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IMPACT OF TEA LEAVES SAPONINS ON SOME RUMEN PARAMETERS OF AWASSI LAMBS

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ABSTRACT

The study was conducted in one of the private farms of Wasit Governorate for the period from 22/1/2019 to 11/5/2019. The investigation focused on the utilization of various levels of saponins to decide their impact on the performance of Awassi lambs. The study included 16 Awassi lambs aged 3-4 months with an average weight of 23.68 kg. The lambs were randomly distributed on four experimental treatments of four animals each. The feed provided as 3% of the lamb body weight was allowed at two times/ day (8 am & 4 pm). Lambs were randomly assigned to the control (without addition), and to treatment groups in which saponins were added either by 60 or 120 or 180 mg/ kg feed. The results showed a significant decrease (P≤0.05) in the pH of the fourth treatment in the first, second and third months, as it reached 6.60, 6.65, and 6.63 respectively compared to the control of 6.93, 6.83 and 6.80, respectively. The results also showed a significant increase (P≤0.05) in the number of total, cellulolytic and lactic acid bacteria in the fourth treatment as it reached (891 x 108, 296 x 107, 687 x 107 CFU / cm^3), respectively, compared with those of control (596.33 x 108, 254.66 x 107, 198 x 107 CFU / cm^3 respectively). In addition, the results showed a significant increase (P≤0.05) in both acetic, propionic and butyric acids of the fourth treatment (69.45, 25.10 and 16.48 mol / 100 cm3 respectively) compared to the control (58.11, 21.73 and 14.23 mol / 100 cm3 respectively). The methane level of different treatments showed nearly similar values. However, the concentration of ammonia of the fourth treatment decreased significantly (P≤0.05) in the 1st, 2nd, and 3rd months (11.62, 13.19 and 14.11 mg/ l, respectively) compared to those of the control (17.97, 19.17 and 19.15 mg / l, respectively).

***Keywords*:** Saponin, Awassi lambs, rumen parameters

Introduction

General concerns about chemical compounds that are used as feed additives have increased, and in this context safer alternatives to nutrition have been found, including the use of secondary compounds from plants such as saponins, essential oils, tannins, etc. (Khalifa *et al.,* 2014). The digestion of feed by microorganisms in the rumen under anaerobic conditions results in volatile fatty acids, ammonia, carbon dioxide and methane, ruminants benefit from volatile fatty acids as a source of energy, gases are excreted by belching and consider as a waste (Martin *et al.,* 2010). There are many strategies by which ammonia and methane gas production can be reduced by altering and improving the efficiency of fermentation in rumen, especially by increasing the concentration of propionic acid, and reducing the rate of protein degradation in rumen, research is ongoing to find a promising feed additive that can improve feed digestion and reduce emission harmful gases in rumen (Azzaz *et al.,* 2012). Among the nutritional solutions developed is the use of saponins, which are usually high in tropical plants, which works to prevent emission of ammonia and methane and to modify rumen fermentation (Budan *et al.,* 2013).

The current study aims to determine the effect of adding different proportions of saponins to the diets of Awassi lambs on rumen parameters that include pH, bacterial counts, methane concentration, ammonia, and volatile fatty acids.

Materials and Methods

Extract saponins from tea leaves

Saponin from tea leaves was extracted following the method developed by (Hussein and Awad, 2014). In brief, tea leaves were collected, gently cleaned, dried, then grinded. Dissolve 30 g of crushed powder with 450 ml of 99.8% methanol for a period of 7 days under room temperature. After filtering, the extracted liquid evaporated under low pressure in a rotary evaporator until thick precipitate is formed. The precipitate formed was washed with petroleum ether to remove chlorophyll and fatty substances, and the process continued until the precipitate became colorless. The process of dissolving the precipitate is repeated in 100 ml of 99.8% methanol and added to the resulting solution, diethyl ether. A yellowish precipitate will be formed from saponins and the process of adding diethyl ether will continue until the sedimentation process stops. The resulted compound was dried by continuous stirring under the laboratory temperature to produce the raw extract and is estimated to be (4.3 g) of saponins.

Feeding groups

Animals were distributed on four feeding groups (table, 1). The first group was a control ration (no addition). The second group was a control ration and 60 mg/kg feed from tea saponin. The third group was a control ration plus 120 mg/kg feed from tea saponin. The fourth group was a control ration plus 180 mg/kg feed from tea saponin.

Rumen liquid pH

The pH of rumen content was measured by a pH digital meter models 9909 PW Philips after taking the rumen liquid directly from the animal.

Rumen Microorganisms

The total bacterial count, cellulolytic and lactic acid bacteria were determined in a specific culture medium according to (Al-Galbi**,** 2013).

Volatile fatty acids

Volatile fatty acids were estimated in the laboratories of the Ministry of Science and Technology by the GC device manufactured by the Japanese company HLMADZU.

Ammonia nitrogen

Ammonia nitrogen was determined by Catalyzed Indophenol Colorimetric Reaction (CICR) method as described by (Chaney and Marbach, 1962) in the Animal Nutrition Laboratory of Baghdad University.

Table 1 : Component of different concentrated ration

Nutrients	Percentage of feed materials included in experimental diets%			
	Control	T1	T2	T3
Barley	50	50	50	50
Wheat bran	20	20	20	20
Corn	20	20	20	20
Soy bean meal	7.5	7.5	7.5	7.5
Vitamin and minerals	2.5	2.5	2.5	2.5
Saponin (mg/kg)	-	60	120	180

T1: adding 60 mg/kg tea saponin or T2: adding 120 mg/kg or T3: adding 180 mg/kg to the control diet.

Methane gas

Shibata *et al*. (1992) developed a methane estimation model in the Friesian heifers, Kuridil sheep and Japanese goats and found that the methane gas concentration could be predicted from the estimate of the amount of dry material intake (DMI) according to the following model, Methane concentration (l / day) = 0.0305 x the amount of dry matter intake (g / day) - 4.441.

Statistical analysis

Data were analyzed with an analysis of variance of a completely randomized design. Treatments effects on studied traits were analyzed using one-way ANOVA design (SPSS, 2016, version 24). The significant differences among treatment means were compared by least significant test within the same statistical packages.

Results and Discussion

pH and different bacteria count

Table (2) shows the average pH, total bacterial count, cellulolytic and lactic acid bacteria. The table indicates a significant decrease (P≤0.05) in the pH value (6.60) of the 4th treatment (180 mg saponin/ kg dry matter) lamb's rumen fluid during the first month in comparison with those of 1st, 2nd and 3rd treatments (6.93, 6.76 and 6.80 respectively). As for the 2nd month, the pH value decreased significantly for the 4th treatment lambs, as it reached 6.56 compared to the 1st and the 2nd treatments, which reached 6.83 and 6.83 respectively, while the 3rd treatment did not differ significantly with all treatments. The value of pH decreased significantly in the third month in lambs of the 4th treatment to which saponins were added 180 mg/ kg dry matter, as the decrease reached 6.63 compared to the 1st, 2nd, and 3rd treatments (6.80, 6.83 and 6.80, respectively). The extent of the decrease in the pH value of this study was within the natural ranges for the growth of most of the microbes in the rumen. It can be concluded from these results that adding saponins in relatively high quantities has significantly changed the pH value, which leads to providing an appropriate environment for the growth of beneficial bacteria that increase fermentation of carbohydrates and thus increase the concentrations of volatile fatty acids. This result was consistent with that of (Mao *et al*., 2010), who noticed a significant decrease (P≤0.05) in the pH of the rumen fluid compared with the treatment of the control when added saponins at (0, 3) grams / day to the diets of lambs. Whereas (Aazami *et al*., 2013) when added saponins at different levels (0, 100, 200) mg/ kg dry matter to the sheep diets, noticed no significant differences.

The table (2) also indicates that there were significant (P<0.05) differences in the total bacteria, cellulolytic and lactic acid bacteria count of lambs receiving no saponin (control). In terms of the total number of bacteria, 891 x 810, 719x10^8 and 596x10^8 CFU/ ml of rumen fluid from control, 1st, 2nd, 3rd, and 4th groups respectively. The 3rd and 4th treatments also showed a significant increase (P≤0.05) in the number of cellulolytic bacteria (294.33 and 296.66 x 10^7 CFU/ml) compared to the control treatment (254.66 × 10^7 CFU/ml). The 2nd treatment showed no significant differences in cellulolytic bacteria (277.3 x 10^7 CFU/ml) compared to other experimental treatments.

Table 2 : Effect of adding different levels of saponins on the pH, total bacterial count (× 10^8 CFU/ ml), cellulolytic bacteria and lactic acid bacteria (× 10^7 CFU/ml) in rumen fluid for different treatments (± standard deviation)

Treatment	pH			Bacteria count		
	1st month	2nd month	3rd month	Total	Cellulolytic	Lactic acid
T1	6.93±0.15 a	6.83±0.15 a	6.80±0.10 a	891.00±28.05 a	254.66±10.11 c	198.00±12.00 c
T2	6.76±0.06 b	6.83±0.11 a	6.83±0.06 a	719.00±41.67 b	277.30±13.01 b	233.33±27.46 b
T3	6.80±0.100 b	6.73±0.057 b	6.80±0.100 a	596.33±56.35. c	294.33±23.71 a	287.33±28.58 b
T4	6.60±.012 c	6.56±0.152 c	6.63±0.057 b	542.00±26.90 c	296.66±14.04 a	687.00±115.25 a
P-value	0.05	0.05	0.05	0.05	0.05	0.05

*T1: adding 60 mg/kg tea saponin or T2: adding 120 mg/kg or T3: adding 180 mg/kg to the control diet. * Means with different letters vertically differ significantly (P<0.05)*

Table (2) shows significant differences (P≤0.05) in the number of lactic acid bacteria. The 4th group lambs (saponins 180 mg/kg) showed the highest count of lactic acid bacteria (687x10^7 CFU/ml), while the values of other groups were (198, 223.33, 187.33) x 10^7 CFU/ ml of rumen liquid for control, 2nd, and 3rd respectively.

Volatile fatty acids

Table (3) shows volatile fatty acids concentration in the rumen fluid of lambs of the studied treatments. There were significant differences (P≤0.05) between experimental treatments in the total fatty acids when adding different levels of saponins. Where the 2nd, 3rd and 4th treatments significantly (P≤0.05) differed from the control treatment in the total fatty acids (2.52, 2.66, 2.69 and 2.34 mmol/L respectively). Significant differences (P≤0.05) appeared in the concentration of acetic acid in the rumen liquid of 2nd, 3rd, and 4th (62.33, 68.22 and 69.45% respectively) from that of the control treatment (58.11%). The table also shows significant differences (P≤0.05) in the concentration of propionic acid, where all experimental saponin treatments (60, 120 and 180%) showed higher concentrations (24.26, 24.89 and 25.10% respectively) compared with the control group (21.73%). Significant differences (P≤0.05) were observed between the experimental treatments and control group in the concentration of butyric acid. Adding different levels of saponin (60, 120 and 180 mg/ kg) exceeded that of control group (16.09, 16.73 and 16.84% respectively vs. 14.23%).

Table 3 : The effect of adding different levels of saponins on the concentration of volatile fatty acids in the rumen fluid (± standard deviation)

Treatment	Total fatty acids mmol/L	Percent (%) of volatile fatty acid Acetic	Propionic	Butyric
T1	2.34 b	58.11c	21.73b	2.34 b
	± 0.31	± 1.21	± 1.02	± 0.31
T2	2.52 a	62.33 b	24.26a	2.52a
	± 0.42	± 2.32	± 1.21	± 0.42
T3	2.66 a	68.22 a	24.89a	2.66a
	± 0.49	± 2.34	± 1.26	± 0.49
T4	2.69 a	69.45 a	25.10a	2.69a
	± 0.50	± 2.41	± 1.3	± 0.50
P-value	0.05	0.05	0.05	0.05

*T1: adding 60 mg/kg tea saponin or T2: adding 120 mg/kg or T3: adding 180 mg/kg to the control diet. * Means with different letters vertically differ significantly (P<0.05)*

The reason may be attributed to the addition of saponins, which increased the concentrations of volatile fatty acids and reduced the pH concentration. The increase in the concentration of glucose as a result of adding saponins caused an increase in the concentration of propionic acid. These results were consistent with those of (Wina *et al*., 2006), who observed a significant increase (P≤0.05) in the concentration of propionic acid and butyric acid when adding saponins (0.48, 0.72 g / kg body weight) to the sheep diets compared with the control treatment. The results also agreed with (Liu *et al*., 2009) when adding saponins at different levels (0, 100, 200, 300) mg/ kg of the sheep diets. They observed significant increase (P≤0.05) in the concentration of propionic acid of the third treatment (200 mg/kg) and the fourth treatment (300 mg/kg) compared with the second treatment (100 mg/kg) and the control. Liu et al (Liu *et al*., 2019) also observed an enhancement in volatile fatty acid when adding teat saponin as 2 gm/ head/ day for Dorper sheep. When feeding lambs diets containing saponins (0, 3 gm/kg body weight), a significant increase (P≤0.05) was observed in the concentration of propionic acid of the saponins treatment compared to the control (Zhou *et al*., 2011). While the results were not in agreement with those (Nasri *et al*., 2011), who observed that there was no significant difference in the concentration of volatile fatty acids when adding saponins (30, 60, and 90 mg/kg dry matter) to sheep diets compared to the control (zero saponin) treatment.

Concentrations of ammonia and methane

Table (4) shows a significant decrease (P≤0.05) in the concentration of ammonia gas, which is associated with an increase in the amount of saponins added. Adding saponins at 120 and 180 mg/kg led to a significant decrease (P≤0.05), in ammonia concentration in the first month (14.27 and 6.60 mg/ l, respectively), compared with the zero and 60 mg/kg of saponin (17.97 and 17.48 mg/l respectively). The concentration of ammonia also showed significant differences (P≤0.05) in the second month. Group received 180 mg/kg saponin exhibited the least value (6.56 mg/L) in comparison with those received 0, 60 and 120 mg/L (19.17, 18.30 and 15.48 mg/L respectively). As for the third month, the concentration of ammonia gas for the fourth treatment also recorded the least value (14.11 mg/L) compared to both the first, the second, and the third treatment (19.15, 18.38 and 16.42 mg/L, respectively).

The reason for the decrease in ammonia gas may be attributed to the possibility that saponins bind to the protozoa cell membranes, leading to the destruction of the membranes of these cells, and thus the number of protozoa in rumen decreased **(**Patra and Saxena, 2009**)**. Liu *et al.*(2019) also observed a significant decrease (P≤0.05) in the concentration of ammonia when adding saponins at different levels (0 and 2 gm/head/day) to sheep diets, due to the low number of proteolytic bacteria in the rumen. The present results agreed with those of (Zhou *et al*., 2011), who observed a significant decrease (P≤0.05) in the concentration of ammonia in the treatment to which saponins were added compared with the control treatment.

Table (4) also shows that the concentration of methane gas in the rumen fluid was not significantly affected (P≤0.05) when adding saponins to the diets of Awassi lambs at different levels 60, 120 and 180 mg/ kg of dry matter compared with the control treatment. These results were similar to the findings of (Liu *et al*., 2019)], as there was no significant difference in methane production in the treatment for which saponins were added 2 g/ head/ day to sheep diets compared with the control.

Table 4 : The effect of adding different levels of saponins on the concentration of ammonia nitrogen (mg/L) and methane (L/ day) in rumen (± standard deviation)

Traits	Ammonia gas 1st mo.	2nd mo.	3rd mo.	Methane 3rd mo.
T1	17.97 a	19.17 bc	19.15 c	27.35
	± 2.22	± 1.63	± 1.35	± 0.60
T2	17.48 b	18.30 bc	18.38 bc	27.59
	± 0.879	± 0.527	± 1.30	± 0.44
T3	14.27 a	15.48 b	16.42 b	28.47
	± 1.44	± 1.01	± 1.13	± 1.62
T4	11.62 a	13.19 a	14.11 a	28.76
	± 0.65	± 0.89	± 0.36	± 0.77
p-value	0.05	0.05	0.05	N. S

*T1: adding 60 mg/kg tea saponin or T2: adding 120 mg/kg or T3: adding 180 mg/kg to the control diet. * Means with different letters vertically differ significantly (P<0.05)*

Conclusions

Adding saponin (60-180 mg/kg) reduced rumen fluid pH within a normal range. The addition of 180 mg saponin/kg body weight increased total, cellulolytic and lactic acid bacteria counts by 49.41%, 16.23% and 246.97% respectively in comparison with control group. The main volatile fatty acid (acetic, propionic and butyric acids) of the group received 180 mg saponin/kg body weight elevated by 19.51%, 15.51% and 15.81% respectively compared with those of control group. Ammonia concentration of rumen fluid increased by 35.34%, 31.19% and 26.32% during 1st, 2nd and 3rd month of the study respectively when saponin was added by 180 mg/kg. Methane emission estimated depending on dry matter consumed of different treatments was similar.

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