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Effect of Pomegranate Peel or Bread Yeast on Rumen Fermentations Characteristics in Awassi Lambs

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Abstract
This study was conducted to determine the effect of pomegranate peel powder and baking yeast (*saccharomyces cerevisiae*) on rumen PH, the concentration of volatile fatty acids, the total number of bacteria, and the concentration of ammonia (NH_3-N) in the rumen fluid.
were selected 20 male iraqiawassi lambs at 3-4 months old, and randomly divided into 4 equal groups. The first group (control) was fed on concentrated dite at a rate of 3% of body weight with green fodder and free grazing, while pomegranate peels were added to the concentrated fodder for the second group (pomegranate peel group) at a rate of 1% of concentration dite, and bread yeast was added to the fodder of the third group (saccharomyces cerevisiae group) at a dose of 4 g for each animal. The control group was added to its diet with 1% pomegranate peel powder and yeast (saccharomyces cerevisiae) at a dose of 4 g per kg of concentrated feed. The results of the study showed a significant increase ($P<0.05$) in the pH value in the third and fourth groups compared to the control group, and the total count of rumen bacteria was significantly increased ($P<0.05$) in the three treatment groups compared to the control group, the concentration of volatile fatty acids(VFA) also increased significantly ($P<0.05$) in the third and fourth groups compared with the control group, while the concentration of rumen ammonia (NH3-N) significantly ($P<0.05$) decreased in the three treated groups compared with the control group.
Conclusion: We conclude that adding pomegranate peels and yeast has improved rumen fermentation in terms of increasing the concentration of volatile fatty acids, reducing the concentration of ammonia, and increasing beneficial microorganisms in the rumen fluid.

Keywords: Bread yeast, Rumen fermentations, Awassi lambs, Pomegranate peel.

Introduction

Pomegranate consumption and production have significantly expanded globally in recent years due to growing awareness of the possible health benefits of this fruit's numerous components[1]. This growing desire has sparked the creation of cutting-edge industrial technologies that offer consumers fresh fruit juices and "ready to eat" pomegranate arils. It is anticipated that these developments will cause a significant buildup of active pomegranate mass. Components of pomegranates have drawn interest due to their purported ability to heal

wounds [2], ruminant nutritionists and microbiologists are looking for natural alternatives to these chemical feed additives for environmentally friendly animal production due to the growing interest in organic farming and the impact of ammonia and methane generated by ruminants to climate change. In recent years, a class of natural compounds known as plant secondary metabolites (PSMs), which include saponins and phenols in the diet of ruminants, have showed some potential as a nutritional tactic [3].Plant secondary metabolites may be employed to control rumen microbial diversity, production, and proportion of volatile fatty acids, as well as methane generation as a nitrogen metabolism. Utilising oils that are essential can be a practical method to increase ruminants' ability to efficiently absorb nutrients [4]. Additionally, it was mentioned that secondary metabolites from plants may have a positive impact on protein metabolism, reducing the amount of dietary protein that is broken down in the rumen and boosting the uptake of amino acids in the small intestine. Despite the fact that medicinal plants are employed for their health-related qualities, including their antibacterial, antioxidant, anti-inflammatory, anti-parasitic, and anticancer capabilities [5]. One of the most crucial tactics in developing nations is the use of cheap, easily accessible, domestic, and abundant sources of feedstuffs. Agro industrial by-products are the initial candidates for these applications. Additionally, these byproducts, which are typically released into the environment, have nutritional value [6]. Addition recently using agricultural waste and industrial by products in ruminant nutrition to reducing costs and reduce of human food in animal consumption [7].

Due to changing climatic circumstances and a lack of water resources, the cost of animal feeds, especially protein supplements, has increased; yet, these supplements may be metabolized less effectively (e.g losses of NH3-N) in the rumen, resulting in a reduction in animal performance [8]. As a by-product of the pomegranate juice industry, pomegranate peel includes significant levels of polyphenols like saponins,flavonoids, ellagic tannins, ellagic acid, and gallic acid [9].Thus, the C and N contents of sheep diet can be changed by the addition of saponins from PP[10]. According to sheep (*Ovisaries* L.) create 8 kilograms of enteric methane (CH4) gas per year.Saccharomyces cerevisiae may be able to operate as a buffering agent in the rumen by absorbing acidic elements, and it may also be able to manipulate bacterial dominance by suppressing the microflora that produces acid [11], saccharomyces cerevisiae Additionally, it may assist bacteria in two ways: first, by creating an ideal environment by reducing acidity and limiting lactate accumulation, and second, by promoting some bacterial strains may aid in boosting VFA synthesis additionally, saccharomyces cerevisiae lessened both the disparities between and daily variations in pH readings. As a result, the rumen environment was more stable[12].

Materials and Methods

This study was conducted to study the effect of supplementation of yeast and pomegranate peel on rumen fermentation aspects. 20 male Iraqi Awassi lambs, aged up to 4 months, were selected and randomly divided into 4 equal groups. The first group (control) was fed a 3% concentrated dite f of body weight with green fodder and free grazing, while pomegranate peels were added to the concentrated feed of the second group (pomegranate peel group) at a rate of 1%, and baking yeast was added to the feed of the third group (saccharomyces cerevisiae group) at a dose of 4 grams of yeast (saccharomyces cerevisiae) for each animal, and as for the fermented group, peel powder was added to its diet. Pomegranate at a rate of 1% and yeast (saccharomyces cerevisiae) at a dose of 4 g per kg of concentrated feed. Pomegranate peels and yeast were prepared from local markets.

Sample Collection.

In accordance with samples were taken from the same lambs during the entire sampling period via an elastic stomach tube attached to a specific vacuum, then to preserve the 10 ml of

subsamples, 0.2 ml of 50% sulfuric acid was added to stop bacterial growth and trap ammonia [13]. The samples were then refrigerated at (-20 °C) until further examination [14]. All parameters were measured monthly for three months

Rumen pH Determination.
The digital Hanna Instruments HI98103 Checker pH Tester, which was calibrated with 4 and 9 common pH buffer solutions, was used to filter the samples and quickly assess their pH[15].

Determination of Total count of bacteria cfu/units.
Bacterial colonies were calculated using [16]. methodology. Samples of rumen fluid were collected at 0, 3, and 6 hours after each meal after being filtered through four layers of cheese cloth. Serial dilutions of the ruminal fluid in sterile buffered saline were performed. Four quarters of the nutritional agar were separated, and the centre of each quarter was covered with 0.02 ml of ruminal fluid that had been dissolved in tube number seven (107) of the experiment. After that, each nutrient agar plate was incubated for 24 hours at 37°C.

CFU per ml = Average number of colonies for a dilution × 50 × dilution factor

Determination of Total Volatile Fatty Acid (TVFA) mg/100ml
The storage freezing ruminal fluid samples were centrifuged and TVFA content was determined using the steam distillation method in accordance with [17].

Assaying the TVFA required adding 1 ml of rumen fluid, 1 ml of arthocholic acid, and 1/2 ml of methyl red as a colour reagent to a tube in which the reaction took place under the influence of water evaporation. Titration was then performed using (0.1 N) sodium hydroxide until the colour changed from red to yellow, at which point the TVFA was calculated using the equation shown below.

$$VFA(mEq) = \frac{titratation - Blank}{wight\ of\ sample} \times 100 \times sodium \text{ hydroxide concentration}$$

Determination of NH3 -N mg/100ml:
Frozen ruminal fluid samples were thawed at room temperature and centrifuged at 4,000g for 20 min. The supernatant was analysed by two stages first the distillation with 7ml MgO, reception flask which contain the NH3-N was obtained after 10 ml subsamples were preserved by the addition of 0.2 ml of 50% sulfuric acid to terminate calculated using the equation below [14]:

$$N - NH_3 \left(\frac{mEq}{100cm3}\right) = \frac{titratation - blanck}{wight\ of\ sample} \times concentration\ of\ acid \times 0.014 \times 100$$

Statistical analysis
One-way ANOVA was used to examine the data, and the least significant differences (LSD) post hoc test was used to determine whether there were significant differences between the means. Statistical significance was defined as P 0.05 [18].

Results

Total pH means/ month.

The PH value in the third and fourth groups increased significantly and was within the normal range of PH values in comparison with the control group during the 30, 60, and 90 days of the experiment (Table 1)**.**

Table (1) Effect of dietary dried pomegranate powder (PPP) and Saccharomyces cerevisiae (Sc) on total pH means / days in Awassi lambs M±SE.

period / groups	1st month	62nd month	93rd month
G1 (control)	6.30±0.021 b	6.29±0.02 b	6.11±0.03
G2 (1%PPP)	6.34±0.011 ab	6.33±0.03 ab	6.15±0.01
G3 (4g SC)	6.37±0.013 a	6.36±0.01 a	6.38±0.01 a
G4 (1%PPP + 4 g Sc)	6.39±0.011 a	6.38±0.03 a	6.40±0.03 a
LSD	0.06		

Means within the same column with different letters differ significantly (P<0.05)

Total means of Total bacterial count/month

The number of total bacteria increased significantly in the three treatment groups compared to the control group, during a period of 1, 2, and 3 months of the experiment period. as seen in Table 3

Table (2) Effect of dietary pomegranate peel powder (PPP) with or without Saccharomyces cerevisiae (Sc) on total mean of Total bacterial count / month (Log_{cfu}/mL) of Awassi lambs M±SE

months / animals/ group	1st month1	2nd month	3rd month
G1 (control)	4.01±0.0006 b	4.02±0.0012 b	4.6±0.0018 b
G2 (3%PPP)	4.14±0.0005 ab	4.17±0.0016 ab	4.16±0.0008 ab
G3 (4g SC)	4.28±0.0010 a	4.27±0.0008 a	4.28±0.0007 a
G4 (3%PPP + 4 g Sc)	4.27±0.0011 a	4.29±0.0014 a	4.27±0.0012 a
LSD	0. 25		

Total of total volatile fatty acids means / month.

The concentration of volatile fatty acids increased significantly in the third and fourth groups compared to the control group, as can be seen in the table during a period of 1, 2, and 3 months of the experiment.as show in table 5

Table (3) Effect of dietary dietary pomegranate peel powder (PPP) and Saccharomyces cerevisiae (Sc) on Total means of total volatile fatty acids / month (mg/100ml) of local Awassi male lambs. (M±SE)

period / animals/group	1st month	2nd month	3rd month
G1 (control)	4.40±0.17 b	4.06±0.47 b	4.40±0.85 b
G2 (1%PPP)	4.58±0.14 ab	4.20±0.34 ab	4.53±0.55 ab
G3 (4g SC)	5.03±0.25 a	4.83±0.47 a	4.95±0.24 a
G4 (1%PPP + 4 g Sc)	5.02±0.21 a	4.96±0.16	5.06±0.60 a
LSD	0.53		

Totalmean of NH_3-N / month

The concentration of ammonia in the stool fluid decreased significantly in the three treated groups compared to the control group, as seen in the table, during a period of 1, 2, and 3 months of the experiment**.**

Table (4) Effect of dietary pomegranate peel powder and Saccharomyces cerevisiae (Sc) on total mean of NH3-N / month between different collections of Awassi lambsmg/100ml. (M±SE)

period / animals/group	1st month	2nd month	3rd month
G1 (control)	3.49±0.21 a	4.10±0.21 a	4.25±0.10 b
G2 (31%PPP)	3.01±0.15 b	3.80±0.11 b	3.93±0.08 b
G3 (4g SC)	3.20±0.14 b	3.73±0.12 b	3.90±0.24 b
G4 (1%PPP + 4 g Sc)	3.13±0.11 b	3.76±0.10 b	3.86±0.16 b
LSD	0.28		

Means within the same column with different letters differ significantly (P<0.05)

Discussion

The significant increase in the pH value in the third and fourth groups compared to the control group, as can be seen in Table 1 in the 1st , 2nd and 3rd month after the begging of the experiment, may be due to the effect of yeast(saccharomyces cerevisiae) in increasing the activity of microorganisms in the rumen [19]. As pH is considered an excellent indicator to knowing the conditions of rumen environment and the stability of the pH value within the normal range gives an indication of the efficiency and development of digestion and fermentation processes.Yeast also works to increase the total count and types of microorganisms in the rumen, which work to consume lactic acid and prevent its accumulation in the rumen fluid, thus increasing the pH value [20,21], as well as bacteria (Selenomanas, Rumintium and Megasphaeran) increase under the influence of yeast and thus raise the pH [22,23]. In addition, yeast lead to increase the concentration of propionic and acetic acid in rumen fluid, as products of volatile fatty acids resulting from the digestion of carbohydrates, and reduce the concentration of lactic acid [24]. Thus, yeast reduce the incidence of cases of rumen acidity in ruminants [25,26].

The superiority of the third and fourth groups in the rumen total bacteria count of over the control group may be due to the effect of yeast, as it works to increase the numbers of cellulolytic bacteria, which includes species *R. flavefaciens and F.succinogenes,* which work to increase and improve the digestion of fiber in the feed, This is an important key to feeding ruminants by increasing the benefit of feeding, in addition to the role of yeast in increasing the numbers and effectiveness of various types of natural bacteria in the rumen [27,28]. Which indicated an increase in the number of bacteria in the rumen of Buffalo bulls after adding yeast (saccharomyces cerevisiae) to their diet [29]. in dairy cows, and in goats[30].

Yeast improve the digestive process in animals by increasing the effectiveness and number of normal flora in the rumen [31,32]. Providing yeast improves the important growth factors for the microflora in the rumen, including vitamins A and B and other important mineral elements [33], and this process leads to Increasing the digestibility of fiber in the feed and thus increasing the production of volatile fatty acids, and some studies have indicated the effect of yeast in increasing the number and effectiveness of *Ruminococcusalbus* and *Fibrobactersuccinogen*es bacteria in the rumen fluid.These bacteria work to increase and improve the digestion of carbohydrates, especially fiber.And ultimately converting them into volatile fatty acids [34], as the high level of volatile fatty acids is evidence of the efficiency of microorganisms in the rumen of ruminants [30,35,36], which indicated an increase in the concentration of volatile fatty acids in goats after adding yeast to their feed.

The concentration of ammonia (NH3-N) in the rumen is used as an indicator of the decomposition of proteins ingested with food by microbes in the rumen and the increase in the absorption of non-protein nitrogenous compounds. The high concentration of ammonia in the rumen fluid causes a group of health problems for ruminants [37].The ammonia (NH3-N) concentration in the lambs of the three treatment groups was significantly lower compared to the control group. may be due to the effect of pomegranate peels, as adding pomegranate peels affects the inhibition of the activity of protozoa [38], and thus reduces the decomposition of food protein and ultimately a decrease in ammonia, as the protozoa are responsible for the decrease in the number of rumen bacteria that work to increase the absorption of ammonia and the formation of microbial protein, so the effect of pomegranate peel by reducing .The activity of protozoa leads to an increase in the activity of bacteria and a decrease in ammonia [39].Or the reason may be due to the effect of pomegranate peels on bacteria [40], as it leads to increasing the activity of rumen bacteria that consume ammonia (NH3-N) as one of the non-protein nitrogen compounds and convert it to microbial protein [38]. As a decrease in ammonia concentration was associated with an increase in microbial protein [25].

Or the reason may be the effect of tannin compounds found in abundance in pomegranate peels, as tannin works to bind with the protein compounds in the feed digested in the rumen and reduces their decomposition into ammonia (NH3-N), which is transported to the abomasum to be digested enzymatically later. This preserves the protein compounds and thus reduces the concentration of ammonia in the rumen, these results are consistent with [41,42,43],who indicated a decrease in the concentration of ammonia (NH3-N) in the rumen fluid of lambs after being fed pomegranate peels at a rate of 1% of the weight of the concentrated feed.

Or the reason for the decrease in (NH3-N) in the third and fourth groups may be due to the effect of yeast (saccharomyces cerevisiae), which works to reduce the speed of decomposition of food protein in the rumen and delay its decomposition when it enters the Dudenium. It has been mentioned [44]that yeast improves the formation of microbial protein in the rumen by using non-protein nitrogenous substances, including ammonia, thus reducing its concentration in the rumen, and these results are consistent with both [45,46], which indicated a decrease in

the concentration of ammonia in the rumen fluid in cows after feeding them on a diet to which yeast was added at a rate of 2, 4, 6 ,8 and 10 percentage.

Conclusions

We conclude that adding pomegranate peels and yeast has improved rumen fermentation in terms of increasing the concentration of volatile fatty acids, reducing the concentration of ammonia, and increasing beneficial microorganisms in the rumen fluid**,** all this lead to improving the animal health.

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