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Research Article

The effect of lemongrass, *Cymbopogon citratus*, on some physiological parameters in male Awassi lambs

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Abstract: This study was conducted to study the effect of dietary supplementation of lemongrass, *Cymbopogon citratus*, on some physiological characteristics of male Awassi lambs. Twelve male Awassi lambs with ages of 3-4 months, and an average weight of 20.13±0.58kg, were randomly divided into four groups (4 animals per group) as (T1) control, T2, T3, and T4 were Added dietary lemongrass at rates of 0.5, 1 and 1.5%/kg dry matter of concentrated feed, respectively, for 90 days. The results revealed a significant increase ($P \leq 0.05$) in the numbers of red blood cells (RBC), hemoglobin (Hb), packed cell volume (PCV) and lymphocytes in the T3 compared to the control group. A significant decrease was also recorded in the numbers of white blood cells, MCV, MCH, and neutrophils in T3 and T4, while the monocytes and basophils and MCHC did not differ significantly ($P \leq 0.05$) in treatments. There was also a significant decrease ($P \leq 0.05$) in the cholesterol, low-density lipoproteins, serum creatinine, liver enzymes AST and ALT in animals treated with lemongrass, whereas, the total protein, albumin, globulin, glucose, testosterone and serum urea were not significantly affected. The results revealed that supplementation of lemongrass at rates of 0.5, 1 and 1.5%/kg dry matter of concentrated feed for male Awassi lambs can improve some blood parameters that are related to the growth and health of lambs.

Keywords: Lemongrass, Physiological parameters, Awassi lambs.

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Introduction

Livestock contributes greatly to providing food security and raising the country's economic level due to the great importance of its products, as they are essential food commodities for human consumption. The environment in Iraq is suitable for raising sheep due to the availability of short grass pastures and an acceptable level of rainfall. But the number of sheep decreased in Iraq (the Arab Organization for Agricultural Development 2001), and their number reached 6.780 million heads. They are a major source of income for the population of the pastoral areas in Iraq (FAO 2003).

Medicinal plants are introduced as food additives to animal diets to improve their reproductive performance and productivity. They have also been used as a substitute for growth stimulants and antibiotics (Ojeu, 2003). Recently, essential oils have attracted attention to their potential as alternatives to antibiotics in livestock (Wallace, 2004) because microorganisms have adapted to those antibiotics (Shehata et al. 2004; Al Bordeny et al. 2005; Al Ashry et al. 2006). Medicinal herbs have also been used to treat various diseases in animals and humans (Tipo et al. 2006). The lemongrass *Cymbopogon citratus*, has antioxidant properties (Akin et al. 2007; Barbosa et al. 2008) and is used in contraindications because it contains volatile fatty acids. It is widely used in tropical countries, especially in Southeast Asia. Citral oil constitutes the highest percentage of

Table 1. The proportions of the feed materials used in the formation of the experimental diets, gm/kg dry matter.

Feed material	T1	T2	T3	T4
wheat bran	35	35	35	35
barley	60	60	60	60
yellow corn	4	4	4	4
salt	0.9	0.9	0.9	0.9
minerals and vitamins	0.1	0.1	0.1	0.1
Crushed lemon grass	---	0.5	1	1.5

the essential oils extracted from lemongrass and is necessary for synthesizing vitamin A.

Researchers in animal science are focused on improving performance by increasing the efficiency of feed conversion. After many years of using antibiotics in feed to improve animal performance, European Union decided to ban antibiotics because of their improper impact on animal products consumed from meat and milk. Therefore, the interest in natural alternatives such as medicinal plants has increased to modify microbial fermentation and improve productive qualities (Rojo et al. 2015; Matloup et al. 2017). Hence, this experiment was designed to study the effect of adding different levels of lemongrass on the physiological performance of Awassi lambs.

Materials and working methods

Experimental animals: The study was conducted in the animal field of the Department of Animal Production, College of Agriculture, Tikrit University, Iraq. 12 male Awassi lambs with an average weight of 20.13±0.58kg and ages of 3-4 months were used in this experiment. They were divided into four groups, with four animals per group, and were placed in cages in a semi-open barn. The average weights of the animals in the four groups were 20.65, 19.55, 20.15, and 20.20kg, randomly assigned to groups. The lambs were fed for a preliminary period of 21 days, then weighed for two consecutive days, and this was the starting weight. During the experiment, the animals were weighed weekly at eight o'clock in the morning using the electronic scale after removing the fodder for 12 hours. The animals were fed during the experiment on concentrated feed (Table 1), with two morning and evening meals at a rate of 3% of the weight of the live animal to meet their nutritional needs and achieve a weight gain of 120g/day/animal (NRC 1985). The concentrated feed was adjusted according to the weekly weight developments of the animals, and the diets were equal in the total food compounds (Table 2). Food was offered freely to the animals, as well as clean water and briquettes of mineral salts all day. The first group was fed a diet free of crushed lemongrass; the second, third, and fourth groups, crushed lemon grass, were added to their diets daily at 0.5, 1, and 1.5% per/kg dry matter of concentrated feed, respectively.

Blood collection: Blood samples were collected from animals at the end of the experiment after cutting off feed and water for 12 hours, directly from the jugular vein by a 10ml wine syringe and divided into two parts; 1ml with tetra acidic acid (E.D.T.A.) and 9 ml was placed in sterile tubes and stored in the refrigerator (4°C) and after their centrifuge for 20min at 3000 rpm, blood serum separated for further studies.

Red blood cells were counted using a special slide for counting the hemocytometer, according to Hughes-Jones et al. (2008). The percentage of PCV was calculated using capillary tubes (Hughes-Jones et al. 2008). The hemoglobin concentration was estimated by the method of Drabkin & Austin (1935) with an Apel Spectrophotometer and according to the instructions of the Indian manufacturer Agappe. Mean Corpuscular Volume (M.C.V), Mean Corpuscular Hemoglobin (M.C.) and Mean Corpuscular Hemoglobin Concentration (M.C.H.C) were calculated according to Coles (1986). The number of white blood cells was calculated using a hemocytometer according to Hean's (1995), and the differential count of white blood cells (lymphocytes and monocytes) was also calculated by light microscopy using an oil lens (100X) (Sood 1985).

The cholesterol in the blood serum was

Table 2. The chemical composition of the feed materials used in the composition of the experimental diets (gm/kg).

Chemical composition	Barley	wheat bran	yellow corn	total
dry matter%	55.71	31.647	3.568	90.925
Protein%	6.432	5.551	0.3612	12.344
ether extract %	0.852	1.4175	0.1736	2.4431
raw fiber %	3.9	3.7205	0.0804	3.8009
ash%	2.292	1.7465	0.0932	4.1317
soluble carbohydrates%	42.234	19.212	2.8592	64.305

Table 3. Effect of treatment with different levels of Lemongrass on some blood parameters of lambs Awassi.

Item	Treatments				Significant level
	First	Second	Third	Fourth	
RBC (x 10^6/µL)	3.92±0.26^b	3.99±0.34^b	5.88±0.24^a	3.73±0.34^b	**
Hb (g/100ml)	9.27±0.38^b	9.94 ±0.29^{ab}	10.27±0.07^a	10.10±0.23^{ab}	*
PCV (%)	13.31±0.75^b	14.24 ± 0.47^{ab}	16.17±1.00^a	14.70±0.67^{ab}	*
MCV (femtoliter)	34.03±1.52^{ab}	36.12±3.02^a	27.47±0.59^b	39.79±2.41^a	**
MCH (pictograms/ cell)	23.72±0.75^a	25.16±1.62^a	17.54 ±0.72^b	27.45±2.21^a	**
MCHC (%)	69.82±1.73^a	69.87±1.38^a	64.01±3.99^a	68.85±1.59^a	N.S
WBC (x10^3/µL)	10.40±0.46^a	9.83±0.63^a	9.15±0.61^a	6.55±0.14^b	**

The values represent the mean ± standard error; (*)Means significant differences (P≤0.05); (**)Means significant differences (P≤0.01).

determined by the enzymatic method using kit number 2160 supplied by the French company Biomeriux. The triglycerides and high-density lipoproteins in the blood were estimated by an enzymatic method using a ready-made kit (Biolabo Company) and low-density lipoproteins in the blood according to Friedewald et al. (1972). For AST and ALT enzymes, the chromatic method of Reitman & Frankel (1957). The serum urea and creatinine were estimated using a ready-made kit (Linear Chemicals Company) by a spectrophotometer (Young 1997). The weight of the animals was calculated weekly by a 40 kg electronic scale (DahongYing, China) after stopping the feed for 12 hours.

Statistical analysis: Data were analyzed according to a Complete Randomize Design (CRD) to study the effect of different concentrations of lemongrass on the studied traits, and the significant differences between the means were compared with Duncan (1955) test. The ready-made statistical program SAS (2003) was used in the statistical analysis.

Results and discussion

Physical properties of blood: The results indicated significant (P≤0.05) differences between the Awassi lambs of the four groups in the number of red blood cells and hemoglobin concentration after 90 days of treatment with lemongrass (Table 3). The third treatment was significantly higher (P≤0.05) red blood cells. The reason for the high hemoglobin in the blood, the percentage of compacted blood cells, and the number of red blood cells in the animals of the second treatment is the effect of lemongrass on the performance of the treated animals in general by increasing the capacity for absorption and utilization of lemongrass (Ademuyiwa & Grace 2015). Anti-oxidant and anti-inflammatory (Al-Jubouri 2008) characteristics of herbs improved the health status by improving hematological variables.

The results showed a significant effect (P≤0.05) between the groups treated with lemongrass and the control group after 90 days in MCV and MCH, as the third treatment significantly decreased. The MCHC

Table 4. Effect of treatment with different levels of Lemongrass on a Differential count of white blood cells in lambs Awassi.

Item	Treatments				Significant level
	First	Second	Third	Fourth	
Neutrophils	48.67±1.67 [a]	49.00±4.62[a]	23.67±2.60[b]	42.67±0.88[a]	**
Eosinophils	7.67±0.33[a]	4.66±0.33[bc]	7.00±0.58[ab]	3.66± 1.45[c]	*
Basophils	0.66±0.33[a]	0.00±0.00[a]	1.00±0.57[a]	0.67± 0.33[a]	N.S
Lymphocyte	38.67±0.33[c]	42.67±3.18[c]	65.67±0.88[a]	50.67±0.88[b]	**
Monocyte	4.33±0.67[a]	4.00±1.73[a]	3.00±0.58[a]	3.00±0.58[a]	N.S

The values represent the mean±standard error; (*) Means significant differences ($P \leq 0.05$); (**)Means significant differences ($P \leq 0.01$).

Table 5. Effect of treatment with different levels of Lemongrass on proteins, glucose and testosterone hormone in blood serum lambs Awassi.

Item	Treatments				Significant level
	First	Second	Third	Fourth	
Total protein (g/dl)	6.80±0.15[a]	7.00±0.15[a]	6.86±0.12[a]	7.00±0.25[a]	N.S
Albumen (g/dl)	3.43± 0.08[a]	3.33±0.12[a]	3.33±0.12[a]	3.10±0.41[a]	N.S
Globulin (g/dl)	3.33±0.18[a]	3.70±0.15[a]	3.53±0.12[a]	3.90±0.26[a]	N.S
Glucose (mg/dl)	44.33±2.02[a]	47.00±6.42[a]	40.33±2.02[a]	47.66±2.96[a]	N.S
Testosterone	2.5±0.31[a]	3.73±0.84[a]	4.03±0.64[a]	3.93±0.49[a]	N.S

The values represent the mean ± standard error; (*)Means significant differences ($P \leq 0.05$); (**)Means significant differences ($P \leq 0.01$).

was not significantly affected ($P \leq 0.05$) among the treatments. A significant decrease ($P \leq 0.05$) was also noticed in their numbers in the fourth treatment compared to the rest of the treatments (Table 3).

The results indicated significant ($P \leq 0.05$) differences between the Awassi lambs of the four groups in the percentages of neutrophils, eosinophils, and lymphocytes after 90 days of treatment with lemongrass (Table 1). The percentage of neutrophils in the blood of the third treated animals decreased significantly ($P \leq 0.05$), and for the eosinophils white cells, the results showed significant differences ($P \leq 0.01$) between the different treatments. The fourth and second treatments significantly decreased, while the third treatment did not differ significantly from the second treatment (Table 4).

The results showed no significant effect ($P \geq 0.05$) between groups treated with lemongrass and the control group after 90 days for both basal and mononuclear cells, while the percentage of lymphocytes increased significantly ($P \leq 0.05$) in the third and fourth treatments compared to the second and control treatments (Table 4). The decrease in the numbers of white blood cells, especially neutrophils, and the high numbers of lymphocytes and mononuclear cells in the blood in treated animals may be due to the role of citral, the main component of lemongrass. In laboratory rats, it has the effect of removing toxins and inhibiting the development of cancer (Rabbani et al. 2006).

Biochemical characteristics of blood: The results revealed no significant effect ($P \geq 0.05$) between lambs treated with lemongrass and the control group for a trait of total proteins, albumin, globulin, and glucose (Table 5). This result agreed with Hosoda et al. (2006) in using (mint, cloves, and lemongrass) and Kholif et al. (2017), who used lemongrass and rosemary on the level of total blood proteins, albumin and globulin.

Lipid profile: The results showed significant differences between the four groups after 90 days in the cholesterol level in the blood serum of male Awassi lambs. The third treatment recorded a significant decrease ($P \leq 0.01$), whereas the fourth did not differ significantly. The decrease in the level of cholesterol in the treated animals may be due to the lemongrass plant containing compounds that may inhibit Hydroxymethyl glutaryl CoA reductase

Table 6. Effect of treatment with different levels of Lemongrass on lipid profile in blood serum of lambs Awassi.

Item	Treatments				Significant level
	First	Second	Third	Fourth	
Cholesterol (mg/dL)	84.33±0.67^{a}	73.33±3.18^{ab}	70.00±5.51^{b}	83.00±1.15^{a}	*
Triglyceride (mg/dL)	58.33±0.88^{a}	54.33±1.45^{a}	55.67±1.76^{a}	54.00±1.00^{a}	N.S
HDL(mg/dL)	29.00±3.06^{a}	31.00±4.73^{a}	33.67±2.60^{a}	35.00±3.79^{a}	N.S
LDL (mg/dL)	39.00±2.55^{b}	27.47±5.43^{b}	58.87±5.57^{a}	72.20±1.33^{a}	**
VLDL (mg/dL)	11.67±0.18^{a}	10.87±0.29^{a}	11.13 ±0.35^{a}	10.80±0.20^{a}	N.S

The values represent the mean ± standard error; (*)Means significant differences (P≤0.05); (**)Means significant differences (P≤0.01).

Table 7. Effect of treatment with different levels of Lemongrass on some indicators of kidney and liver function in lambs Awassi.

Item	Treatments				Significant level
	First	Second	Third	Fourth	
Urea (g/dL)	40.33±1.20	40.67±1.86 ^{a}	39.00±1.73^{a}	40.5±1.44^{a}	N.S
Creatinine (µ mol\L)	1.26±0.02^{a}	1.18±0.02^{a}	0.79±0.03^{b}	1.05±0.14^{a}	*
AST (U/L)	114.00± 0.58^{a}	106.5±2.02^{ab}	100.00±2.87^{b}	102.00±4.04^{b}	*
ALT (U/L)	23.00±1.15^{a}	20.50±0.86^{ab}	18.00±1.00^{b}	21.50±0.86^{a}	*

The values represent the mean ± standard error; (*)Means significant differences (P≤0.05); (**)Means significant differences (P≤0.01).

enzyme responsible for building cholesterol (Stryer 2000), or may be attributed to the cause of low cholesterol to the active components of this plant, including citral and geraniol and their role as an antioxidant. They contain compounds that may inhibit the enzyme methyl hydroxy glutaryl COA reductase (MHG CoA), reducing cholesterol levels (Stryer 2000; Kumar et al. 2011). The compounds in lemongrass oil may inhibit the lipase enzyme in fat cells and thus reduce the amount of cholesterol entering the blood (Rahman 2003; Ghule et al. 2006). The lemongrass may remove the resulting toxins, because it contains citral, which can remove toxins and is anti-cancer through its ability to activate the second phase of the GST enzyme (Nakamura et al. 2003). Kholif et al. (2017) found that adding 10g/day of lemongrass to the diet of milking Shami goats for 12 weeks led to a significant decrease in the level of total cholesterol. Lee et al. (2018) also noted that the treatment of male rabbits suffering from hypercholesterolemia (with lemongrass essential oil) led to a significant decrease in the total cholesterol level.

Regarding the effect of dietary supplementation of lemongrass on male lambs during 90 days on the triglycerides, the differences were not significant (P≥0.05) between the treatments. HDL results showed an increase in male lambs of the fourth and third treatments compared to the first treatment (Fig. 6). This may be due to the active substances in the lemongrass plant, including flavonoids, which act as an antioxidant to prevent the oxidation of LDL-C, and increases the beneficial HDL-C, preventing the deposition of LDL-C inside the arteries and veins e (Baratta 1998; Orrego et al. 2009). This result agreed with the findings of Ewenighi et al. (2013) who found a significant increase in high-density lipoproteins in the blood serum of alloxan-induced diabetic in male rats treated with lemongrass extract. Al-Qaisi et al. (2013) also reported treating adult male white rats with lemongrass oil for 120 days leading to a significant increase in HDL-C high-density lipoproteins. Lee et al. (2018) observed that treating male rabbits with hypercholesterolemia (feeding a high-cholesterol diet for four weeks) with lemongrass essential oil led to an increase in the level of high-density lipoproteins.

LDL after 90 days of treatment with lemongrass indicated a significant effect on different treatments, and its level decreased significantly (P≤0.05) in the fourth and third treatments (Table 1). In the first and second groups, the results showed low-density

lipoprotein (VLDL) i.e. no significant effect of lemongrass on these treatments. Our results agreed with the findings of Santhosh Kumar et al. (2011), who confirmed that the treatment of male rats with induced hypercholesterolemia with dexamethasone with lemongrass oil at a concentration of 100 and 200mg led to a significant decrease in the level of low-density lipoproteins (LDL). Ewenighi et al. (2013) showed that the treatment of male albino rats with diabetes mellitus induced with alloxan with the alcoholic extract of lemongrass led to a significant decrease in the level of low-density lipoproteins after one week.

Indicators of kidney and liver function: The results showed a significant effect ($P \leq 0.05$) of lemongrass on the blood creatinine level, as the third treatment, recorded a significant decrease ($P \leq 0.05$) compared to other treatments and no effect in all treatments in blood urea level in male Awassi lambs after 90 days (Table 7). These results agreed with Wajda Al-Obaidi & Wardam (2012) who observed that the treatment of male rats with experimental diabetes induced with alloxan with an alcoholic extract of lemongrass plant led to a significant decrease in the level of urea and creatinine. Haggag (2015) showed a decrease in the level of urea and serum creatinine when treating nephrotoxic male rats by injecting Cisplatin subcutaneously with an aqueous extract of lemongrass for six weeks compared.

For AST and ALT, the results showed a significant decrease ($P \leq 0.05$) in the AST in the fourth and third treatments, while it did not differ significantly from the second group. The first and fourth treatments did not differ significantly from the second treatment. The reason for the decrease in these enzymes is transporting the amino group in the serum of treated animals may be due to the lemongrass active compounds such as Citral and Murecene, or other phenolic compounds and carotenoids that can scavenge free radicals and maintain the composition of biomolecules, especially those involved in the synthesis of membranes cellular (Rabbani et al. 2006), the effectiveness of citral in enhancing the activity of the GST (Glutathione-s-Transferase) (Nakamura et al. 2003 or the activity of the Geranail, which enhances the activity of effective GSH in the anti-free radical scavenging cycle (Metha et al. 2012). These results agreed with Al-Mohammadi et al. (2014) in a study on male and female domestic rabbits affected by oxidative stress induced by hydrogen peroxide at a concentration of 2% and alcohol 20% consumed in drinking water at a double dose for 60 days, that had a significant decrease in the ALT and AST and an improvement in the condition of hepatocytes when treated with aqueous extract of lemongrass. Haggag (2015) also reported that the addition of an aqueous extract of lemongrass for six weeks induced nephrotoxic male rats by subcutaneous Cisplatin injection led to a decrease in the level of liver enzymes ALT and AST. Ozims et al (2017) showed that treatment of white rats (males and females) with hepatotoxicity with Paracetamol with aqueous extract of lemongrass at a concentration of (200, 300, and 400mg per kg of body weight) led to a decrease in the level of AST, ALT enzyme in infected.

Conclusions

Treatment with dietary lemongrass showed a significant improvement in liver function by decreasing the level of AST and ALT enzymes in the blood serum and a positive effect t on some physiological blood parameters (RBCs, PCV and Hb) and a significant decrease in the level of cholesterol, total and low-density lipoproteins, in the treated lambs.

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