ±0.08	2.14 ^a ±0.16	1.34 ^b ±0.18	1.80 ^{ab} ±0.28
T4 (nmol/L)	103.6 ^b ±2.42	87.92 ^c ±3.37	94.60 ^{bc} ±5.58	235.00 ^a ±4.47
Antioxidant status				
TAOC(mmol/L)	0.39 ^d ±0.02	1.29 ^a ±0.07	0.97 ^b ±0.02	0.72 ^c ±0.04
H ₂ O ₂ (mmol/L)	0.20 ±0.001	0.20 ±0.01	0.20 ±0.012	0.21 ±0.006

Each value represents an average of 7 samples ± SE.

a,b, means at the same row with different superscript are significantly different (P<0.05).

Con=control diet; LG, control diet +4g Lemongrass /kg of DM ; RO, control diet+4g dried Roselle/kg of DM and LGRO, control + 2g of Lemongrass and 2g dried Roselle /kg of DM. TAOC: Total antioxidant capacity and H₂O₂: Hydrogen peroxide.

Total antioxidant capacity were significantly increased in treated ewes compared to untreated ewes (Table 2), while the concentration of H₂O₂ was not significantly affected by treatment. This result is confirmed that Lemongrass have antioxidant capacities such as free-radical scavenging metal ion chelation, and singlet oxygen quenching capacity (Velasco and Williams, 2011). In the same context, several studies both in vitro (Serrano *et al.*, 2007) and in vivo (Mossalam *et al.*, 2011) found antioxidant effect of Roselle extract. Such antioxidant activity is due to its strong scavenging effect on reactive oxygen and free radicals (Rocha De Souza *et al.*, 2007 and Sia *et al.*, 2010), protection of cell from damage by lipid peroxidation (Farombi and Fakoya, 2005), decrease of glutathione depletion, rise of the liver and reduction blood activity of superoxide dismutase and catalase (Farombi and Fakoya, 2005) even though in the liver it improved superoxide dismutase, catalase, and glutathione and declined malondialdehyde (Mossalam *et al.*, 2011). The effects were detected for both water and ethanolic extracts from Roselle flowers, as well as from the leaves or seeds (Mohd-Esa *et al.*, 2010)(Sia *et al.*, 2010)(Sia *et al.*, 2010). (Hosoda *et al.*, 2006) found that fed Lemongrass at 5% in the diet of Holstein steers affect the immunity system resulted from increasing antioxidant activity in plasma of steers. As well, Ojo *et al.* (2006) found that the extracts of Lemongrass produced significant (P<0.05) antioxidative effect by inhibiting the elevation of serum levels of malondialdehyde and catalase in rats.

Parasitic existence in fecal samples

In this study, the microscopical examination of fecal samples for parasitic protozoa detected two types of coccidia sp. were *Eimeria bakuensis* (*ovina*) and *Eimeria ahsata*.(Fig. 1). These results agrees with Arslan *et al.* (1999) who reported that *E. ahsata* in Bursa province and *E. bakuensis* in the Aegean region. All fecal samples from all groups were found to be infected with *Eimeria* species. No samples were infected with only one species, the severity of infection tend to be the same in all groups except in LGRO group, which had a slight decrease in oocyst output.



a) Oocyst of *Eimeria bakuensis* (x40)



b) Oocyst of *Eimeria ahsata* (x40)

Fig. (1): Parasitic existence in fecal samples

The morphology of *Eimeria* sp: 1-*Eimeria bakuensis* (Fig. 1a): ellipsoidal in shape, the wall with smooth outer layer thick yellowish brown in color, micropyle present, it measured in average (8-5) width \times (12 - 7.5) length. sporulation time :3 days. 2-*Eimeria ahsata* (Fig . 1b): Avoid in shape, smooth wall, yellowish brown in color, micropyle with distinct polar cap and polar body, it measured (10-5) width \times (17.5 – 10) length sporulation time: 3 days.

Actually, there is no sufficient data on the effect of Lemongrass or Roselle as an anticoccidial agent in feeding. Furthermore, clinically, normal sheep and goats often shed coccidial oocyst (Khodakaram-Tafti and Hashemnia, 2017). The dimension of coccidial output in LGRO had a positive anticoccidial effect on ewes, so we advise further research to study the ascending grade of these compounds as an anticoccidial agent.

CONCLUSION

Dietary Lemongrass or Roselle may improved some of blood metabolites, antioxidant status and had positive anticoccidial effect on Saidi ewes.

ETHICAL APPROVAL

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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تأثير إضافة حشيشة الليمون والكرديه في عليقة النعاج على خصائص مكونات الدم و كمضادات للكوكسيديا

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أجريت هذه التجربة بهدف دراسة تأثير إضافة حشيشة الليمون والكرديه كمصادر لمضادات الأوكسدة في عليقة النعاج الحلابة. تم استخدام 28 نعجة في نهاية الحمل بمتوسط وزن 45 ± 2 كجم قسمت الى اربعة مجاميع (7 نعاج في المجموعة) لمدة 75 يوم (15 يوم قبل الولادة و 60 يوم بعد الولادة) وكانت العليقة المقدمة لهذه المجموعات كآلاتى: مجموعة الكنترول: المخلوط المركز: تين القمح (60:40 على أساس المادة الجافة).

1- مجموعة حشيشة الليمون: عليقة المجموعة الكنترول+4 جرام حشيشة الليمون/ كجم من المخلوط المركز.
2- مجموعة الكركديه: عليقة المجموعة الكنترول+ 4 جرام بتلات زهرة الكركديه/ كجم من المخلوط المركز.
3- مجموعة خليط حشيشة الليمون والكرديه: عليقة المجموعة الكنترول + 2 جرام حشيشة الليمون + 2 جرام بتلات زهرة الكركديه/ كجم من المخلوط المركز. تم متابعة وزن الحيوانات كل 15 يوم لتحديد المقررات الغذائية حسب حالة الحيوان. تم تسجيل الماكول. وتم تجميع عينات الدم في نهاية التجربة لتقدير بعض مكونات الدم وأيضاً تم أخذ عينات روث لتقدير أعداد الكوكسيديا. وتم تحليل النتائج أحصائياً باستخدام برنامج (SPSS).

والنتائج تشير الى الآتى:

- هناك انخفاض في مستوى البروتين والالبومين في بلازما الدم في المجموعات المعاملة بالمقارنة بالمجموعة الكنترول.
- هناك زيادة معنوية في مستوى الجلوبيولين في بلازما الدم في مجموعة حشيشة الليمون.
- هناك انخفاض معنوى في تركيز الكرياتينين في بلازما الدم في المجموعات المعاملة بمقارنة بمجموعة الكنترول.
- هناك زيادة معنوية في مستوى الكوليسترول في بلازما الدم في مجموعتى حشيشة الليمون والكرديه بينما انخفض في المجموعة الرابعة التى تم مزج بها كل من حشيشة الليمون و بتلات زهرة الكركديه.
- هناك زيادة معنوية في نشاط انزيمات الكبد في المجموعة الرابعة التى تم بها التغذية على كل من حشيشة الليمون و بتلات زهرة الكركديه.
- هناك تحسن في مستوى مضادات الأوكسدة الكلية في المجموعات المعاملة بمقارنة بمجموعة الكنترول.
- هناك انخفاض معدل اخراج الكوكسيديا في المجموعات المعاملة بمقارنة بمجموعة الكنترول.

و كانت الخلاصة أن التغذية على كلا من حشيشة الليمون أو بتلات زهرة الكركديه بمستوى 4 جرام/ كجم مخلوط مركز ليس له اى تأثيرات سلبية على الاداء الانتاجى والفسىولوجى النعاج الحلابة بل أدى الى تحسن فى مناعة النعاج.

الكلمات المفتاحية: حشيشة الليمون، الكركديه، مضادات الأوكسدة، النعاج الحلابة