UNIVERSITY OF FORESTRY – SOFIA
FACULTY OF VETERINARY MEDICINE
Department "Anatomy, Physiology and Animal Sciences"

GEORGES HANNA AL HANNA

IMPACT OF DIFFERENT HERBS ON BODY PERFORMANCE AND MEAT QUALITY IN AWASSI MALE LAMBS

ABSTRACT

for awarding of educational and scientific degree "Doctor"

Professional field: 6.3.Livestock breeding
Scientific specialty: "Breeding of farm animals, biology and biotechnology of reproduction"

Scientific supervisors: Prof. Dr. Zapryanka Nikoolaeva Shindarska
Prof. Dr. Boulos Aljammal

Reviewers:

Prof. Krum Vladimirov Nedelkov, DVM, PhD
Prof. Teodora Lyudmilova Popova, PhD

Sofia, 2024

The dissertation contains 113 pages of text, 12 tables and 35 figures. The sources used include 252 titles.

The dissertation is divided into six sections:

I. Introduction
II. Aim and tasks
III. Materials and methods
IV. Results and discussion
V. Work Contributions
VI. Conclusion and recommendations

The defense of the dissertation will be held at 11.30 a.m. on June 14, 2024, in the „Acad. Mako Dakov" hall of the University of Forestry - Sofia, 10 Kliment Ohridski Blvd at an open meeting of the Scientific Jury composed of:

Chairman: Assoc. Prof. Georgi Ivanov Georgiev, DVM, PhD

Members: Prof. Krum Vladimirov Nedelkov, DVM, PhD
Prof. Eng. Teodora Lyudmilova Popova, PhD
Prof Maya Mitkova Ignatova, PhD
Assoc. Prof. Kalin Yordanov Hristov, DVM, PhD

The materials on the defense (dissertation, abstract, reviews and opinions) are available to those interested on the website of the University of Forestry (www.ltu.bg) and in the dean's office of FELA - Sofia, Blvd. Kliment Ohridski №10.

I. INTRODUCTION

Most animal breeders rely on theoretical information regarding the composition of feedstuffs specifically regarding amino acids and energy content (FAO, 2000). Major challenges in such agricultural businesses are to ensure an enhanced net yield and to minimize high expenditure on feed. Many recent research data has been done regarding this aspect, and various strategies have been studied in introducing new feed supplements and feed additives. An additive that has been addressed severe criticism is antibiotics. There are some important reasons behind the restriction of the use of antibiotics, some of which are the drug resistance established in bacteria and the drug residues found in meat. On the other hand, elimination of antibiotics from the diet has resulted in poor performance and in an increase in the susceptibility to diseases. Attempts were made to find other alternatives to overcome such challenges. One of which has shown immense interest in recent years is the utilization of growth promoters of natural origin like aromatic plants.

Odoemelam *et al.* (2013) observed that there are strong indications that herbs, spices and their products exert antioxidative, antimicrobial and growth promoting effects in livestock. The antioxidative effects of some of the herbs and spices in protecting the quality of feed as well as that of food derived from animals fed these substances cannot be ruled out. For antimicrobial actions, observations in vivo support the assumption that they possess the potential to contribute to the final reduction of intestinal pathogen pressure.

Herbs and spices, in other words, aromatic plants, have been used since ancient times as folk medicine and as preservatives in foods. The best-known aromatic plants, such as oregano, rosemary, chamomile, sage, anise, basil, etc., originate from the Mediterranean area. They contain many biologically active compounds, mainly polyphenolics, which have been found to possess antimicrobial, antioxidant, antiparasitic, antiprotozoal, antifungal, and anti-inflammatory properties. Currently, the demand for these plants and their derivatives has increased due to the fact that they are natural, eco-friendly and generally recognized as safe products. Therefore, aromatic plants and their extracts have the potential to become new generation substances for human and animal nutrition and health (Christaki *et al.*, 2012).

Lee *et al.* (2004ab), Ciftici *et al.* (2005), Erats *et al.* (2005) and Zhang *et al.* (2005) have reported that in the new century the use of antibiotic growth promoters as a feed additive has been banned. Such antibiotics have been added to animal feed rations for improving growth performance, disease prevention and for proliferation of useful microorganisms in the intestinal microflora. After banning the use of most feed additives antibiotic growth promoters by the European Union, because of their secondary effects like bio resistance and the reminiscence of trace antibiotics in tissues, scientists looked for alternatives to antibiotics. Therefore, they focused on herbs, spices and plant extracts as possible antibiotic alternatives. Traditionally, essential oils extracted from plants have been used in the therapy of some diseases, and they had an important role in maintaining human health. The effect of essential oils is widely known in human and animal use. Their addition to the feed ration or

water improved the feed intake, feed conversion ratio, and carcass yield action as well as improved utilization of digestive products by enhancing liver functions.

From these aromatic plants, we worked with the *Salvia officinalis* (garden sage, common sage), Chamomile or *Matricaria chamomilla* and the thyme or *Thymus vulgaris*. Beneficial effects of herbal active substances in animal nutrition may include the stimulation of appetite and feed intake, the improvement of endogenous digestive enzyme secretion, activation of immune response and antibacterial, antiviral, antioxidant and anthelminthic actions (Al-Kassie, 2010).

The growing interest of consumers in substances of natural origin in addition to the increasing concern surrounding potentially harmful synthetic additives has resulted in the use of aromatic plants, their extracts and essential oils, as functional ingredients in the pharmaceutical, food and feed industries. Such industries are currently looking for efficacious, safe and cost-effective substances with clearly defined modes of action and proven benefits. Plant derived components have considerable potential to fulfill such demands. Although there is still a lack of knowledge, especially regarding the consistency of in vivo trial results and mechanisms of action of various components within the aromatic plants, they could be applied as new generation compounds for human and animal health and nutrition. It is also important to take into consideration that improved animal health can translate to improved food safety and quality, which benefits the consumer (Sacchetti *et al.*, 2005).

II. AIM AND TASKS

The general aim of this study is to investigate the potential of local natural herbs (Chamomile, sage and thyme) as growth promoters of Awassi male lambs targeting the substitution of antioxidants and antibiotics conventionally used in the Lebanese lambs industry. Therefore, to evluate the impact of tested natural herbs on Awassi breed physiology and growth and to evaluate the impact of tested natural herbs on sheep meat production and quality. The aim was achieved through conducting a first trial where 4 groups of Awassi sheep are fed by chamomile as supplement to the basal diets to evluate their effects on health status, body performance and meat quality, conducting a second trial where 3 groups of Awassi sheep are fed by sage as supplement to the basal diets to evluate their effects on health status, body performance and meat quality, conducting a third trial where 4 groups of Awassi sheep are fed by thyme as supplement to the basal diets to evluate their effects on health status, body performance and meat quality.

III. MATERIALS AND METHODS

1. Experimental site: Animals, Housing and Diets

The following methodology was used to conduct the study. A total of forty-seven Awassi Sheep, 3 months old, were purchased from Beqaa' valley (Lebanon) and were divided into three groups: group A form 20 sheep, group B from 15 sheep and group C from 12 sheep for this experiment. Wood shavings were used as bedding materials. The site was composed of an opened barn with windows and curtains to study the impact of feeding male sheep different supplementation levels on body

performance during the growth period exclusive of antibiotics and antioxidants as basal ration, mixed with chamomile flowers dry meal for group A and with dried leaves of *Salvia Officinalis* for the second group B and with thyme dry meal for the third group C.

The farm was equipped with drinkers, feed troughs, automatic ventilation and lighting systems. Ambient temperature was kept at 25 °C. Water and feed for the duration proceeding the experimental period were offered *ad libitum*.

On arrival to the farm, the lambs were dipped in antiparasitic water solution and weighed individually where live body weight (LBW) at the beginning of the experiment was 23.37±2.5 kg (P>0.05). The ears were tagged, the lambs have been shorn and they were vaccinated against FMD disease and Toxemia, and they were given Antithelmic doses in water against worms and other internal parasites. All animals were fed the basal diet for 5 days and with Chamomile (group A), Sage (group B) and Thyme (group C) supplementation for another 5 days after allotting them randomly by 4 sub-groups of 5 heads each within the group A and to 3 sub-groups of 5 heads each within the group B and to 4 sub-groups of 3 heads each within the group C.

Basic Ration (BR) in mashed form based on yellow corn-soybean meal mixture containing neither antibiotics nor antioxidants was fed to all experimental groups of lambs with an exception that all rations of control groups of A (CG1), B (CGM) and C (CGTH) were supplemented with Premix- Vital Kondo (15 g daily/head), Antioxidant- Ethoxyquin (150 ppm= 0.015% of ration) and Antibiotic-Chlortetracycline (100 mg/head/day) as recommended by FDA and National Research council.

Animals of all other experimental groups were fed the basic ration (BR) exclusive of any antibiotics and antioxidants but supplemented with different levels of herbs. Feeding of all experimental groups ended after 8 weeks. Animals were fed twice daily, in the morning and in the evening. Experimental lambs of group A were fed the following: (Table 1):

- Control group (CG 1): BR + Premix + antioxidant + antibiotic.
- Experimental group (EG 2): BR with 2% Chamomile flowers dry meal.
- Experimental group (EG 4): BR with 4% Chamomile flowers dry meal.
- Experimental group (EG 6): BR with 6% Chamomile flowers dry meal.

For group B (Table 2):

- CGM (control group) was fed the basic ration (BR) + Premix + antioxidant + antibiotic.
- EGM1 - 1 % *Salvia officinalis* dry meal added to BR.
- EGM3 - 3 % *Salvia officinalis* dry meal added to BR.

For group C, Animals of the Control group (CGTH) were fed BR+ Premix + antioxidant + antibiotic. The remaining 3 groups were fed the following experimental rations (Table 3):

- Experimental group (EGTH 2): BR with 2% dry Thyme meal.
- Experimental group (EGTH 4): BR with 4% dry Thyme meal.

- Experimental group (EGTH 6): BR with 6% dry Thyme meal.

All rations were supplemented with NaCl and Dicalcium Phosphate. Lambs had an opened access to free consumption of wheat straw and clean water. The amount of concentrate-mix fed levels was adjusted on weekly basis on lambs live body weight (LBW). Chemical compositions of rations were calculated (Table 4) in reference to the National Research Council (NRC, 1985), where it shows that all experimental rations fed to animals of the three groups A,B, and C as seen in the table 4 were iso-proteinic and isoenergetic not exceeding 18.4% of crude protein and 5.05 NEg MJ/kg of feeds in reference to the National Research Council (NRC, 1985), and the difference was in the amount of different herbs that were used in this experiment.

Table 1. Ration formation for group A

Ingredients	Cost price	BR (Control)	BR + 2% Chamomile	BR + 4% Chamomile	BR + 6% Chamomile
		CG1	EG2	EG4	EG6
	LBP/1 kg	Kg			
Chamomile	3000	0	20	40	60
Barley (ground)	360	400	380	360	340
yellow corn (ground)	380	540	540	540	540
Soybean meal	700	360	360	360	360
Wheat bran	340	320	320	320	320
Cotton seed meal	580	240	240	240	240
NaCl	250	40	40	40	40
Dicalcium Phosphate	1500	40	40	40	40
Wheat straw	600	60	60	60	60
Mixing cost	4000				
Total, kg		2000	2000	2000	2000
Cost price, $/ton (1$=1507 LBP aaprox.)		320	337	355	373

Table 2. Ration formation for group B

Ingredients	Cost price	BR (Control)	BR + 1% Sage dry meal	BR + 3 % Sage dry meal
		CGM	EGM1	EGM3
	LBP/1 kg	Kg		
Chamomile	2000	0	20	60
Barley (ground)	360	400	380	370
yellow corn (ground)	380	540	540	540
Soybean meal	700	360	360	360
Wheat bran	340	320	320	320
Cotton seed meal	580	240	240	240
NaCl	250	40	40	40
Dicalcium Phosphate	1500	40	40	40
Wheat straw	600	60	60	60
Mixing cost	4000			
Total, Kg		2000	2000	2000
Cost price, $/ton (1$=1507 LBP prox..)		319.73	325.20	336.13

Table 3. Ration formation for group C

Ingredients	Cost price	BR (Control)	BR + 2% Thyme	BR + 4% Thyme	BR + 6% Thyme
		CGTH	EGTH 2	EGTH 4	EGTH 6
	LBP/1 kg	kg			
Thyme	4000	0	20	40	60
Barley (ground)	360	400	180	160	140
yellow corn (ground)	380	540	270	270	270
Soybean meal	700	360	180	180	180
Wheat bran	340	320	160	160	160
Cotton seed meal	580	240	120	120	120
NaCl	250	40	20	20	20
Dicalcium Phosphate	1500	40	20	20	20
Wheat straw	600	60	30	30	30
Mixing Cost	4000				
Total, kg		1000	1000	1000	1000
Cost price, $/ton (1$=1507 LBP approx..)		319.07	366.93	415.47	464.00

2. Health and mortality

Daily observations for the lambs showed that no health problems were noted. All lambs were in good health. No signs of indigestion or diarrhea or any blood signs in manure were observed.

3. Data Collection

3.1. Live body weight measurements

Individual LBW of the lambs was measured at weekly basis and at slaughter using electronic balance (kg). Average live body weight at the initiation of the experiment (aLBW) of the sheep was 26 ± 1.5 Kg.

3.2. Feed intake (FI), carcass weight and meat sampling

The quantity of concentrate residues left behind and not eaten by the lambs were collected from each pen daily and weighed and subtracted from the given quantity to calculate feed intake (FI). Lambs apparent feed intake (aFI/head), live body weight gain (LBWG/head) and apparent feed conversion ratio (aFCR/head) were calculated weekly. At the end of the experiment (after 8 weeks) 3 heads from each group were sacrificed by vein incision till complete bleeding and then skinned and eviscerated to calculate carcass weight. Meat cuts of around 100 g of loin eyes (at 9^{th} -12^{th} ribs) were taken at slaughter and kept in refrigerator at 3-7 ˚C and chilled for 24 hours. Physical analysis of meat was studied. Parameters studied were before and after freezing and cooking: Luminosity (L), redness (a), yellowness (b), pH and tenderness. Water thawing capacity and cooking loss capacity were also studied.

3.3 Live body weight gain (LBWG) and feed conversion ratio (FCR)

Weekly and cumulative live body weight gain (LBWG) and FCR were calculated at the end of each feeding period, all animals were individually weighed.

4. Meat analysis

4.1. Physical properties

For further investigation on physical analyses, ribs sections of mutton between 9^{th} and 12^{th} rib location (Fig. 5) of the left half (Loin eye or HH section) were collected (about 2 x 100 g) from each animal. after skinning and eviscerating and packing after immediate weighing by 100g in 2 polyethylene sheets. One of the two sheets was stored in refrigerators for 24 hours of cooling at 4°C - 5°C while the other polyethylene sheet was stored below -27ºC to freeze for 7 days. The meat physical properties were studied at the animal production laboratory of the faculty of agriculture and veterinary medicine of the Lebanese University.

4.1.1. Meat color (L*, a* and b*) after cooling and freezing

At Fresh slaughter and at 24 hours of cooling post mortem, and after 1 month of deep freezing, meat color was determined using a chromameter (ADCI-60- C instrument). This instrument was set to measure using the CIElab color space (Commission Internationale de l'Eclairage or International Commission on Illumination) system values of luminance (L*), redness (a*), and yellowness (b*) using illuminate D.

All measurements (3 repetitions/sample) were carried out on the surface of meat, where the area was free of obvious color defects (bruises, blood spots, and hemorrhages).

4.1.2. PH

At Fresh slaughter and at 24 hours of cooling post mortem, and after 1 month of deep freezing, the PH was measured with a portable pH meter (Hanna Instruments HI 931,000). Measurement of each sample was repeated 3 times for average accuracy by a direct insertion of a combined electrode in the meat and the mean value was calculated. The electrode was rinsed with distilled water after each use.

4.1.3. Drip loss

Drip loss was determined after 24 hours of cooling by the method of offer and Knight (1988). Meat is dried with paper towel and reweighed and calculated as percentage of initial weight.

4.1.4. Cooking loss

Cooking loss was examined according to Honikel (1998). Meat samples were packed in polyethylene bags and cooked in a water bath at 80 - 82°C for 15 minutes and then were cooled for 45 minutes at room temperature. After they were removed from bags, dried with filter paper and weighed. Cooking loss was expressed as the percentage loss relative to the weight immediately before cooking.

4.1.5. Thawing loss

Samples of meat were packed in polyethylene bags and frozen for 1 month to determine thawing and cooking losses. After 30 days they were removed from the fridge and kept for thawing slowly in a refrigerator at 4 °C. After 12 hours thawing,

meat were taken from bags, dried with filter paper and reweighed. Thawing loss was expressed as a percentage of the frozen weight. And the same samples were used for cooking.

4.2. Meat texture

After cooking meat, hardness (tenderness) was estimated using a penetrometer (interface RS232C) with a needle of 2.5 g based on a weight of 47.5 g, thus attaining a total weight of 50 g. The penetration was carried out on cooked meat slices (3cm x 2cm x 1cm) prepared such that the longest dimension was parallel to the fiber axis. The slice was placed on a horizontal support and the force of the needle was applied perpendicularly to the muscle fibers for 5 seconds (El Rammouz *et al.*, 2013). The penetrometer needle depth (PND, mm) was recorded and calculated at an average of 3 replications by sample.

5. Economical effect

Cost prices of meat obtained from the 3 experimental groups where Chamomile, Sage and Thyme were added were calculated and compared to that received from control group fed commercial basal diet.

6. Statistical analysis

One way analysis of variance (one way ANOVA) of the results obtained were calculated to evaluate the statistical differences between treatments and replicate means using "Sigmastat software V. 3.5". Significant effects were further explored using Tukey's significant difference test to assert the interaction among treatment means. The results are presented as Mean and standard deviation (X +/- SD). A significance level of $P < 0.05$ was used. Pearson Correlation was used to study the positive and negative independence of the results of different variables among all experimental groups with different confidence levels as $P<0.001$***, $P<0.01$** and $P<0.05$*.

IV. RESULTS AND DISCUSSION

1. Feeding and Feed intake (FI)

Rations constructed for feeding the lambs were given according to the levels of nutritive values suggested in the publications issued by the NRC (1985). We noted that the calculated differences related to the metabolic energy (2671.7±40.1 MEkcal/kg of feeds) were insignificant ($P=0.071$).

The results obtained for overall feed intake (FI) at the end of the experiment were significantly different ($P<0.05$) in group A attaining its maximal level (112.8 ± 12.9 kg/ group) in control group (CG1) and its minimal (89.9 ±9.13 kg/group) in EG6 whose animals were fed basic ration supplemented with 6% chamomile. Note that, palatability of rations and appetite of animals were positively accepted in all animal groups where no feed rejection was noticed (figure 1).

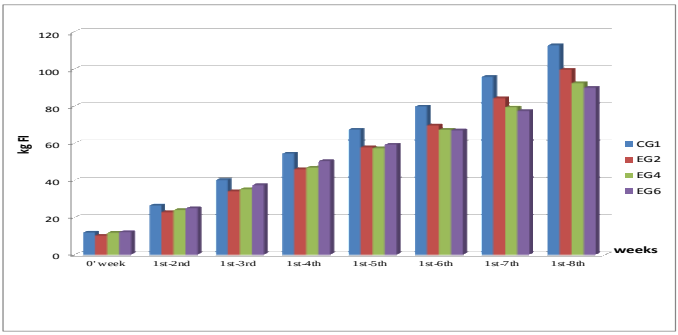

Figure 1. Estimated cumulative average of Feed intake among lambs of the experimental group A, kg/group

Apparent cumulative feed consumption in group A at the end of the experiment were lower by 20.3%, 18.1% and 12.6% in groups EG6, EG4 and EG2, respectively as shown in Fig.1. This can be explained by the fact that decreased feed consumption has attributed to higher flavoring effects of 4% and 6% chamomile flower meal supplementation to the basic ration decreasing the palatability of feed and minimizing the feed intake.

These results were in opposite to those findings obtained by Kamel (2001) who applied essential oils of 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 (Alçiçek *et al.,* 2003) having anti-inflammatory, antiseptic, diaphoretic and sedative properties (Panda, 2005) by killing and inhibiting the harmful intestinal Microorganisms in the intestinal tract of the animals (Kolacz *et al.*,1997).

Concerning group B, figure 2 shows the estimated cumulative feed intake (ecFI, kg/head) by the lambs of all sub-groups during the eight weeks of the experiment.

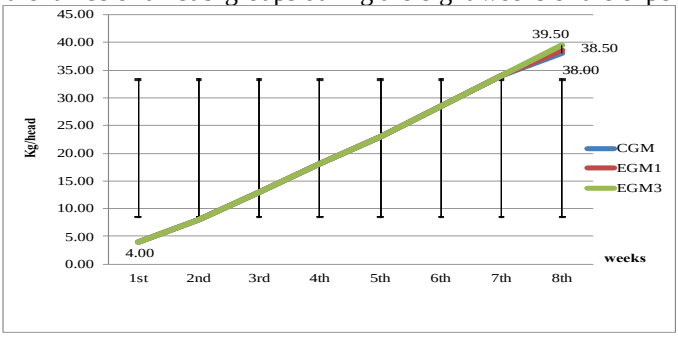

Figure 2. Estimation of cumulative Feed Intake among experimental male lambs of group B, kg/head

Figure 2 and table 4 show an equal intake of feeds distributed on the experimental animals. The results obtained demonstrate that the overall feed intake at the end of the 8th week of the experiment increased proportionally with the increase of LBW, attaining a small increase (P>0.05) in group EGM3 (197.5 kg/group) by 3.9 % and EGM1 (192.5 kg/head) by 1.3 % in comparison with the control group (CGM) where it consumed the amount of 190 kg/group.

The observation during the experiment showed that the lambs finish taking the ration containing sage faster than the control group, this can be due to the beneficial effects of the sage in the animal nutrition, and which may include the stimulation of appetite and feed intake.

Table 4. The overall Feed Intake (FI) among experimental lambs of group B, kg/group

	BR	**BR+ 1% *Salvia officinalis***	**BR+ 3% *Salvia officinalis***
At the end of week	**Basic ration (CGM)**	**EGM1**	**EGM3**
1st	**20.00**	**20.00**	**20.00**
2nd	**40.00**	**40.00**	**40.00**
3rd	**65.00**	**65.00**	**65.00**
4th	**90.00**	**90.00**	**90.00**
5th	**115.00**	**115.00**	**115.00**
6th	**142.50**	**142.50**	**142.50**
7th	**170.00**	**170.00**	**170.00**
8th	**190.00**	**192.50**	**197.50**

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.

Results obtained in figure 3 for group C at the end of the trial (after 8 weeks) show statistically an insignificant difference between lambs at week 0 with the highest for EGTH6 5.07 kg, and the lowest EGTH4 4.21 kg, this value increases in all sub-groups to reach after 8 weeks the highest level ~100 kg in EGTH4 and lowest value was for CGTH, ~90 kg (P>0.05).

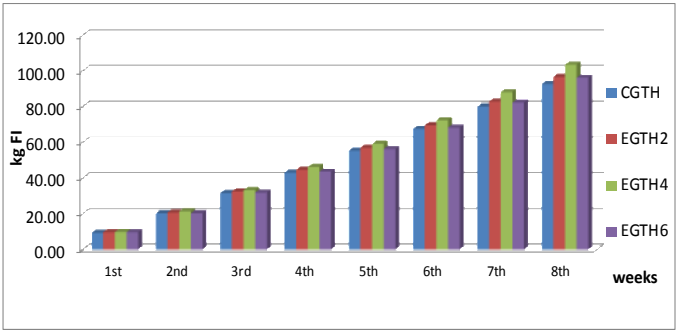

Figure 3. Cumulative Feed intake among lambs of the experimental group C, kg/group

These results were in opposite to those findings obtained by Wiley & Sons (2009) who applied essential oils of 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 thyme (AL-Kassie, 2009) having anti-inflammatory, antiseptic, diaphoretic and sedative properties (Nieto, 2016) by killing and inhibiting the harmful intestinal Microorganisms in the intestinal tract of the animals.

2. Live body weight (LBW, kg)

The average live body weight of the experimental lambs of group A at the age of 3 month (0 Week of experiment) was calibrated insignificantly (P=0.938) between 26.4±1.13 kg, 26.1±0.67, 25.72±2.1 and 26.2±2.2 kg in CG1, EG2, EG4 and EG6, respectively (Table 5).

Table 5 shows the variations in weekly LBW among the experimental animal groups. The highest LBW was recorded in group EG2 (42.5±0.89 kg) after 8 weeks of feeding basal diet, with no antibiotics nor antioxidants, and with 2% chamomile supplementation followed by control group CG1 (41.2±1.62 kg), EG6 (40.2±2.43 Kg) fed 6% chamomile supplemented to the basic ration and EG4 (39.42±2.99 kg) with 4% supplementation. This is explained by the positive correlation (0.928***) of LBW with FI since animals of CG1 and EG2 consumed more and hence there LBW was higher.

These results match with the ones obtained by Al-Kassie (2010) and 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 play a role to enhance the activity of thyroxin hormone that accelerates the nutrients metabolites and biochemical reaction in the animal body (Mahmmod, 2013).

Table 5. Weekly variations in live body weights among animals of group A (LBW/week), kg

At the end of each week	BR	BR + 2% Chamomile flower meal	BR + 4% Chamomile flower meal	BR + 6% Chamomile flower meal
Groups	CG1	EG2	EG4	EG6
0' week	26.42 ± 1.13	26.08 ± 0.66	25.73 ± 2.07	26.25 ± 2.16
1st	27.35 ± 1.26	26.58 ± 0.66	26.0 ± 2.06	27.01 ± 2.4
2nd	29.48 ± 1.36	28.37 ± 0.77	27.77 ± 2.38	28.77 ± 2.60
3rd	31.03 ± 1.51	29.68 ± 0.51	29.23 ± 2.81	30.83 ± 2.51
4th	33.15 ± 1.58	32.67 ± 0.83	32.28 ± 2.49	32.73 ± 2.54
5th	35.15 ± 1.59	34.99 ± 0.64	34.05 ± 2.28	34.78 ± 2.51
6th	37.25 ± 1.08	37.17 ± 1.01	36.68 ± 1.95	37.13 ± 2.70
7th	39.58 ± 1.28	39.09 ± 0.74	38.35 ± 1.94	38.48 ± 1.96
8th	41.25 ± 1.62	42.58 ± 0.90	39.43 ± 2.99	40.28 ± 2.44
Results in rows of each week were statistically insignificant among experimental groups (P>0.05)				

The results on LBW of the experimental group B where the effect of Salvia officinalis in free-antioxidant basic ration is studied are summarized in figure 4.

The results in figure 4 show that there is no significant difference (P>0.05) between the 3 sub-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 (control) and EGM1 (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) sub-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, this result was agreeing with the other experiment done before.

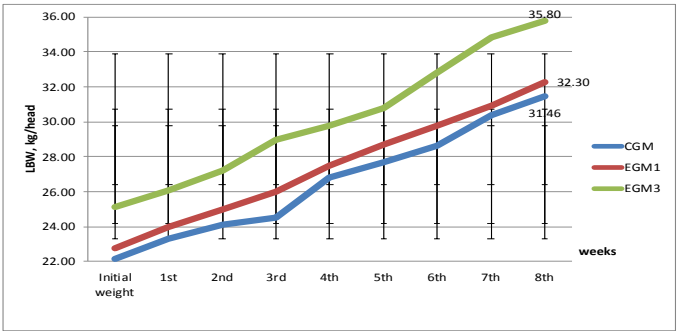

Figure 4. Live body weight among experimental groups of Males, kg/head

This obtained result 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.

Table 6. Weekly variations in live body weights among experimental groups (LBW/week), kg

At the end of each week	BR	BR + 2% Thyme meal	BR + 4% Thyme meal	BR + 6% Thyme meal
Groups	CGTH	EGTH2	EGTH4	EGTH6
0' week	13.00 ± 2.00a	13.67 ± 0.58a	11.67 ± 1.16a	14.33 ± 0.58a
1st	13.93 ± 2.16a	15.03 ± 0.29a	13.13 ± 1.23a	15.77 ± 0.40a
2nd	16.00 ± 2.00a	17.00 ± 1.00a	16.00 ± 1.00a	17.33 ± 0.58a
3rd	18.33 ± 2.08a	19.33 ± 1.53a	19.00 ± 1.00a	19.67 ± 0.58a
4th	20.33 ± 1.53a	22.00 ± 1.73a	22.67 ± 1.53a	21.67 ± 0.58a
5th	23.00 ± 1.73a	24.00 ± 2.65a	25.00 ± 1.00a	24.33 ± 0.58a
6th	25.00 ± 1.73a	25.67 ± 2.08a	25.50 ± 1.00a	24.83 ± 0.58a
7th	26.67 ± 1.16a	27.67 ± 2.08a	30.00 ± 1.00a	28.00 ± 1.00a
8th	27.67 ± 1.16a	29.33 ± 1.53ab	32.00 ± 1.00c	30.00 ± 1.00cb
abc Mean values with different letters between columns in the same row are significantly different (P<0.05)				

Table 6 shows the variations in weekly LBW among the experimental animal groups. Live body weight LBW increases in all group from week 0 to week 8. At week 0 the highest LBW is 14.33±0.58kg in EG6 and the lowest one is 11.67±1.16 kg in EG4 compare to week 8 with highest LBW to EG4 32±1.00 kg and the lowest one in CG1 27.67 ± 1.155 kg. This is in support with the results obtained by AL-Kassie *et al.* (2008) and Frankie (2009), and Christaki (2012), that showed a significant improvement of body weight (BW) due to the main constituents of the herbs; the supplementation of 2% thyme dry meals plays a role to enhance the activity of thyroxin hormone that accelerates the nutrients metabolites and biochemical reaction in the animal body (Mahmmmod, 2013).

3. Live body weight gain (LBWG)

Figure 5 shows the weekly variation in the average LBWG, kg/head in group A. Significant differences (P<0.05) were obtained only among animal groups at the end 1st and 8th weeks of the experiment. Although better LBWG was observed in CG (1.02±0.18 kg/week/head) at the end of the 1st week of the experiment (P<0.05), this image was1 reversed at the end of the 8th week where LBWG was significantly (P<0.05) the best in group EG2 (3.42±0.48 kg/week/head). Note that no significant differences were observed in LBWG between EG6 (1.74±0.68 kg/week/head) and CG1 (1.73±1.09 kg/week/head). The minimal level was attained in group EG4 at a level of 1.38±1.21 kg/week/head). The recorded results of the experiment agree with the data presented by Spernakova *et al.* (2007), who had demonstrated that the addition of Chamomile developed higher body weight gain compared to control group whose animals were fed basic diets with no herb addition due to the active health stimulating elements found in the herbs that show their positive effect on performance.

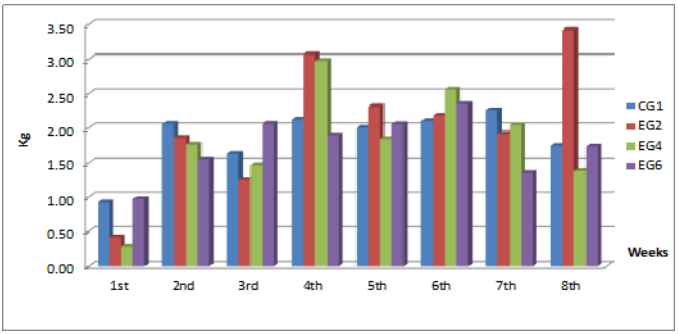

Figure 5. Weekly average variations in live body weight gain among experimental groups (LBWG/week/head), kg

In group B, the weekly variation in the average LBWG, kg/head is shown in figure 6. The figure shows that at the end of 1st week group EGM3 supplemented with 3% of *salvia officinalis* in the basic ration 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 this group (3rd) 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 ± 0.00 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.

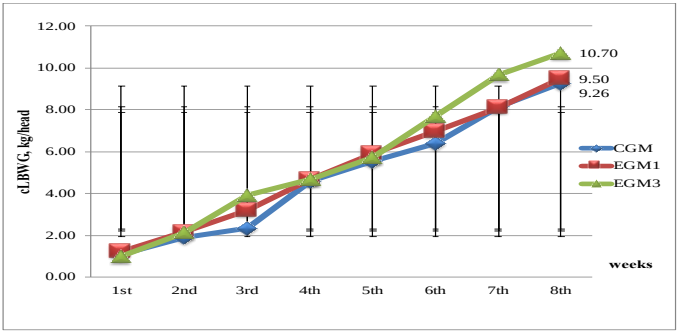

Figure 6. Weekly-cumulative Live body weight gain variations among experimental groups of Male lambs, kg/head

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 this study the results showed that the LBWG is lower in the two sub-groups of group B (CGM1-CGM3) with Salvia officinalis having lower BWG than the control group (CGM) which agree with results of Abd El-Maksoud *et al.* (2002).

Table 7 shows the weekly variation in the average LBWG, kg/head of the group where the lambs were supplemented with different levels of thyme. The recorded results of the trial show the live body weight gain LBWG change largely between group in the different week of the experiment and the LBWG is highest in EGTH4 in many stages of the experiment.

Table 7. Daily average variations in live body weight gain among experimental groups (LBWG/day/head), kg

Groups	CGTH	EGTH2	EGTH4	EGTH6
1st	0.15±0.0256a	0.06±0.037bc	0.04±0.0275c	0.11±0.0644ab
2nd	0.29±0.08	0.27±0.08	0.25±0.12	0.25±0.1
3rd	0.23±0.04	0.18±0.1	0.21±0.11	0.29±0.08
4th	0.3±0.14	0.44±0.12	0.43±0.2	0.27±0.06
5th	0.29±0.09	0.33±0.13	0.26±0.05	0.29±0.18
6th	0.3±0.11	0.31±0.06	0.37±0.12	0.34±0.22
7th	0.32±0.1	0.27±0.18	0.29±0.22	0.19±0.15
8th	0.25±0.0972b	0.49±0.0681a	0.2±0.174b	0.25±0.157b
	abc Mean values with different letters between columns in the same row are significantly different (P0.05)			

4. Feed conversion ratio (FCR)

Figure 7 shows the cumulative weekly average data of feed conversion efficiency (FCR). It is worthy to mention that FCR at the beginning of the trial was extremely high in all groups (P>0.05). The highest was in EG2 (16.71±3.25) whose animals were fed with 2% chamomile supplemented to daily ration followed by CG1 (13.48±2.37) fed basic ration, EG6 (13.44±3.37) basic rations supplemented with 6 % chamomile and EG4 (13.33±5.16) with 4% Chamomile flower dry meal.

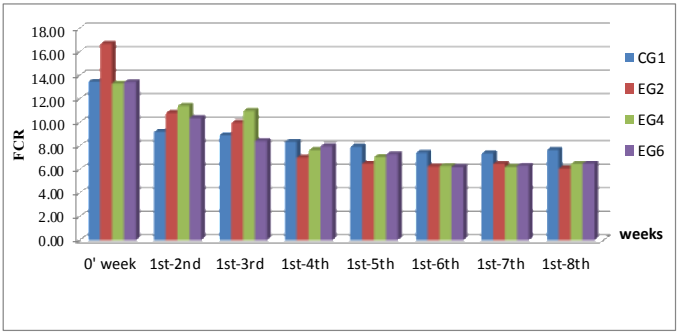

Figure 7. Cumulative average of weekly variations in Feed conversion ratio among experimental animals of group A (FCR/week/head)

This can be explained by the fact that animals in all groups needed more time to accommodate with the new management conditions including feeding dry rations supplemented with chamomile flower dry meal in different proportions which prevented them from effectively converting food into product. Nevertheless, lambs had 10 days duration before the setting of the experiment to acclimatize with the new conditions of keeping and feeding. Beginning from week 2 we recorded an enhancement in the FCR by getting more weight gains in all groups after being accommodated to the new management conditions by converting effectively what was consumed. At the end of the trial animals of EG2 group had the most effective (P<0.05) FCR level (6.09±0.40) in comparison with all other groups- CG1 (7.69±1.11), EG4 (6.50±0.46) and EG6 (6.52±0.84).

Feed conversion was improved significantly (P<0.05) with the addition of chamomile dry flower meal to the basic ration. The obtained results are identical with the findings of Santurio *et al.* (2007) who concluded that when chamomile flowers are added at a level of 1kg/100kg of the diet the body weight gain and feed conversion ratio increased due to the positive impact of the biochemical active components of the herb in the form of antimicrobial, antifungal and antioxidant defense property against harmful body microorganisms. It was noted that FCR is in negative correlation with LBWG (r=-0.575***) and FI (r=-0.28***).

In group B, table 8 shows that there is no significant difference (P>0.05) among lambs in the weekly-cumulative FCR of the three experimental sub-groups during all the period of the experiment.

As it is shown from table 8 that FCR of the 3rd group- 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.

Table 8. Weekly-cumulative FCR variations among experimental lambs of group B

	BR	BR + 1% *Salvia officinalis*	BR + 3% *Salvia officinalis*
At the end of week	CGM	EGM1	EGM3
1st	4.89 ± 2.79	3.61 ± 0.96	5.9 ± 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
5th	4.28 ± 0.81	4.28 ± 1.33	4.29 ± 1.48
6th	4.49 ± 0.53	4.34 ± 1.13	3.9 ± 1.13
7th	4.24 ± 0.65	4.37 ± 0.89	3.67 ± 1.02
8th	4.14 ± 0.53	4.15 ± 0.64	3.83 ± 0.97
All mean values of different columns in the same row are significantly different (P>0.05)			

Table 9 shows that there is no significant difference (P>0.05) of the weekly cost price variations between all the 3 sub-groups during the experiment except group EGM3 during the 4th week with a cost price of (3.55± 1.74$/1kg of mutton) which was significantly (P<0.05) higher compared to CGM and EGM1. This table demonstrates that the week-by-week cost price of 1kg of meat of group CGM where no antioxidants were added was 0.72 $/1kg of mutton during the 4th week of the trial, whereas the results of this indicator at the end of the 2nd month (8th week) is doubled (1.89 $/1kg of mutton).

Table 9. Weekly cost price variations among the experimental group B of Males, $/1 kg of mutton

	BR	BR + 1% *Salvia officinalis*	BR + 3% *Salvia officinalis*
At the end of week	CGM	EGM1	EGM3
1st	1.57 ± 0.89a	1.37 ± 0.36a	2.65 ± 1.60a
2nd	1.97 ± 0.86a	2.59 ± 1.72a	2.12 ± 1.25a
3rd	3.99 ± 1.15a	3.17 ± 1.60a	1.91 ± 1.91a
4th	0.72 ± 0.19a	1.75 ± 1.15a	3.55 ± 1.74b
5th	2.30 ± 1.15a	2.55 ± 1.49a	2.53 ± 1.06a
6th	2.36 ± 1.17a	1.96 ± 0.39a	1.45 ± 0.61a
7th	1.14 ± 0.57a	1.96 ± 0.41b	1.3 ± 0.35a
8th	1.89 ± 1.57a	1.34 ± 0.51a	2.48 ± 0.22a
ab Mean values of different columns in the same row with different letters are significantly different P<0.05			

As for group EGM1 the cost of 1kg of meat for the same periods of the experiment was higher than CGM (1.72 $/1kg of mutton) and lower than EGM3 (3.55 $/1kg of mutton). Note that the cost price of 1 kg of mutton for the 8th week of the experiment was lower and more economical than CGM and EGM3 attaining the levels of 1.89 and 2.48 $/1kg of mutton. The results also showed that during the 8th week of the experiment supplementing the basic ration with 3% *Salvia officinalis* was least efficient where the cost price of 1 kg of mutton was 2.48 $. This high cost for the group EGM2 can be accepted because the higher production of meat for this group even the quality of meat is better and with low rancidity. The control group has a low cost but low meat production and low meat quality. The group EGM1 have a moderate cost but also a good meat quality and meat production.

Figure 8 shows that there is no significant difference (P>0.05) of the overall cost price of 1 kg of meat among the different groups during all the period of the experiment.

As shown from the figure 8 that the overall cost-price of 1kg of meat at the end of the 1st month of the experiment higher in EGM3 group attaining the level of 1.88 $/1kg of meat in comparison with CGM and EGM1 reaching more economical levels, 1.28 and 4.54 $/1kg of meat respectively. The same tendency was seen also at the end of the 2nd month of the trial (8th week) whereas it attained in animal-groups EGM3, CGM and EGM1 the levels of 1.73, 1.30 and 1.58 $/1kg of meat respectively.

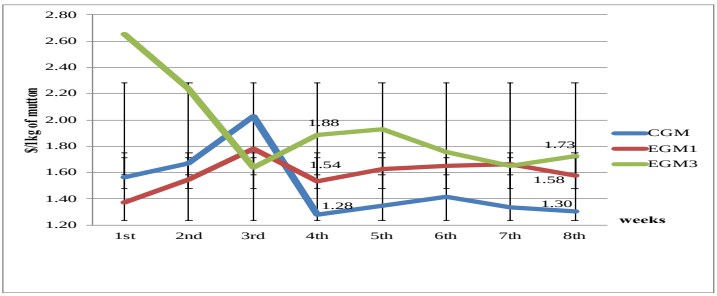

Figure 8. Overall cost price variations among experimental groups of Males, $/1 kg of mutton

Table 10 shows the weekly average data of feed conversion efficiency (FCR) of animals belonging to group C. The results can be explained by the fact that animals in all groups needed more time to accommodate with the new management conditions including feeding dry rations supplemented with thyme dry meal in different proportions which prevented them from effectively converting food into product. Nevertheless, lambs had 10 days' duration before the setting of the experiment to acclimatize with the new conditions of keeping and feeding.

Table 10. Weekly average variations in Feed conversion ratio among experimental groups (FCR/week/head)

Group	CGTH	EGTH2	EGTH4	EGTH6
1st	5.00±1.24	3.60±0.81	2.97±0.64	3.62±0.76
2nd	2.61±0.61	3.14±0.98	1.97±0.35	3.78±0.55
3rd	2.74±0.62	2.86±0.45	2.24±0.72	2.87±0.59
4th	3.91±2.18	2.68±0.57	1.98±0.22	3.40±0.09
5th	2.79±0.60	4.45±2.17	3.57±0.87	2.98±0.70
6th	3.93±0.272a	5.47±2.841a	16.63±0.446b	15.40±0.358b
7th	5.63±2.71	4.36±0.33	2.13±0.07	2.99±0.83
8th	8.85±0.37	6.33±3.09	5.15±0.16	4.97±0.17
abc Mean values with different letters between columns in the same row are significantly different (P<0.05)				

Cumulative data on Feed conversion ratio as shown in Table 11 was Feed conversion was improved significantly (P<0.05) with the addition of thyme dry flower meal to the basic ration.

Table 11. Weekly-average of cumulative variations in Feed conversion ratio among experimental groups (FCR/week/head)

Groups	CGTH	EGTHT2	EGTH4	EGTHT6
1st	5.00±1.24	3.60±0.81	2.97±0.64	3.62±0.76
2nd	3.27±0.452b	3.18±0.41b	2.28±0.35a	3.63±0.133b
3rd	3.02±0.489b	3.05±0.43b	2.20±0.271a	3.28±0.367b
4th	3.08±0.547b	2.91±0.416b	2.12±0.212a	3.31±0.268b
5th	2.99±0.48	3.13±0.61	2.34±0.19	3.18±0.08
6th	3.14±0.439ab	3.33±0.409ab	2.86±0.212a	3.77±0.095b
7th	3.38±0.51	3.47±0.33	2.68±0.17	3.56±0.33
8th	3.75±0.524a	3.68±0.24a	2.92±0.165b	3.73±0.283a
ab Mean values with different letters between columns in the same row are significantly different (P<0.05)				

The obtained results are identical with the findings of Smeed (2001) who concluded that when thyme flowers are added at a level of 3kg/100kg of the diet the body weight gain and feed conversion ratio increased due to the positive impact of the biochemical active components of the herb in the form of antimicrobial, antifungal and antioxidant defense property against harmful body microorganisms.

5. Physical properties of meat

5.1. Meat PH

The PH of the meat is related to glycogen level in the muscle tissues. The PH of group A is shown in figure 9 and have increased in CG1 meat from 5.57 to 5.69 after 24 hours of cooling and then attained the level of 5.79 after 1 month of freezing (P>0.05).

However, as shown in figure 9, PH of fresh meat at slaughter was significantly (P<0.05) more acidic (5.57±0.054) in CG1 and EG6 (5.59) than EG2 (5.75) and EG4 (5.67). After 1 month of freezing CG1 became less acidic (5.79) in comparison with EG2, EG4 and EG6 averaging the level of 5.72, 5.77 and 5.67, respectively (P>0.05).

The slow freezing process leads to development of crystals in muscle tissues. When defrosting, crystals tear the up cells, leading to a greater inter muscular distribution of salinity, thus a decrease in the acidity (P>0.05) was noticed in sub-groups CG1 (5.79±0.045), EG4 (5.77±0.08) and EG6 (5.67 ± 0.06) and only a slight decrease in salinity in EG2 (5.77±0.02).

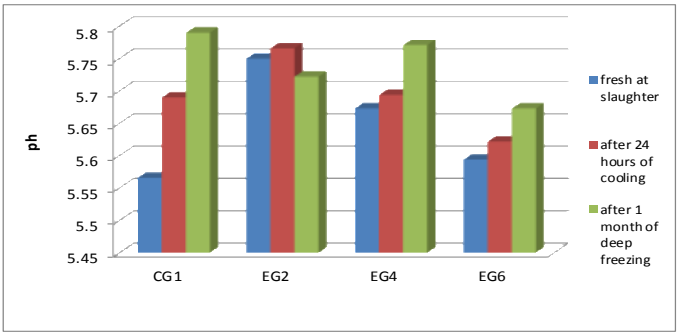

Figure 9. Average Variations in pH variable in Group A

Figure 10 summarizes the variations in PH results of group B, where it calibrated insignificantly (P>0.05) after 24 hours of cooling at 5-7 °C between 6.03 ± 0.10 in sub-group CGM and 6.02 ± 0.28 in sub-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.

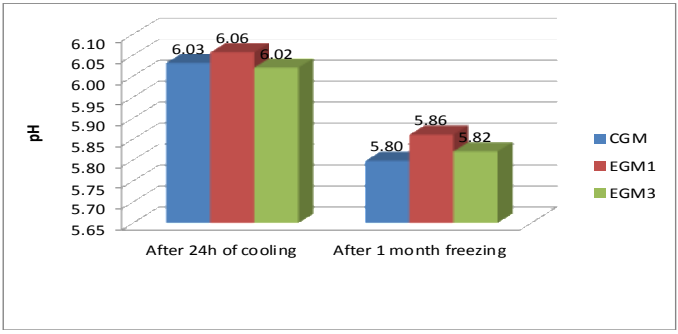

Figure 10. pH variations before and after freezing of mutton in Group B

More basic insignificant values (P>0.05) were obtained in the sub-group EGM1 before (6.06 ± 0.21) and after freezing (5.86 ± 0.10) in which lambs were fed a supplementation of 1 % *Salvia officinalis* to the basic ration of sheep. The results shown in figure 10 shows that the PH of the meat at 24h cooling after slaughter is more basic than the PH at 1month of freezing, whereas after this duration of time, the PH of meat became more acidic due to the maturation of meat. 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 better results, lower PH such (5-6) is preferred for sage EO (Gutierrez *et al.*, 2008).

As for the results of group C, pH of the control group after cooling (5.68±0.17) was lower than after freezing (5.78±0.03), unlike the experimental groups EGTH2, EGTH4, EGTH6 where pH after cooling (6.43±0.17, 5.78±0.17, 5.86±0.17) actually was higher than pH after freezing (5.73±0.03, 5.77±0.03, 5.70±0.03). pH for group thyme 2% 24h after cooling (6.43±0.18) was significantly (P<0.05) different than those in control group after 24h of cooling (5.68±0.18) and pH for the group 6% thyme after 1 month of freezing (5.71±0.03).

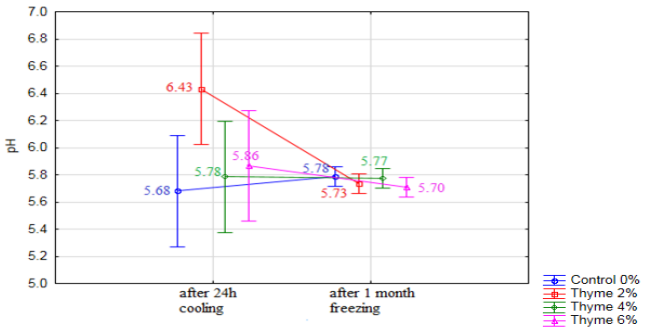

Figure 11. The variation of pH with the concentration of thyme and storage methods.

The fact that adding 2% thyme to the feed has significantly raised the meat pH after cooling (fig. 11). This means that thyme stopped the degradation process that leads to the accumulation of lactic acid in the meat. This could be due to the antioxidative action and improved stress response of the thyme and its essential oils (Jordán *et al.*, 2007; Nieto *et al.*, 2011a).

5.2 Color changes

Product's color is affected by the interaction of myoglobin pigment in meat with the absorbance and reflectance of light (AMSA, 2012).

5.2.1. Luminance L*

Luminance (L*) of meat reveals the reflection of water on the surface of samples. The figure 12 revealed the variation of L*, after 24 hours of cooling L* decreased in all of the sub-groups of group A. CG1 (43.667 ±2.68 to 42.5± 1.64), EG2 (54.08 ± 12.8 to 50.24 ±3.3) EG4 (45.73 ±4.53 to 43.29 ± 0.45), EG6 (40.3 ± 2.5 to 40.118 ± 0.9), (P<0.05) there is a significant difference between sub-groups, where the highest one is for EG2 followed by EG4, CG1, EG6.

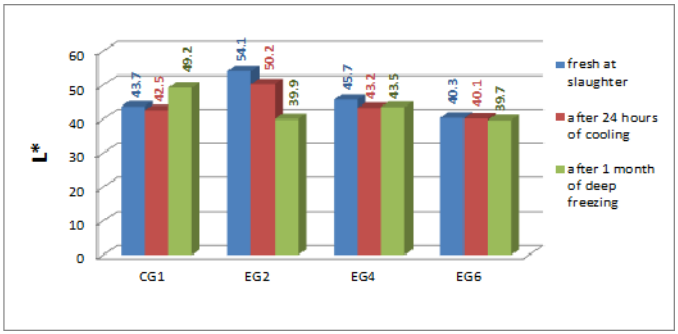

Figure 12. Average variations in L variable after slaughter

After one month of freezing, L* of mutton increased in CG1 (42.5 ± 1.64 to 49.19 ± 4.9) and EG4 (43.2 ± 0.45 to 43.5 ± 3.42), yet in contrast it decreased in EG2 (50.24 ±3.34 to 39.92 ± 1.07) and EG6 (40.118 ±0.9 to 39.65 ±1.64) but ($P<0.05$) and the higher is for CG1 (49.19 ± 4.9) followed by EG4 (43.5 ± 3.42) and EG2 (39.92 ± 1.07) ($P < 0.05$). Note that the L* is negatively correlated with tenderness ($r = -0.408$*) and positive correlated with PH ($r = 0.415$*).

Figure 13 shows the obtained results ($P>0.05$) of *L* after slaughter (cooling and freezing) in the group B.

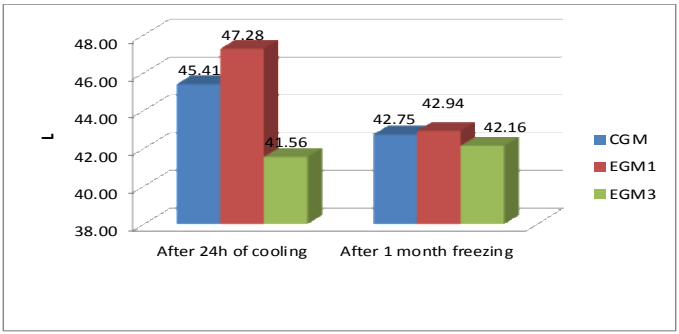

Figure 13. *L* color variations before and after freezing of mutton

The luminance decreases in all groups after freezing due to the loss of surface water. 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 EGM1 whereas in EGM3 it was 47.28 ± 5.63, lower than control. This tendency decreased after 1 month of freezing

in CGM (42.75 ± 2.34) and EGM1 (42.94 ± 2.57) and increased 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 darker colour which results in an intense colour of meat (Liu *et al.*, 2009).

Figure 14 shows the different variations in "L*" level of group C. After cooling EGTH2 (38.51±0.88) scored the lowest value, followed by CGTH (control group) by 39.65±0.88 and EGTH6 by 45.87±0.88. The highest level of "L*" was recorded in EGTH4 (46.45±0.88). After freezing, the order changed where this indicator dropped in CGTH to 33.68±2.67 followed by EGTH6 by 40.85±2.67. Moreover, EGTH2 attained the level of 41.87±2.67 surpassing EGTH6. The highest value was obtained in group T4 (44.22±2.67). Statistically, values obtained for "L*" of CGTH 1 month after freezing (33.68±2.68) was significantly different ($P<0.05$) than those obtained in EGTH6 (45.88±0.89) and EGTH4 (46.45±0.89) after 24h of cooling. This variation could be attributed to both the presence of thyme in the ration and the capacity of cooling to maintain a high "L*". These findings agree with the work of Nieto *et al.* (2010) that found thyme to delay color deterioration. This activity could be dose dependent. The small dose of 2% could be very small to produce any effect on color.

The study also found that (L*) after 24h of cooling was positively correlated ($P<0.05$) to live body weight gain (LBWG) (annex I). This correlation means that as LBWG increased so did (L*) after cooling since high LBWG leads to high body weight, then (L*) was higher with higher body weight. The obtained results did not agree with the findings of Tejeda *et al.* (2008) who stated that weight has no effect on meat color.

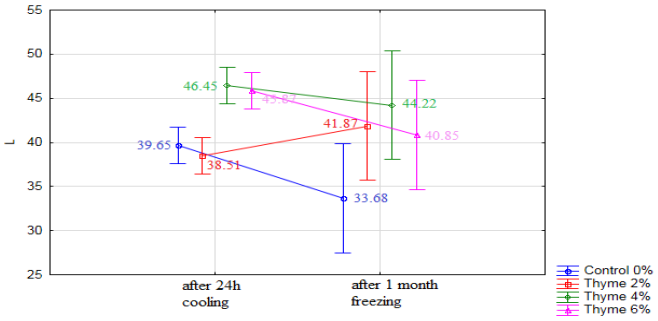

Figure 14. The variation of L* with different concentrations of thyme

5.2.2 Redness a*

Variation of redness is shown in figure 15. Redness of lambs' meat slaughtered decreased after 24h of cooling in all sub-groups of group A. CG1 (18.99 ± 4.53 to 15.78 ± 2.9), EG2 (21.6 ± 7.8 to 15.4 ± 1.14), EG4 (17 ± 2.7 to 14.01 ± 1.97), EG6 (20.2 ± 4.43 to 18.32 ± 2.16), ($P > 0.05$). And then after 1 month of freezing the redness continue decreasing in EG4 to (13.86) and EG6 to (14.95) in contrast a* value increase in CG1
to (17.5) and in EG2 to (19.36). The denaturation of myoglobin proteins due to the high temperature effect and the formation of metmyoglobin, the oxidized form of myoglobin, is the reason of this drop in redness a* of meat (Sen *et al.*, 2014).

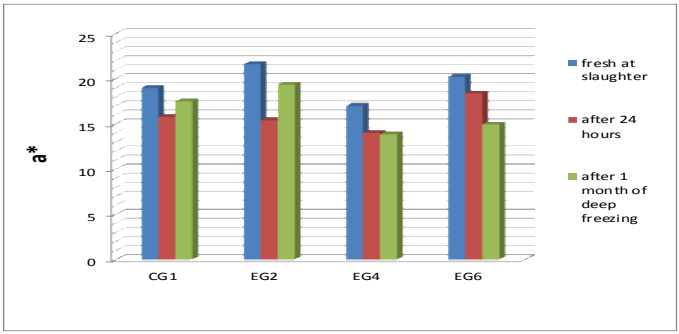

Figure 15. Average Variations in a* variable after slaughter

For the group B, the figure 16 above demonstrates the variation of the results after cooling and freezing of different sub-groups. The redness depends on the type of fibre in muscle and the presence of myoglobuline in tissue where iron is abundant.

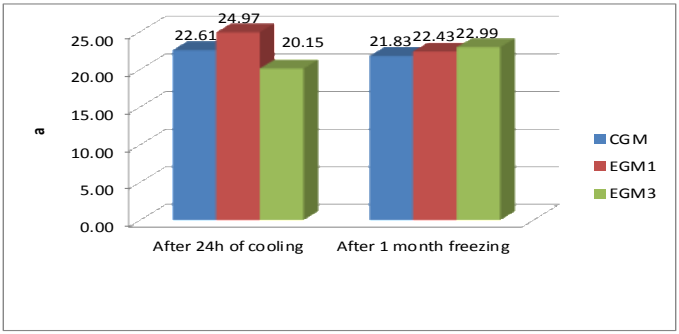

Figure 16. *a* color variations before and after freezing of mutton

There is high difference in redness in the results obtained before and after freezing among all sub-groups (P>0.05). The lowest level of *a* before and 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 till after freezing for sub-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* is negatively correlated (P<0.05) with thawing loss (%) after 1 month of freezing. The addition of natural antioxidants may improve oxidative stability of meat what can be the reason for smaller changes in meat color (Hanczakowskam *et al.*, 2015).

Level of "a*" after 24h of cooling (fig. 17) was lower (P>0.05) in animal groups where thyme was supplemented to BR with 4% (20.82±2.09), 6% (22.66±2.09) and 2% (27.19±2.09) vs. control group CGTH (32.70±2.09). However, after 1 month of freezing CGTH and EGTH2 attained lower levels (24.01±4.61and 19.42±4.61, respectively) and was higher (P>0.05) in EGTH4 and EGTH6 (25.28±4.61, 25.40±4.61, respectively). This is in conflict with the results obtained by Nieto *et al.* (2010) concluding that thyme increased meat color (delayed color deterioration) thus increasing "a*". This could be explained due to the fact that the meat was refrigerated and frozen. These conditions could have hidden any changes caused by thyme. Another explanation to this contradiction is that it is due to the quantity used, in this experiment, was not high enough to produce any effects.

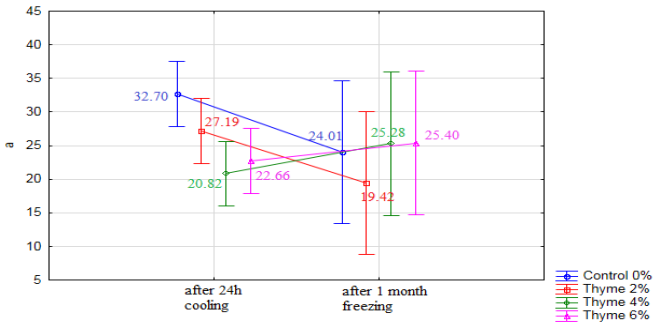

Figure 17. Variation of "a" with the concentration of thyme and storage methods.

Redness "a*" after 24h cooling was negatively correlated (P<0.05) with live body weight (LBW), weekly feed intake (wFI), cumulative feed conversion ratio (cFCR) and cumulative feed intake (cFI). This is in disagreement with what Santos-Silva *et al.* (2002) stated, that as slaughter weight increased so did redness "a*". These results suggest that as FI and FCR increase, naturally

LBW also increased and “a*” decreased. This could mean that as weight increased, iron concentration (meat content) decreased. This is reflected by a decrease in “a*” redness. This could be due to the composition of the feed, the breed (genotype and conformation) or a number of different parameters.

5.2.3. Yellowness b*

Yellowness refers mainly to the intramuscular fat tissues. Variation in yellowness b* decreased 24 hours after cooling in all sub-groups of group A, from (10.53 to 9.34) in CG1, (9.56 to 8.98) in EG2, (8.57 to 6.8) in EG4 and from (9.58 to 8.1) in EG6 without significant difference (P>0.05) as shown in figure 18.

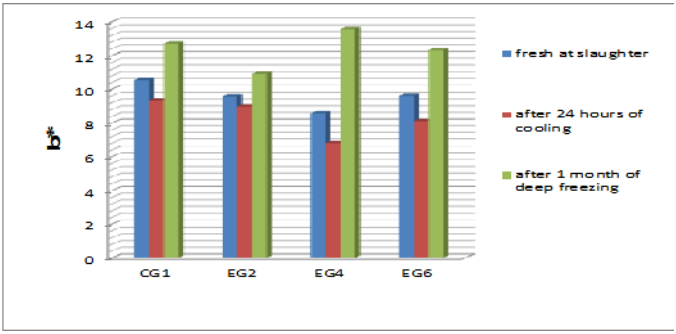

Figure 18. Average Variations in b variable after slaughter

After 1 month of freezing b* value increase in all sub-groups of group A respectively in EG4, CG1, EG6 and then EG2 (13.5, 12.68, 12.3, 10.9) with a significant difference with EG2. This result is logical because hardness of meat after 1 month of freezing, and then the animals of the EG2 have the more tender meat.

In group B, the results show that yellowness increased before and after freezing and the difference is statistically insignificant (P>0.05).

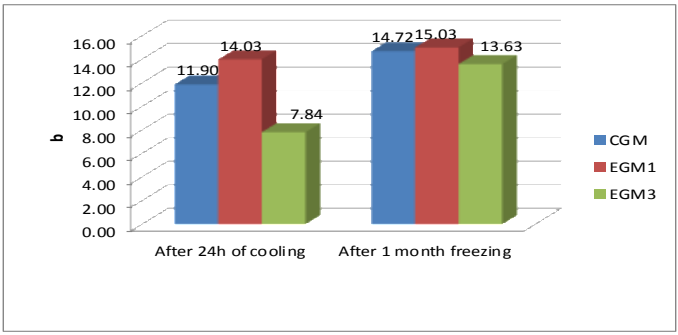

Figure 19. *b* color variations before and after freezing of mutton

Figure 19 shows the level of *b* during the whole experiment. According to the mentioned results groups was characterized as samples with insignificant colour changes and the observer perceived a clear similar colour. It means that observer can perceive one colour 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.

The highest effect was shown in EGM1 (14.03 ± 4.88) followed by CGM (11.90 ± 4.47), whereas the lowest was attained in EGM3 (7.84 ± 1.38). The same tendency was followed by the same manner during the state of after freezing but on lower levels, 15.03 ± 2.04, 14.72 ± 1.82 and 13.63 ± 2.00, respectively. The results obtained showed that b* after 1 month of freezing is positively correlated ($P<0.05$) with the achieved results after 24 h of cooling.

In the thyme trial "b*" was higher ($P>0.05$) after 24h cooling than after 1 month freezing. "b*" after cooling for the groups CGTH, EGTH2, EGTH4, EGTH6 attained 14.82±1.73, 12.82±1.73, 12.05±1.73, 16.47±1.73, respectively (figure 20). In comparison for after freezing "b*" attained lower values CGTH (11.12±1.11), EGTH2 (12.48±1.11), EGTH4 (11.36±1.11), EGTH6 (8.16±1.11). Therefore, thyme, at these concentrations, had no effect on "b*".

"b" after 24h cooling was negatively correlated ($P<0.05$) with LBW before slaughter which is in agreement with Santos-Silva *et al.* (2002), who found that as slaughter weight increased, yellowness "b*" decreased, which is expected since as LBW increases, so does muscle mass and thus fat would therefore decrease. It was also negatively correlated ($P<0.05$) with cFCR and cFI. It makes sense that as FCR increases so does FI. The increase of FCR means that more muscle (meat) is produced by the animal and therefore fat would decrease. The previous causes yellowness to decrease (yellow pigment, b*).

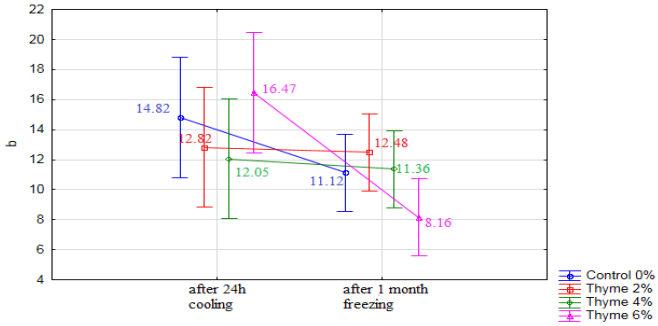

Figure 20. Variation of "b" with concentration of thyme and storage methods.

5.3. Water holding capacity

Changes in weight of meat samples are due to the surface evaporation because of differences in temperature and relative humidity between the environment and meat sample (Cano-Muñoz, 1991).

5.3.1 Drip loss & thawing loss

The water released by the meat when it is cooled in the refrigerator is expressed as drip loss. The drip and thawing losses is due to the breakdown of the cell membrane and the diffusion of water outside the cell.

After cooling in the refrigerator for 24 hours from slaughter, the mutton loss of their initial weight was shown in figure 21 for group A with a significant difference ($P<0.01$) where EG2 loss was (28.1% ± 2.79) followed by EG4 (27.64% ±0.76) and then CG1 (20.23% ±2.72). The minimal loss in water is showed in EG6 where the loss is (17.86 % ±1.47). After 1 month of thawing the loss increased in all subgroups respectively EG4 (38.12 %), EG2 (31.17%) and then CG1 (24.86 %) followed by EG6 (20.47 %), and the difference was highly significant ($P<0.01$), where the difference between the sub-groups EG4 and EG6 was clear. The drip loss is correlated with PH ($r = 0.729^{**}$) and negative correlated with the tenderness ($r = -0.632^{*}$), in another way the thawing loss is negatively correlated with the drip loss ($r = - 0.734^{**}$).

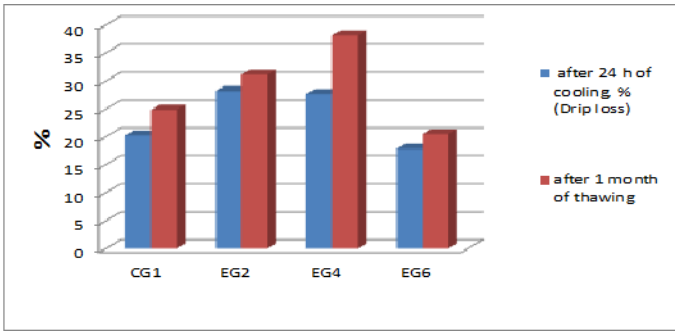

Figure 21. Water loss after cooling and freezing

What concerns the group B, Figure 22 illustrates the variation in drip and thawing losses among the three sub-groups (P>0.05).

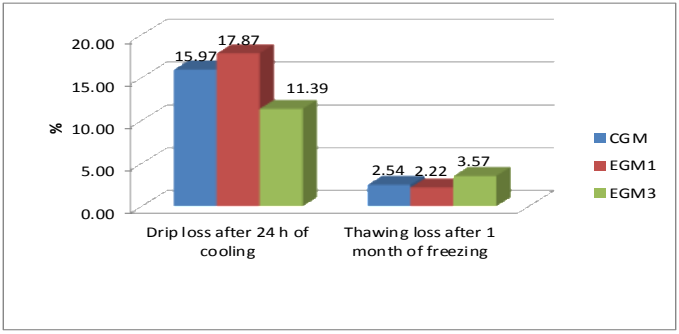

Figure 22. Drip and Thawing losses before and after freezing of mutton, %

The figure 22 shows EGM3 as the least in water loss after cooling, followed by CGM and EGM1 reaching the levels of 11.39 ± 2.39%, 15.97 ± 10.73 and 11.39 ± 2.39%, respectively. Consequently, the loss in the thawing water after freezing was lower than the obtained drip loss but in a reversed order where the lower was in EGM1, followed by CGM and EGM3, with a value of 2.22 ± 0.28%, 2.54 ± 0.26 and 3.57 ± 1.68%, respectively. These results are insignificantly different between the sub-groups after freezing and cooling. The drip loss is high after cooling or the thawing loss is relatively low after freezing.

In the thyme trial, high concentrations of thyme seemed to decrease drip loss. Drip loss attained levels of 6.09±3.29, 7.92±3.29, 5.31±3.29, and 1.98±3.29 for the groups CGTH, EGTH2, EGT4H, and EGTH6 respectively (figure 23).

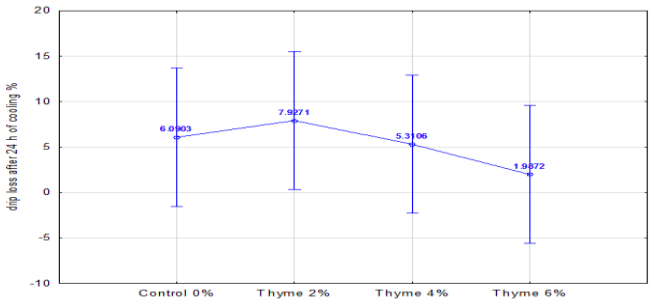

Figure 23. Variation of drip loss with thyme concentration

After statistical analysis, none of these variations was significant (P>0.05). Thyme did not affect drip loss.

In the thyme trial, thawing loss is represented in figure 24. Thawing loss seemed to decrease (P>0.05) with high levels of thyme attaining 0.95 ±0.16, 0.9±0.16 for EGTH4 and EGTH6 respectively. With low inclusion of thyme (EGTH2) in the rations, thawing loss attained higher level than the control group (CGTH) by 1.30±0.16 and 1.21±0.16 respectively. Thyme does not appear to affect thawing loss.

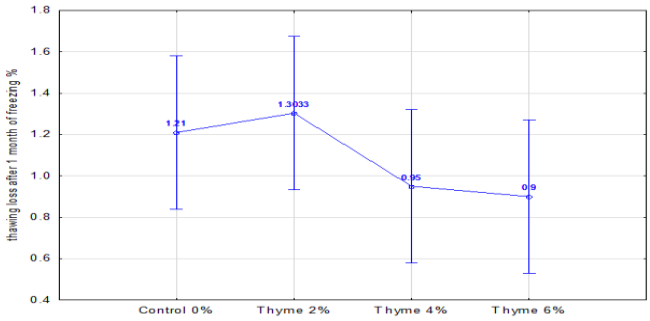

Figure 24. Variation of thawing loss with thyme level.

Thawing loss was positively correlated (P<0.05) with LBW, WFI, cFCR and cFI. Increase in FI leads to an increase in FCR consequently, leading to an increase in LBW. This causes a bigger volume of water to be present in the muscle. When this water quantity of water is lost. Thawing loss is negatively correlated (P<0.05) with "a" and "b" after 24h cooling.

5.3.2 Cooking loss

Cooking loss is the amount of water lost due to the cooking process. Cooking losses of cooled and freezed samples from group A are shown in figure 25. This figure shows that the cooking loss of frozen samples was higher (P > 0.05) than the loss of cooled samples (32.8% > 30.8% in EG4), (32.2 % > 23.1% in EG2), (31.9 % > 30.3% in CG1), and (28.4% > 22.4 % in EG6).

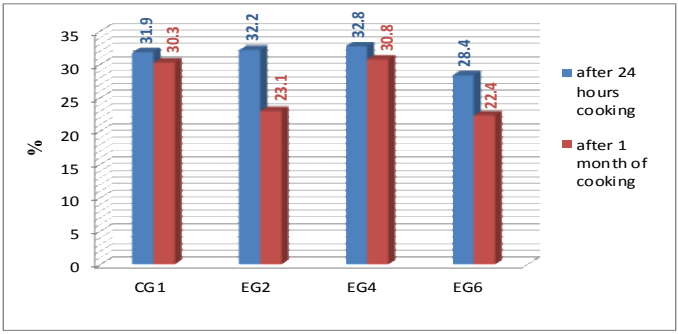

Figure 25. Water loss after cooking

Nevertheless, the loss of water in samples after cooking frozen mutton samples, were significant (figure 25) (P < 0.05) and it's clear between CG1 (higher loss) and EG6 (less loss). 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'. Cooking loss and LBWG are found to be negatively correlated (r = - 0.468*).

In group B, the result of cooking loss is shown in figure 26 where the cooking loss decreased in all sub-groups of group B after freezing comparing to the results obtained 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 ± 6.52%, 29.40 ± 2.06 and 27.79 ± 1.02%, respectively.

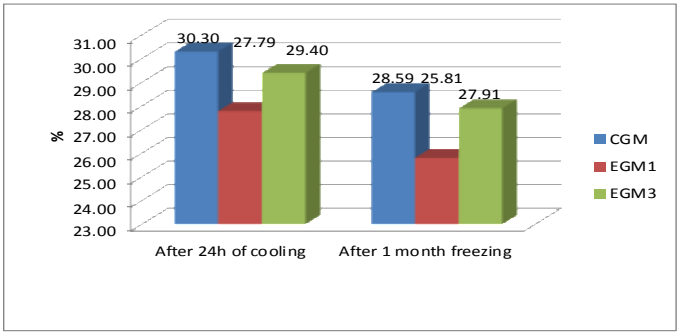

Figure 26. Cooking loss before and after freezing %

The cooking loss after freezing resulted in the same sequence where the lowest was in EGM1 (25.81 ± 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 as shown in group A as well.

Figure 27 shows that cooking loss in Thyme experiment after freezing was higher than it was after cooling. After freezing, cooking loss reached levels of 26.20±1.97 for T0, 20.85±1.97 for T2, 36.04±1.97 for T4, 31.77±1.97 for T6. Moreover, after cooling, cooking loss was lower for T0 (21.14±3.40), T2 (19.95±3.40), T4 (30.45±3.40), T6 (30.70±3.40).

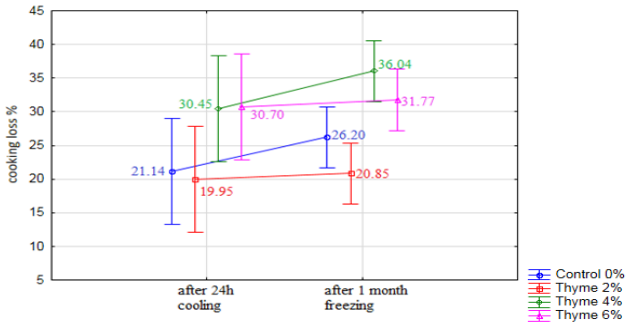

Figure 27. The variations of cooking loss with thyme concentration and method of storage.

Statistically, cooking loss after cooling for T2 (19.96±3.40) was significantly different ($P<0.05$) than cooking loss for T4 after 1 month of freezing (36.04±1.97). This change could be due to the fact that freezing and the formation

of large ice crystals leads to fiber damage. This fiber damage will be exacerbated by the heat treatment (cooking). This will lead to a bigger water loss while cooking. This could also be explained by the different concentrations of thyme that could have contributed to this variation in cooking loss or both.

Cooking loss after 24 h of cooling was positively correlated ($P<0.05$) with LBW and "L" after 24h cooling. Cooking loss after 24 h of cooling was negatively correlated ($P<0.05$) with "a" after 24h cooling. Water is a very important parameter in determining color, notably luminosity and redness. When water content is high, light is reflected more. This makes the meat appear glossy (high L*) and have a lighter color (lower a*).

5.4. Tenderness

Tenderness level depends on the level of maturation of muscle. Figure 28 shows the variation of PND values thus the tenderness of all cooked samples. In group A, the tenderness of cooked meat 24 hours after cooling was higher in EG6 (8.4mm±0.7) followed by EG2 (5.64 mm± 2.1) than CG1 (5.5mm ±0.4), and EG4 (5.2 mm ±0.9) ($P<0.05$). PND values of the meat 24 hours after cooling are significantly different between the sub-groups whereas for the values of EG6 was the highly significant.

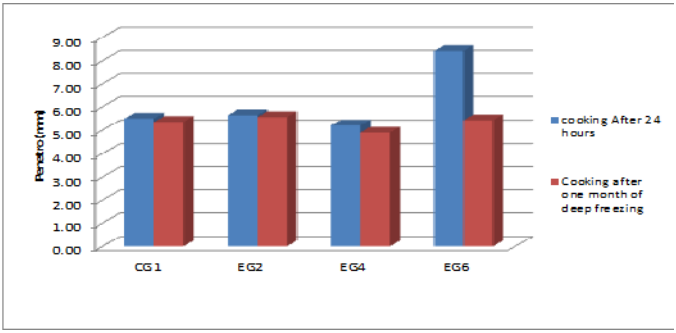

Figure 28. Average Variations in Penetro variable

One month after thawing and cooking, the meat became heavier in all samples with no significant difference ($P>0.05$), but it was more tender consecutively in the sub-group EG2 (5.56mm), EG6 (5.43mm), CG1(5.36mm) and then EG4 (4.889). The tenderness of the meat is negatively correlated with the results of the variable L in the colour ($r = -0.408$*). These results agree with those of Hergenreder (2011) stating that after freezing, cooked meat is tougher than the cooked meat after cooling and in contradiction with the results achieved by Koohmaraie *et al.* (1998)

stating that frozen samples cooked after thawing are tenderer than those cooked after cooling which matches with our results.

In group B, figure 29 shows the variation in penetration levels after using Penetrometer (P>0.05). The results show that after 24 h of cooling, the most tender meat obtained after cooking was obtained from sub-group EGM1 (4.87 ± 0.44mm) fed 1% *Salvia* with the basic ration in comparison to the meat of control group CGM (3.3 ± 0.64mm) fed no Salvia, whereas EGM3 occupied the 1st place in cooked meat tenderness after 1 month of freezing (5.4 ± 0.8mm) followed by both CGM and EGM1 (4 ± 0.63mm).

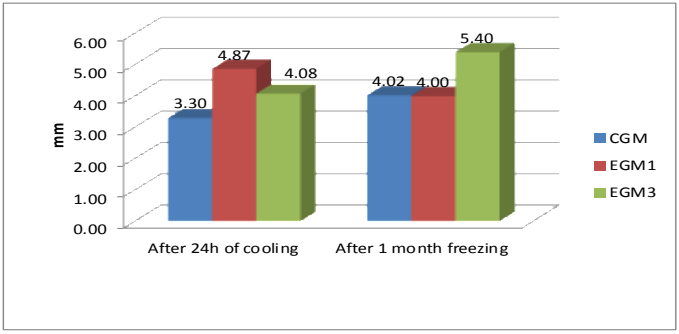

Figure 29. Tenderness of mutton before and after freezing of group B, mm

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

Figure 30 shows the variation of tenderness in the thyme trial. Tenderness after freezing was higher than after cooling. In this trial tenderness after freezing was higher than after cooling.

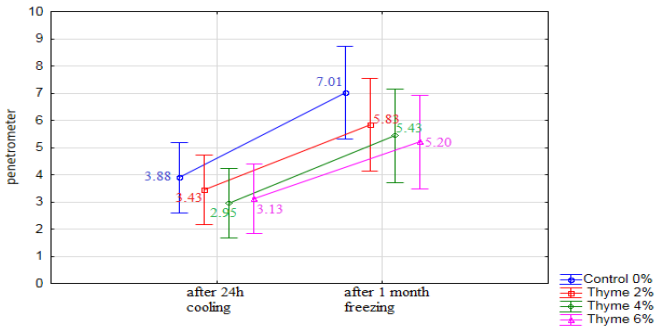

Figure 30. The variation of tenderness with thyme concentration and storage method

Tenderness after freezing for T0, T2, T4, T6 attained levels of 7.01±0.74, 5.83±0.74, 5.43±0.74, 5.20±0.74 respectively, whereas tenderness after cooling for these groups reached lower levels T0 (3.88±0.55), T2 (3.43±0.55), T4 (2.95±0.55), T6 (3.13±0.55). Statistically, tenderness for T0 after freezing (7.01±0.74) was significantly different ($P<0.05$) than that of T2 (3.43±0.55), T4 (2.96±0.55), T6 (3.13±0.55), after cooling. This could be explained by attributing the change to the temperature and the presence of thyme in the feed, or none of the previous mentioned factors. Penetrometer measurements after freezing was negatively correlated ($P<0.05$) to "L*" and cooking loss after cooling. It was also positively correlated ($P<0.05$) to "a*" after cooling. This could not be explained as there is no relation between any of these parameters because meat for each of these measurements was subjected to different temperatures.

V. WORK CONTRIBUTIONS

This work presents both scientific and practical contributions:

- The study presented cost-effective methods to ameliorate Awassi male lambs' performance using natural herbs widely available at almost zero cost in their feed, therefore reducing the cost of meat production in Lebanon.
- It offers a strategy to ameliorate the production conditions of the sheep sector in Lebanon, coping with the high cost of traditionally imported antibiotics, antioxidants and their scarcity in the Lebanese market due to the economic crisis nowadays.
- The study showcased how natural herbs in Awassi sheep feed can provide a growth promoting effect, essential for improving production parameters.
- Add to this, it determined positive effects of natural herbs inclusion in the Awassi male lambs' diet, such as enhanced meat quality.
- Moreover, it showed that supplementing the basal diet with different inclusion rates of different studied herbs is an effective method to ameliorate body growth of Awassi male lambs.

VI. CONCLUSION AND RECOMMENDATIONS

The Awassi lambs breed is one of the most popular sources of lamb meat in the Lebanese market for human consumption, characterized by higher fat and saturated fatty acids content, thus representing a healthier meat choice for the local consumer, and making it a preference among other products of animal origin. Scarce results were found on local as well as international practical applications on comparing the influence of chamomile, sage and thyme replacer to antioxidant in feed with different percentages.

Beneficial effects of chamomile, sage or thyme in small percentages mixed with basic rations and fed to Awassi sheep may arise from activation of feed intake and secretion of digestive secretions, immune stimulation, anti-bacterial, antiviral or anti-inflammatory activity and antioxidant properties. This situation requires the world to restrict using antibiotic growth promoters in sheep feed and push the Lebanese farmers to follow a new method of feeding their lambs rations supplemented with natural herbs & spices such as chamomile and sage as growth promoters without using synthetic antioxidants. Our results showed the following conclusions: Meat taken from EG2 was better in the aspect of zoological results and in meat quality. Meat taken from EG6 was slightly better in meat quality based on the physiochemical properties and in economical traits. Meat taken from EG4 was good, but the control showed to be better that EG4 meat. As a result, this study shows that it is highly recommended to use 2% chamomile flower meals as a supplementation to 100 kg of basal diets in the daily feeds of Awassi sheep during the growth period, as a replacement to antibiotic and antioxidant additives, which has proved to give the meat a better quality mostly similar to that of organic meat which is a major demand of customers nowadays. In addition to that, we recommend using sage in a quantity of 1% salvia supplemented as part of the basic ration in Awassi lambs feeding. In the thyme trial, EGTH2 after cooling had better results in luminosity index, and cooking loss. It also had a higher pH. This study recommends the use of 2% thyme as a supplementation to 100 kg of basal diets in the daily feeds of Awassi sheep in the growing period, replacer to antibiotic and antioxidant and giving the sheep better performance. Such a quantity produces meat of a quality that is comparable to the highly thought-out organic meat. Moreover, better feed conversion ratio was obtained in group EGTH4 where the lambs were supplemented with 4% thyme in addition to the basal diet. Lastly, we suggest performing this practice on a commercial level to determine the real economic effect of using natural herbs & spices, such as chamomile, sage and thyme, on body performance and meat quality. As future work, this study should be conducted on Awassi male sheep fed basic rations with more and less concentrated supplementation (%) revealing the appropriate concentration that leads to higher animal performance.

Conference papers

1. **Al Hanna G**, Genova K, Shindarska Z, Jammal B (2018) Effect of feeding thyme dry meal to weaned Awassi male lambs on body performance. AGROSYM proceedings: 1700-1705.

2. **Al Hanna G**, Genova K, Shindarska Z, Jammal B (2018) Effect of supplementing chamomile dry flower meal in weaned Awassi male lambs daily rations on body performance and meat quality. AGROSYM proceedings: 1693-1966.

Journal papers

3. **Al Hanna G (2022).** Effect of feeding sage meal to weaned Awassi male lambs on body performance and meat quality. Online J. Anim. Feed Res., 12(5): 314-323. DOI: https://dx.doi.org/10.51227/ojafr.2022.42**.**