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EFFECT OF FEEDING SAGE MEAL TO WEANED AWASSI MALE LAMBS ON BODY PERFORMANCE AND MEAT QUALITY

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Supporting Information

ABSTRACT: An experiment was conducted for 8 weeks on 15 Awassi lambs using sage dry meal. Animals were randomly allocated into 3 groups: control group meal (CGM: 0% sage; Salvia officinalis), experimental group meal 1 (EGM1: 1% sage) and experimental group meal 3 (EGM3: 3% sage) by 5 heads. Feed intake at the 8th week increased proportionally with live body weight (LBW). Cumulative live body weight gain increased slightly more in EGM3 after the 5th week to attain 10.7 Kg at 8th week. Feed conversion ratio (FCR) was most effective and attained 3.83 ± 0.97 in EGM3 vs 4.14 ± 0.53 and 4.15 ± 0.64 in CGM and EGM1, respectively. After cooling, luminance ranged between 45.41 ± 2.97 in CGM and 47.28 ± 5.63 in EGM1 whereas in EGM3 it was 47.28 ± 5.63 . Redness, a*, after cooling was lowest in EGM3 (20.15 ± 3.29) followed by CGM (22.61 ± 3.41) and EGM1 (24.97 ± 1.24). Yellowness b* after 1 month of freezing is positively correlated with the achieved results after 24 h of cooling. The least losses in water after cooling was in EGM3 attaining $11.39 \pm 2.39\%$. Meat of CGM loses more water after cooking ($30.30 \pm 6.52\%$) than other groups. Furthermore, after 24 hours of cooling, the most tender meat was in EGM1 (4.87 ± 0.44 mm) in comparison to CGM (3.3 ± 0.64 mm), whereas EGM3 occupied the 1st place in cooked meat tenderness after 1 month of freezing (5.4 ± 0.8 mm). It is concluded and recommended to use rations containing sage meal in the daily feeding of Awassi sheep.

Keywords: Awassi lambs, Feeding, Meat quality, Performance, Sage dry meal.

INTRODUCTION

The productivity loss in small ruminant farming is a significant issue, and food scarcity, which is primarily brought on by high costs, has been highlighted as one of its primary reasons. The maximization of low concentrate feed costs while ensuring an increased net yield are major issues in such agricultural enterprises (De Roest et al., 2018; Hanrahan et al., 2018). This topic has been the subject of a great deal of recent research (Jakubowska and Karamucki, 2021; Jordán et al., 2020), and different introduction tactics have been examined. Antibiotics are a substance that has received harsh criticism (Oberoi et al., 2019). The use of antibiotics is restricted for a number of significant reasons, two of which being the development of drug resistance in bacteria and the presence of drug residues in meat (Bacanli and Başaran, 2019; Patel et al., 2019).

Antibiotics have been removed from the diet, which has led to subpar performance and an increase in illness susceptibility. To get over these difficulties, efforts were undertaken to develop different solutions. Use of growth boosters with a natural origin is one technique that has garnered a lot of interest lately. Strong evidence suggests that herbs, spices, and their products have antioxidative, antibacterial, and growth-promoting benefits on animals, according to Odoemelam et al. (2013). It is possible that some herbs and spices have antioxidant properties that protect both the quality of feed and food made from animals fed these substances (Odoemelam et al., 2013). Aromatic herbs have been employed as food preservatives and in traditional medicine since ancient times (Christaki et al., 2020). The Mediterranean region is the origin of many of the most well-known fragrant plants, including oregano, rosemary, chamomile, sage, anise, basil, etc. Because they are organic, natural, and usually regarded as safe products, the demand for these plants and the derivatives they produce has recently surged. As a result, aromatic plants and their extracts have the potential to lead to new developments in food and health goods for both humans and animals (Giannenas et al., 2020; Christaki et al., 2020). The use of antibiotic growth promoters as a feed additive has been prohibited since the turn of the century, according to Rahman et al. (2022), Wang et al. (2020) and Nm et al. (2018). These antibiotics have been included in animal feed rations to promote the growth of beneficial microorganisms in the intestinal microflora and to avoid disease. Scientists began seeking for alternatives once most antibiotic growth promoters were banned. They concentrated on using herbs, spices, and plant extracts (essential oils-E0) as potential antibiotic substitutes as a result. They increased feed intake, feed conversion ratio, and carcass yield activity when added to the feed ration or water (Patel et al., 2015). Sage is one of the fragrant plants that can be highlighted sage (*Salvia officinalis*). According to Caesar and Cech (2019) sage's bioactive components work together synergistically to exert pharmacodynamic effects that are mostly salt secretive, antiseptic, antidiarrheal, anti-inflammatory, immuno-stimulating, and analgesic.

This study seeks to explore the effects of substituting sage dry meal for antibiotics and antioxidants in basic rations when supplementing sheep for this purpose. Sage dry meal contains natural phytogenic substances at varied quantities. In comparison to the control group of antibiotic-free lambs, the body performance and meat quality of groups of Awassi male lambs are noted.

MATERIALS AND METHODS

Ethical approval

The research was approved by the Bioethics Committees of the Lebanese University, Faculty of Agriculture, Department of Animal Production, and the University of Forestry, Sofia, Bulgaria. and strictly conformed with the internationally accepted standard ethical guidelines for laboratory animal use and care as described in the European Community guidelines; EEC Directive 86/609/EEC, of November 24, 1986.

Animals and study field

A total of fifteen Awassi lambs, sixty to seventy days old, were purchased from Bekaa valley in Lebanon and divided into three groups of five animals each. The site was designed to study the impact of feeding male sheep different supplementation levels of sage meal on body performance and meat quality during the fattening period. Conditions included availability of ambient temperature of 25°C, water and feed were offered *ad libidum* for the duration preceding the experimental period.

Methods

At the start of the experiment, all animals were fed a basic ration (BR) for 5 days after which sage was added after allotting them into 3 groups, of 5 lambs each. Animals of the control group (CGM) were fed free choice antibiotic-free and antioxidant-free basal diet in mashed form based on yellow corn-soybean meal mixture and hay. The remaining 2 groups were fed the following experimental rations: Animals of group (EGM1) were fed BR supplemented with 1% sage dry meal and EGM3 had BR with 3% sage dry meal. All experimental rations fed to animals were not exceeding 18% crude protein and 2718 ME kcal/kg of feeds. Daily observations of the lambs revealed no signs of illness. Every week, a live body weight (LBW) scale was used to measure each participant (kg). On a weekly basis and at slaughter, feed conversion rate (FCR) and live body weight growth (LBWG) were calculated (after 8 weeks of the experiment). After 24 hours of cooling and one month of deep freezing, the physical characteristics of the meat were examined (post mortem), and the L*, a*, and b* colours as well as the pH level were measured using a chromometer and pH-meter in accordance with Offer and Knight's procedure (1988). Measurements were made on the surface of the meat, free of any visible colour flaws, three times each sample (bruises, blood spots, and haemorrhages). According to Honikel (1998), cooking loss was evaluated, thawing loss was calculated, and hardness (tenderness) was estimated using a penetrometer.

Statistical analysis

Using the "SigmaStat software V. 3.5," one way analysis of variance (ANOVA) of the results was used to assess the statistical differences between the treatments. Results were shown as Mean values with standard deviation. The significance level was set at P 0.05. With different confidence levels as P0.001***, P0.01**, and P0.05*, Pearson Correlation was used to examine the positive and negative independence of results of the various variables among all experimental groups.

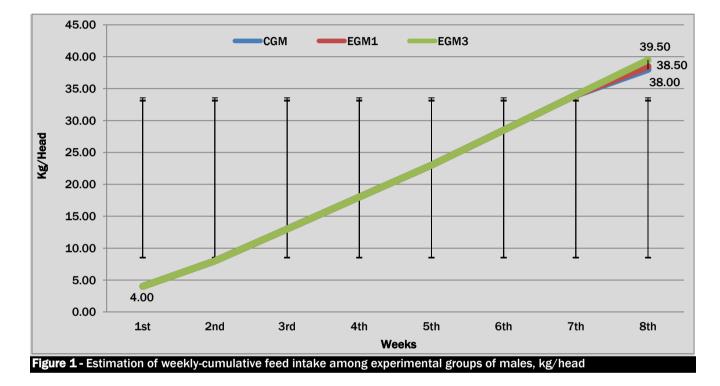
RESULTS AND DISCUSSION

Table 1 shows the amount of the cumulative feeds consumed (cFI, kg/group) and Figure 1 estimated cumulative feed intake (cFI, kg/head) by animals of all groups during the eight weeks of the trial. Both Figure 1 and Table 1 revealed an equal intake of feeds distributed on the experimental animals. The results obtained demonstrate that the overall feed intake at the end of 8th week of the trial increased proportionally with the increase of LBW, attaining a small increase (P>0.05) in group EG3 (197.5 kg/group) by 3.9 % and EGM1 (192.5 kg/head) by 1.3 % in comparison with the negative control group (CG) where it consumed the amount of 190 kg/group.

The observation of animal during the experiment showed that animal finish taking the ration containing sage faster than the control group, this due to the beneficial effects of sage in animal nutrition may include the stimulation of appetite and feed intake. This result agrees with the findings obtained by the experiment done before. This result agrees with the findings obtained by the experiment done before. This result agrees with the findings obtained by the experiment done before. This result agrees with the findings obtained by Kamel (2001), who applied essential oils of different herbs and spices in animal feeding resulting in increased feed intake in comparison with rations exclusive of any herb supplementations. In addition, this improvement in feed consumption was observed may be due to the appetizing effect of active ingredient (borneol) in chamomile having anti-inflammatory, antiseptic, diaphoretic and sedative properties (Srivastava et al., 2019) by killing and inhibiting the harmful intestinal microorganisms in the intestinal tract of the animals (Nazarizadeh et al., 2019).

Table 1 - Estimation of the overall Feed Intake (FI) among experimental groups of Males, kg/group

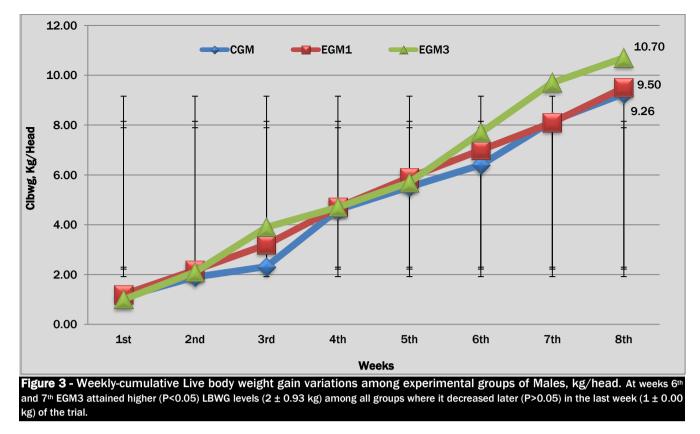
| At the end of week | BR + commercial antioxidants + antibiotics + vitamin premix + mineral premix CGM | 1% Salvia officinalis to BR + vitamin premix + mineral premix EGM1 | 3% Salvia officinalis to BR + vitamin premix + mineral premix EGM3 |
|------------------------|---|---|---|
| | | | |
| 2 nd | 40.00 | 40.00 | 40.00 |
| 3 rd | 65.00 | 65.00 | 65.00 |
| 4 th | 90.00 | 90.00 | 90.00 |
| 5 th | 115.00 | 115.00 | 115.00 |
| 6 th | 142.50 | 142.50 | 142.50 |
| 7 th | 170.00 | 170.00 | 170.00 |
| 8 th | 190.00 | 192.50 | 197.50 |
| | 190.00 ontrol group meal, EGM1: experimental group | | |





week between the 3rd and the 2 other groups with a slight increase in LBW for the 3rd group).

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The results on LBW of the experimental groups where the effect of Salvia officinalis in free-antioxidant basic ration is studied are summarized in Figure 2. The results showed that there is no significant difference (P>0.05) between the 3 groups during the first 6 weeks. Meanwhile, there was a difference (P<0.05) that appeared at the end of the 7th week between the 3rd and the 2 other groups with a slight increase in LBW for the 3rd group (34.8 ± 3.15 kg vs 30.36 ± 1.6 and 30.90 ± 2.64 kg in groups CGM or control) and CGM1 or 1% sage), respectively. This difference (P<0.05) continued to show up in the 8th week between the 1st (31.46 ± 1.29 kg), 2nd (32.30 ± 2.16) and 3rd (35.80 ± 3.15 kg) groups attaining higher LBW levels than the control (CGM). These results showed that introducing Salvia officinalis to the ration has increase the LBW of sheep slightly more than the control group with no significant difference between the 3 groups these results agreed with the other experiment done before. Another study was conducted by LenuNa and Leonte (2015) to determine the effect of sage essential oil vs antibiotics. They reported a best average body weight at broilers fed 8% sage (E3 group), which at the age of 42 days had an average weight of 2919 g, with the best daily live weight of 69, 50 g. Compared to the key group, the other groups treated with various levels of sage oil had daily average intakes higher with 1% (E1), 2% (E2) and 8% (E3) (P<0.05). The results obtained by LenuÑa and Leonte (2015) agrees with the study conducted by Windisch et al. (2009) revealing that feed additives derived from plants, also called phytogenics or phytobiotics or botanicals, can be included in animals' diets to improve their productivity including live body weight. This is also in support with the results obtained by Kolacz et al. (1997) that showed a significant improvement of body weight (BW) due to the main constituents of the herbs; the supplementation of 2% chamomile flowers dry meals plays a role to enhance the activity of thyroxin hormone that accelerates the nutrients metabolites and biochemical reaction in the animal body (Zhian, 2013).

The weekly variation in the average LBWG, kg/head is shown in Figure 3, so that at the end of 1st week group, diet supplemented with 3% of *Salvia officinalis* in the basic ration (EGM3) has the least (P>0.05) weekly LBWG (1 ± 0.7 kg) in comparison with 1st (1.1 ± 0.65 kg) and 2nd (1.2 ± 0.44 kg) groups. Whereas, at weeks 6th and 7th, EGM3 attained higher (P<0.05) LBWG levels (2 ± 0.93 kg) among all groups where it decreased later (P>0.05) in the last week (1 ± 0 kg) of the trial. The group (EGM1) with 1% of *Salvia officinalis* had a fixed rate of weekly LBWG, whereas control group (CGM) and 3rd group (EGM3) followed an irregular weekly rate of LBWG showing a low LBWG at 3rd week, followed by high LBWG at 4th and 7th weeks (Figure 3).

The weekly cumulative live body weight (wcLBWG) increased equally in the different groups but slightly more in EGM3 after the 5th week to attain 10.7 Kg at 8th week (P>0.05). This suggests that the use of *Salvia officinalis* in the diets of ruminants may modulate ruminal fermentation by reduction of methane production, thus potentially involving productive and environmental benefits; however, in vitro dry matter digestibility was decreased linearly. And we should notice that the temperature at the 8th week was more than 45 °C, and this might be the reason in the reduction of feed intake causing stress to animals. And as result a decrease in the BWG at this week to the 2 group with *Salvia officinalis* in the ration. Abd El-Maksoud et al. (2002) observed that the highest weight gain of Nile tilapia (*Oreochromis niloticus*) fingerlings was obtained when fed with 3% marjoram leaves of the total diet. This also resulted in the best protein and

energy utilizations apart from having a significant effect on body composition. On the contrary, results obtained by Abd El-Maksoud et al. (2002) demonstrated that Nile tilapia (*Oreochromis niloticus*) fingerlings fed diets contained 0.5-1% of chamomile flowers, Nigel seed or marjoram leaves alone showed lower performance than the control group. In my study the results showed that the LBWG is lower in the 2 groups (IGM1-IGM3) with S. *officanalis* have lower BWG than the control group (CGM) which agree with results of Abd El-Maksoud et al. (2002).

Table 2 showed that there was no significant difference (P>0.05) among animals all in the Weekly-cumulative FCR of the three experimental groups during all the period of the experiment. As it is shown from Table 2 that FCR of EGM3 (5.9 ± 3.56) at the end of the 1st week was insignificantly (P>0.05) higher than the other 2 groups (CGM = 4.89 ± 2.79 and EGM1 = 3.61 ± 0.96). Nevertheless, this indicator attained the lowest level and most effective conversion of feeds into body gain attaining 3.83 ± 0.97 in animal group EGM3 Vs 4.14 ± 0.53 and 4.15 ± 0.64 in groups CGM and EGM1, respectively. Similarly, Agarwal et al. (2009) demonstrated that plant extracts may decrease the digestibility of feeds because of the inhibition of cellulolytic bacteria metabolism. Moreover, Patra et al. (2006) highlighted that the inhibitory effects of plant extracts on rumen cellulolytic bacteria may cause a decrease in feed digestibility. On the other hand, the supplement increased the total bacteria population even by 23.4%, which can be explained by ecological succession. Inhibition of the protozoa population probably induced a new ecological niche for bacteria.

| Diet Time | BR + commercial antioxidants + antibiotics + vitamin premix + mineral premix | 1% S. officinalis to BR + vitamin premix + mineral premix | 3% S. officinalis to BR + vitamin premix + mineral premix |
|--------------------|--|---|---|
| At the end of week | CGM | EGM1 | EGM3 |
| 1 st | 4.89±2.79 | 3.61±0.96 | 5.90±3.56 |
| 2nd | 5.22±2.45 | 4.06±1.40 | 4.97±2.78 |
| 3rd | 6.44±2.55 | 4.69±1.98 | 3.65±1.52 |
| 4th | 4.06±0.80 | 4.04±1.00 | 4.19±1.75 |
| 5 th | 4.28±0.81 | 4.28±1.33 | 4.29±1.48 |
| 5 th | 4.49±0.53 | 4.34±1.13 | 3.90±1.13 |
| 7th | 4.24±0.65 | 4.37±0.89 | 3.67±1.02 |
| 8 th | 4.14±0.53 | 4.15±0.64 | 3.83±0.97 |

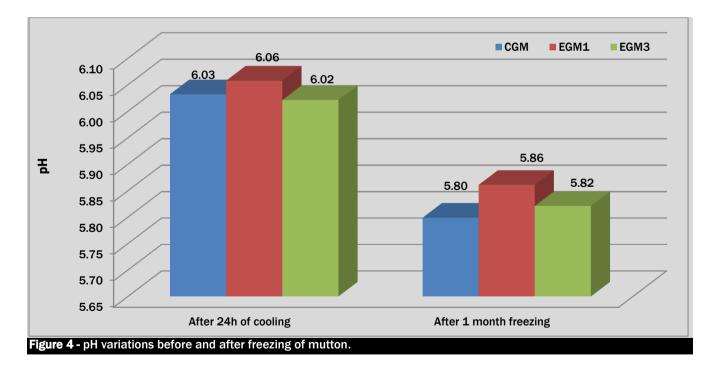


Figure 4 summarizes the variations in pH results. pH of meat is related to glycogen level in muscle tissues. The results of pH level where it calibrated insignificantly (P>0.05) after 24 hours of cooling at 5-7 °C between 6.03 ± 0.10 in group CGM and 6.02 ± 0.28 in group EGM3 and decreased after 1 month of freezing to calibrate insignificantly (P>0.05) between 5.8 ± 0.04 and 5.82 ± 0.07 in CGM and EGM3, respectively. More basic insignificant values (P>0.05) were obtained in EGM1 group before (6.06 ± 0.21) and after freezing (5.86 ± 0.10) whose animals were fed a supplementation

of 1 % Salvia officinalis to the basic ration of sheep. Figure 4 shows that pH of meat at 24h of cooling after slaughter is more basic than the pH at 1month of freezing where after this duration of time pH of meat becomes more acidic and this due to maturation of meat where with maturation the meat becomes more acidic. Moreover, the effect of sage EO *in vivo* could be significantly different than that reported *in vitro*. This difference could be related to the pH in the media where the oil is supposed to exert its effects. For example, the pH of milk varies between 6.4 and 6.6, but in the case of an infection, it increases to pH of 7.4 (Ziv, 1980). For best results, lower pH such (5-6) is preferred for sage EO (Gutierrez et al., 2008).

Color of meat is affected by the interaction of myoglobin pigment in meat with the absorbance and reflectance of light (AMSA, 2012). Figure 5 showed the obtained results (P>0.05) of *L* after slaughter (cooling and freezing). Luminance (L*) of meat reveals the reflection of water on the surface of meat samples. After 24 h of cooling Luminance *L* ranged insignificantly (P>0.05) between 45.41 ± 2.97 in CGM and 47.28 ± 5.63 in group EGM1 whereas in group EGM3 it was 47.28 ± 5.63 , lower than control. This tendency was lowered after 1 month of freezing in CGM (42.75 ± 2.34) and EGM1 (42.94 ± 2.57) and higher in EGM3 (42.16 ± 0.67). The polyphenols contained in *Salvia officinalis* are likely to be oxidized to corresponding quinines by polyphenol oxidases, which are widespread in plant materials. Such quinines may condense to form darkened which results in an intense color of meat (Liu et al., 2009).

Figure 6 demonstrates the variation of the results after cooling and freezing of different groups. The redness depends on the type of fiber in muscle and the presence of myoglobulin in tissue where iron is abundant. There is high difference in redness in the results obtained before and after freezing among all groups (P>0.05). The lowest level of *a* before after cooling was seen in EGM3 (20.15 ± 3.29) followed by CGM (22.61 ± 3.41) and EGM1 (24.97 ± 1.24). This sequence was continued for after freezing for groups CGM (21.83 ± 6.22) and EGM1 (22.43 ± 3.03) but not for EGM3 where this indicator was higher (22.99 ± 4.27) during this physical state. It was noted that meat color a* was negatively correlated (P<0.05) with thawing loss (%) after 1 month of freezing (Figure 6). Addition of natural antioxidants may improve oxidative stability of meat what can be the reason for smaller changes in meat color (Hanczakowska et al., 2015).

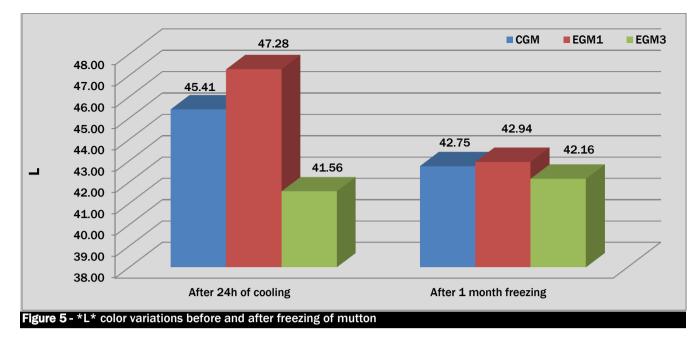
Yellowness refers mainly to the intramuscular fat tissues. Figure 7 shows the level of *b* during the whole trial. The results showed that yellowness increased before and after freezing and the difference is statistically insignificant (P>0.05). The highest effect was shown in EGM1 (14.03 \pm 4.88) followed by CGM (11.90 \pm 4.47), whereas the lowest was attained in EGM3 (7.84 \pm 1.38). The same tendency was followed by the same manner during the state of after freezing but on lower levels (15.03 \pm 2.04, 14.72 \pm 1.82 and 13.63 \pm 2.00), respectively. The results obtained showed that b* after 1 month of freezing was positively correlated (P<0.05) with the achieved results after 24 h of cooling (Figure 7). According to the mentioned results groups was characterized as samples with insignificant color changes and the observer perceived a clear similar color. It means that observer can perceive one color only in all simple. This obtained result agrees with the study conducted by Hanczakowska et al. (2015) revealing that addition of natural antioxidants may improve oxidative stability of meat what can be the reason for smaller changes in meat color.

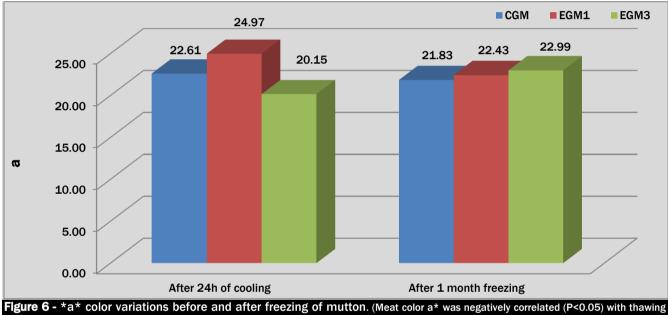
Drip loss is the leakage of microfibers along with increased loss of water, nutrients and proteins from meat. The drip and thawing losses are due to the breakdown of the cell membrane and the diffusion of water outside the cell. Figure 8 illustrates the variation in drip and thawing losses among the three groups (P>0.05). Figure 8 showed the least losses in water after cooling in EGM3 followed by CGM and EGM3 attaining the levels of 11.39 \pm 2.39%, 15.97 \pm 10.73 and 11.39 \pm 2.39%, respectively. Consequently, the loss in the thawing water after freezing was lower than the obtained drip loss but in a reversed order whereas the lower was in EGM1, followed by CGM and EGM3 (2.22 \pm 0.28%, 2.54 \pm 0.26 and 3.57 \pm 1.68%), respectively. These results are insignificantly different between groups after freezing and either after cooling, the drip loss is high after cooling or thawing loss is relatively low after freezing.

Cooking loss is the amount of water lost due to the cooking process. The result of cooking loss in Figure 9 shows that the cooking loss after freezing was lower in all groups if to compare to the obtained results attained after cooling P>0.05). It is worth to note that meat of CGM loses more water after cooking than EGM3 and EGM1 ($30.30 \pm 6.52\%$, 29.40 ± 2.06 and $27.79 \pm 1.02\%$), respectively. The cooking loss after freezing resulted in the same sequence where the lowest was attained in EGM1 ($25.81 \pm 1.58\%$). This decrease in weight or the high cooking loss was very natural due to the decrease of water holding capacity by the effect of proteins' denaturation.

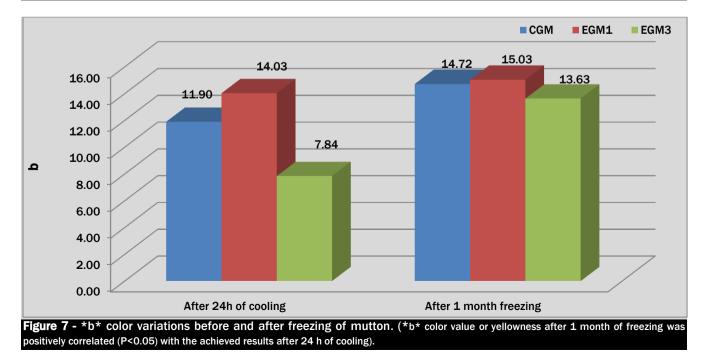
Tenderness level depends on the level of maturation of muscle. Figure 10 shows the variation in penetration levels after using Penetrometer (P>0.05). Results showed that after 24 h of cooling the most tender meat after cooking was obtained from animal group EGM1 (4.87 \pm 0.44mm) fed 1% S. officinalis with the basic ration in comparison to the animals of control group CGM (3.3 \pm 0.64mm) fed no S. officinalis, whereas EGM3 occupied the 1st place in cooked meat tenderness after 1 month of freezing (5.4 \pm 0.8mm) followed by both CGM and EGM1 (4 \pm 0.63mm).

It was noted that meat tenderness was negatively correlated (P<0.01) with b* results after 24 h of cooling and 1 month of freezing (Figure 10).

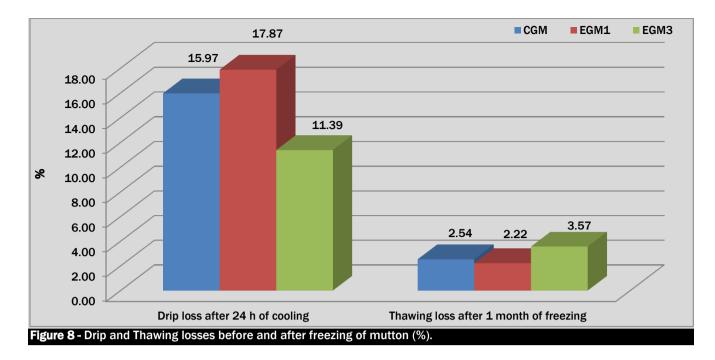


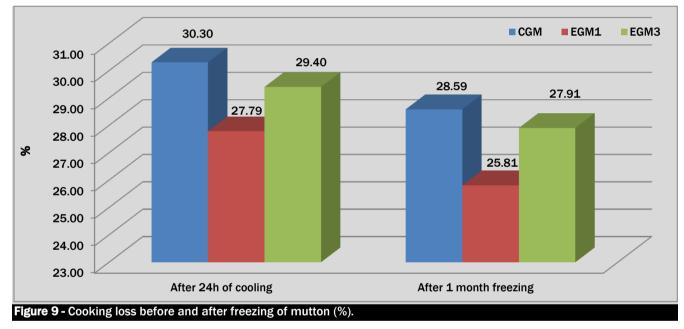


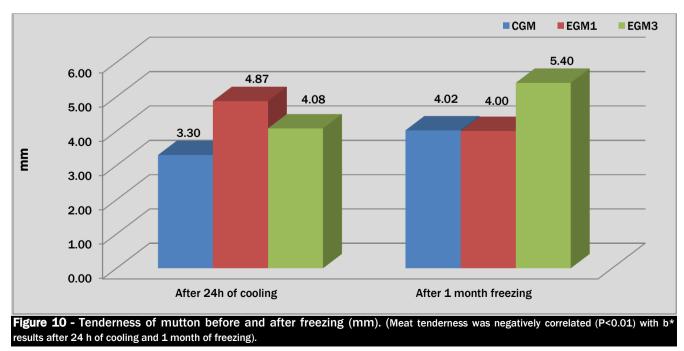
loss (%) after 1 month of freezing).



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CONCLUSION AND RECOMMENDATIONS

Mutton (Awassi), a common meat source for human consumption on the Lebanese market, is characterized by lesser fat that is concentrated in the fat tail, making it a healthier meat option for the Lebanese customer who prefers it above all other products of animal origin. In practical comparisons comparing the effects of Sage replacer to antioxidant in feed with varied percentages of 1 and 3 percent, there are few data from both local and global practical applications. Sage may have positive effects on Awassi sheep through stimulation of the immune system, stimulation of feed intake and digestive secretions, anti-bacterial, antiviral, or anti-inflammatory action, and antioxidant qualities when added in small doses to basic rations. In order to feed their herds rations laced with sage and other natural herbs as growth promoters without using synthetic antioxidants, Lebanese farmers must adopt a new way. To ascertain the true economic impact of utilizing sage on body performance and meat quality, we advise carrying out this practice in a commercial herd. Additionally, Awassi male sheep fed basic rations with sage supplements of varying concentrations (percent) should be used in future studies to determine the optimal concentration that improves animal performance. As a result of our findings, we advise using sage as a basic ration addition in Awassi sheep feeding in a proportion of 1% S. officinalis.

DECLARATIONS

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Conflict of interests

The author did not declare any conflict of interest.

Data availability statement

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

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