

## Influence of adding sage leaves on growth performance, nutrient digestibility, and rumen fermentation in Awassi lambs

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### ABSTRACT

This study was conducted to determine the effect of adding different levels of sage leaf powder (SLP) to the diet of local Awassi lambs on growth performance, feed intake, digestibility and rumen fermentation. Sixteen Awassi male lambs 4-5 months of age were randomly distributed into four treatment groups: no feed additive (SLP0), 5g (SLP5), 10g (SLP10) and 15 g (SLP15) of sage leaves powder/kg of dry matter (DM) were added to the diet. Results revealed that adding SLP did not affect feed intake. However, growth performance, dry matter and organic matter digestion were recorded higher significances ( $P \leq 0.05$ ) in lambs fed SLP5 and SLP10 than in lambs fed SLP15 and SLP0. Total volatile fatty acids concentrations were significantly increased ( $P \leq 0.05$ ) in lambs fed SLP5 than other lambs. In conclusion, the addition of SLP at 5g/kg DM in the diet of Awassi lambs improved growth performance and nutrient digestibility with a positive effect on ruminal fermentation.

**Keywords:** *Salvia officinalis*; Awassi lambs; Growth performance; Nutrient digestibility; Rumen fermentation

### INTRODUCTION

Limiting antibiotics as feed additives is one of the main objectives of the ruminant industry nowadays. There is a critical demand for feed additives acceptable to milk or meat consumers and can manipulate rumen fermentation to enhance digestibility and reduce environmental pollution with nitrogen and methane<sup>1,2,3</sup>. The aromatic medicinal plants and their extracts with high concentrations of secondary metabolism compounds such as terpenes, tannins, phenols, flavonoids, and essential oils are good candidates for these requirements<sup>1,4,5</sup>. They are safe and natural plant additives that do not hurt human and animal health while improving the health and performance of animals<sup>6,7,8</sup>.

Sage (*Salvia officinalis* L.) is a fragrant perennial, evergreen subshrub of the *Lamiaceae* family that is indigenous to the Mediterranean and Middle East but has naturalized in other regions of the world<sup>8,9</sup>. Sage was cultivated worldwide for its culinary, medicinal, and flavor properties. Secondary metabolites in sage leaves include essential oils (monoterpenoids) like  $\alpha$ -Thujone,  $\beta$ -Thujone, 1,8-Cineole,  $\alpha$ -Pinene,  $\beta$ -Pinene, Camphor, Camphene, sabinene and borneol<sup>10,11,12</sup> as well as polyphenolics like rosmarinic acids and carnosol<sup>1,13</sup>. Some of these compounds possess antioxidant, anti-inflammatory, antimicrobial, antifungal, anticancer and antibacterial properties<sup>8,10,14</sup>. Numerous studies have evaluated sage leaves and their essential oil as a feed additive, focusing on feed digestibility and methane production using *in vitro* ruminal fermentation<sup>15,16,17</sup>.

Therefore, the current study investigated the effect of different levels of sage leaf powder on growth performance, nutrient digestibility, and rumen fermentation in local Awassi lambs.

## MATERIALS AND METHODS

This experiment was conducted at a private field in Al-Dujaili/Al-Kut District, Wasit Governorate, Iraq. Awassi lambs were purchased from the local market in Al-Kut and transferred by car to the location of the study. The animals were treated with internal and external parasites before starting the experiment. The sage (*Salvia officinalis* L.) leaves were also purchased from the local market in Al-Kut, finely ground and kept in airtight bags.

A sample of 20 g of dried sage leaves powder was soaked in water for 24 h, and 20 mL of hexane was added to separate oil from the water using a Clevenger-type system for 3 h at 4 °C. Shimadzu 2010 (Japan) gas chromatography-mass spectroscopy (GC-MS) with a flame ionization detector and a DM-5Ms capillary column with lengths (30m × 0.25 um × 0.25 mm), where injection area and detector temperature were 280 °C and 340 °C, respectively. The temperature of the separation column was gradually increased, starting from 100 – 300°C. Inert nitrogen gas was used as a carrier gas at a rate of 100 KPa to identify components in sage essential oil according to the method<sup>18</sup>. In a completely randomized design, a feeding trial was conducted using sixteen local Awassi male lambs with an average initial body weight of 22.38 ±0.57 kg and ages 4-5 months. The lamb was housed in a semi-shad barn with an individual pen (2×1.5 m) equipped with feeding and drinking facilities. The concentrate mixture contained (per kg DM) 450g barley grain, 180g yellow corn, 240g wheat bran, 100g soybean meal, 10g limestone, 10g common salt and 10g premix. All lambs received a concentrated mixture of 3% of their live body weight twice daily, while wheat straw was offered *ad libitum* as roughage for 63 d, preceded by a 14-d adaptation period. Lambs were randomly divided into four equal treatment groups (4 lambs each) and fed a concentrated mixture without additive (control, SLP0), concentrate + 5g/kg DM (SLP5), concentrate + 10g/kg DM (SLP10), and concentrate+ 15g/kg DM (SLP15). The SLP was supplied into one dose mixed with 0.25 kg of concentrate before morning feeding to ensure the whole dose was received<sup>19</sup>. Fresh and clean water was available *ad libitum* to all lambs during the experimental period. Feed intake was recorded daily, and lambs were weighed weekly. The average daily gain and feed conversion ratio were calculated. The digestibility trial was conducted on the <sup>ninth</sup> week of the experiment. Daily feces samples of each lamb were collected before morning feeding for 5 d using collection bags. Feces samples were dried at 60° C for 48 h in a hot air oven and kept for further analysis. Representative samples of concentrate mixture, wheat straw, sage leaves powder, and dried feces were finely ground to pass through a 1mm screen sieve, and analyses for dry matter (DM), ash, crude protein (CP), ether extract, (EE) and crude fiber (CF) contents were performed according to AOAC<sup>2</sup>. The organic matter (OM) content was calculated by subtraction of 100-ash. The nitrogen-free extract was calculated: NFE (g/kg DM) = OM-(CP+CF+EE). The metabolizable energy was calculated as ME (MJ/kg DM) = 0.012CP + 0.031EE + 0.005CF + 0.014 NFE<sup>21</sup>. Ingredients and chemical composition of concentrate mixture, wheat straw and sage leaves powder are shown in Table 1.

Ingredients (g/kg DM)	Concentrate mixture	Wheat straw	Sage leaves powder
Barley grain	450	-	-
Yellow corn	180	-	-
Wheat bran	240	-	-
Soybean meal	100	-	-
Limestone	10	-	-
Common salt	10	-	-
Premix	10	-	-
<b>Chemical composition (g/kg DM)</b>			
Dry matter (DM) (g/kg wet)	935.7	913.3	898.1

<b>Organic matter (OM)</b>	936.1	927.6	903.1
<b>Crude protein (CP)</b>	141.5	21.0	83.5
<b>Ether extract (EE)</b>	35.9	14.1	92.8
<b>Crude fiber (CF)</b>	68.4	395.7	143.9
<b>Nitrogen-free extract (NFE)<sup>a</sup></b>	690.3	496.8	582.9
<b>Metabolizable energy (ME) (MJ/kg DM)<sup>b</sup></b>	12.82	9.62	12.76

<sup>a</sup> calculated NFE (g/kg DM) = OM-(CP+CF+EE)

<sup>b</sup> calculated ME (MJ/ kg DM) = 0.012CP + 0.031EE+0.005CF +0.014NFE <sup>21</sup>

**Table 1. Ingredients and chemical composition of the concentrate mixture, wheat straw and sage leaves powder used in the experiment.**

For rumen fermentation variables, rumen fluid samples were collected at 3 h after morning feeding using a flexible esophageal tube on the last day of the digestibility trial. After discarding the first 50 mL of rumen fluid to minimize saliva contamination and then collecting about 50 mL of rumen fluid, the pH was determined immediately using a portable electronic pH meter (HANNA). The collected rumen fluid was filtrated through 4 layers of cheesecloth, and a few drops of HCl and H<sub>3</sub>PO<sub>4</sub> were added to stop the microbial activity. It was then centrifuged at 3000 rpm for 15 min. Then, the samples were kept frozen at -20°C for subsequent analysis of ammonia nitrogen concentration (NH<sub>3</sub>-N) according to AOAC<sup>20</sup>, and total volatile fatty acids (TVFA's) concentration was determined using Markham distillation apparatus following the distillation method<sup>22</sup>. Data from feed intake, growth performance, nutrient digestibility and rumen fermentation variables were statistically analyzed using a completely randomized design with one-way ANOVA in SAS software<sup>23</sup>. The post-hoc Duncan multiple comparison test was used to compare among the means <sup>24</sup>.

## RESULTS

### Essential oils in Sage leaves

Table 2 shows the chemical compounds found in sage leaves extracted essential oils. The GC/MS analysis of the oil revealed the presence of 21 constituents, which account for 97.4% of the oil. As seen in the same table, the major constituents (up 8 %) of the oil were 1,8-Cineole (12.3%), Camphor (11.2%),  $\alpha$ -Pinene (9.8%) and  $\alpha$ -Thujone (8.5%).

<b>Compounds</b>	<b>Concentration (%)</b>
<b>1,8-Cineole</b>	12.3
<b>Camphor</b>	11.2
<b><math>\alpha</math>-Pinene</b>	9.8
<b><math>\alpha</math>-Thujone</b>	8.5
<b><math>\alpha</math>-Terpinene</b>	6.3
<b><math>\beta</math>-Thujone</b>	5.9
<b>Myrcene</b>	5.1
<b>Camphene</b>	5.1
<b><math>\beta</math>-Pinene</b>	4.5
<b>Borneol</b>	4.3
<b><math>\alpha</math>-Terpinene</b>	4.1
<b>Gama-Terpinene</b>	3.9
<b><math>\alpha</math>-Terpinol</b>	3.7
<b><math>\alpha</math>-Humulene</b>	3.2
<b><math>\alpha</math>- Terpinolene</b>	3.2
<b>Linalool</b>	2.6
<b>Sabinene</b>	2.2
<b>Limonene</b>	0.8

<b>Trance-caryophyllene</b>	0.4
<b>Borneol-acetate</b>	0.2
<b>Thymol</b>	0.1
<b>Total</b>	97.4

**Table 2.** The GC-MS analysis of essential oils in sage leaves.

### Feed intake and growth performance

The addition of different levels of SLP did not affect ( $P \geq 0.05$ ) dry matter intake of roughage ( $361.35 \pm 5.07$  g/d), concentrate ( $723.04 \pm 15.03$  g/d) and total ( $1084.39 \pm 14.85$  g/d) in lambs. The final weights were similar in all groups and ranged from 30.83 to 33.00 kg, but total weight gain was higher ( $P \leq 0.05$ ) for lambs fed SLP5 and SLP10 (10.33 and 9.33 kg) compared to the lambs fed SLP0 (8.50 kg) and SLP15 (8.75 kg). A similar trend happened with average daily gain (147.62 and 133.33 vs. 121.43 and 125 g/d, respectively) (Table 3). The feed conversion ratio improved significantly ( $P \leq 0.05$ ) when lambs were fed SLP5 (7.57) and SLP10 (8.23) during the fattening period compared to those fed SLP15 (8.56) and SLP0 (8.77).

Item	SLP0	SLP5	SLP10	SLP15	SEM	P-value
<b>Feed intake</b>						
<b>Roughage intake (g/d)</b>	352.14	363.80	376.80	352.67	5.07	NS
<b>Concentrate DM intake (g/d)</b>	709.75	751.86	714.05	714.50	15.03	NS
<b>Total DM intake (g/d)</b>	1061.89	1115.66	1090.85	1069.16	14.85	NS
<b>Growth performance</b>						
<b>Initial weight(kg)</b>	22.33	22.67	22.17	22.33	0.57	NS
<b>Final weight (kg)</b>	30.83	33.00	31.50	31.08	0.70	NS
<b>Total weight gain (kg)</b>	8.50 <sup>b</sup>	10.33 <sup>a</sup>	9.33 <sup>ab</sup>	8.75 <sup>b</sup>	0.26	$P \leq 0.05$
<b>Average daily gain(g/d)</b>	121.43 <sup>b</sup>	147.62 <sup>a</sup>	133.33 <sup>ab</sup>	125.00 <sup>b</sup>	3.77	$P \leq 0.05$
<b>Feed conversion ratio</b>	8.77 <sup>a</sup>	7.57 <sup>b</sup>	8.23 <sup>ab</sup>	8.56 <sup>a</sup>	0.21	$P \leq 0.05$

<sup>a,b</sup> within a row with different superscripts different significantly ( $P \leq 0.05$ ); SEM= Standard error of the means; NS= not significant; SLP0=Lambs fed without additive; SLP5=Lambs fed 5 g sage leaves powder /kg DM; SLP10=Lambs fed 10 g sage leaves powder /kg DM; SLP15=Lambs fed 15 g sage leaves powder /kg DM.

**Table 3.** Feed intake and growth performance in Awassi lambs fed different sage leaf powder levels.

### Nutrient digestibility

The apparent digestibility of dry matter and organic matter of the diet in lambs fed SLP5 and SLP 10 were improved significantly ( $P \leq 0.05$ ) compared with those fed SLP0 and SLP15. However, increasing levels of sage leaf powder did not affect other nutrient digestibility of crude protein, ether extract, crude fiber, and nitrogen-free extract (Table 4). However, there is a numerical increase of 10g/kg DM.

Nutrient digestibility (%)	SLP0	SLP5	SLP10	SLP15	SEM	P-value
<b>Dry matter digestibility</b>	60.49 <sup>c</sup>	67.83 <sup>a</sup>	66.40 <sup>ab</sup>	62.78 <sup>bc</sup>	1.02	$P \leq 0.05$
<b>Organic matter digestibility</b>	68.12 <sup>c</sup>	74.58 <sup>a</sup>	72.54 <sup>ab</sup>	69.19 <sup>bc</sup>	0.89	$P \leq 0.05$
<b>Crude protein digestibility</b>	66.10	69.99	69.61	66.40	1.10	NS
<b>Ether extract digestibility</b>	79.16	82.03	80.22	79.28	1.09	NS
<b>Crude fiber digestibility</b>	67.10	73.24	68.47	67.75	1.50	NS
<b>Nitrogen-free extract digestibility</b>	83.86	87.00	86.74	83.69	0.71	NS

<sup>a,b</sup> within a row with different superscripts different significantly ( $P \leq 0.05$ ); SEM= Standard error of the means; NS= not significant; SLP0=Lambs fed without additive; SLP5=Lambs fed 5 g sage leaves powder /kg DM; SLP10=Lambs fed 10 g sage leaves powder /kg DM; SLP15=Lambs fed 15 g sage leaves powder /kg DM.

**Table 4. Nutrient digestibility of the diet in Awassi lambs fed different levels of sage leaves powder.****Rumen fermentation variables**

There were no significant differences in ruminal pH values or ammonia nitrogen concentrations as the levels of addition with sage leaves powder were increased. However, the concentration of total volatile fatty acids (TVFA's) showed superiority ( $P \leq 0.05$ ) in lambs fed SLP5 and SLP10 compared to those fed SLP0 and SLP15.

Rumen fermentation variables	SLP0	SLP5	SLP10	SLP15	SEM	P-value
Ruminal pH	6.71	6.44	6.53	6.67	0.72	NS
Ammonia-N (mg/dL)	23.21	22.97	22.69	23.11	0.37	NS
TVFA's (mmol/L)	81.84 <sup>b</sup>	89.77 <sup>a</sup>	84.25 <sup>ab</sup>	81.49 <sup>b</sup>	1.71	$P \leq 0.05$

<sup>a,b</sup> within a row with different superscripts different significantly ( $P \leq 0.05$ ); SEM= Standard error of the means; NS= not significant; TVFA's= Total volatile fatty acids; SLP0=Lambs fed without additive; SLP5=Lambs fed 5 g sage leaves powder /kg DM; SLP10=Lambs fed 10 g sage leaves powder /kg DM; SLP15=Lambs fed 15 g sage leaves powder /kg DM.

**Table 5. Rumen fermentation variables in Awassi lambs fed different sage leaf powder levels.****DISCUSSION**

Sage leaves used in the current study were rich in essential oils, and the major compounds were 1,8-Cineole, Camphor,  $\alpha$ -Pinene and  $\alpha$ -Thujone. It is within the range reported by <sup>10,25</sup>. Previous research has revealed that the significant constituents of sage essential oil are  $\alpha$ -Thujone, 1,8-Cineole,  $\alpha$ -Pinene,  $\beta$ -Thujone, Camphor, and Borneol<sup>11,12</sup>. The amounts of active compounds of sage vary by region due to environmental factors (climate, water availability, agronomical treatments), processing, drying method and extraction methods<sup>8,9,14,25</sup>. Literature on the effect of sage leaves and their extracts, such as essential oils, on ruminants' feed intake and growth performance is very scarce. To our knowledge, this study is the first study to utilize *Salvia officinalis* leaves but not essential oils as a source of Phyto factors in ruminant nutrition. Regarding feed intake in the current study, the dry matter intakes of roughage (wheat straw), concentrate, and total intake were not affected by feeding Awassi lambs' different levels of SLP. This could be that feeding sage leaves did not affect the animals' appetites, and the lambs did not experience any adverse post-ingestive effects. These findings are consistent with those reported in lambs<sup>26,27,28</sup> and goats<sup>29</sup>, with no difference in feed intake when rosemary leaves were included in the diet. Furthermore, <sup>30</sup> reported that roughage and concentrate feed intake were not alerted when Holstein calves fed on a mixture of leaves, including sage leaves<sup>31</sup> obtained similar results with no change in feed intake when fed sage essential oil to lambs.

Regarding growth performance, the positive effect on the total weight gain and average daily gain of lambs in the current study may be due to sage leaves' containing flavonoids and phenolic compounds which scavenge free radicals<sup>13,32</sup> or maybe the essential oil in sage leaves act as an anti-intestinal worm and improve the health status of the lambs<sup>33</sup>. Furthermore, sage leaves are rich in vitamins A, B1, B2, and C and minerals<sup>34</sup>. They may work together with antioxidants and essential oils<sup>35</sup> to improve the rumen environment and the weight gain of lambs. Lambs fed SLP5 gained about 26 g/d than lambs fed SLP15 and SLP0, and lambs fed SLP10 gained 12 g/day more. This supports the idea that some medicinal herb enhances weight gain and decrease feed efficiency. These results are in harmony with results reported by <sup>27</sup> when 0.2 g of rosemary leaves /kg live body weight/ day were added to the diet of Barki lambs. <sup>28</sup> also obtained similar results when rosemary and laurel leaves were added to the diet of Rahmani lambs at 0.5, 1%. The feed conversion ratio in the current study has the lowest value, and this indicates the efficiency of benefiting from the additive because it is one of the critical economic characteristics. Sage leaves modify rumen fermentation and increase the production of volatile fatty acids, which benefits the host animal (ruminant) by providing it with more significant quantities of microbial protein and increasing the efficiency of feed utilization<sup>26</sup>, which coincides with a reduction in methane production in the rumen measured *in vitro*<sup>3</sup>. However, growth performance was not affected when sage essential oil was added to the diet of lambs<sup>31,36</sup>. The discrepancies in fattening performance in different

studies might be due to the differences in concentrations of essential oils extracted, method of adding herbs, diet, animal and experimental condition<sup>s26,37,29,30</sup>.

Data needs to be more effective of SLP on nutrient digestibility *in vivo*. The superior increase in the diet digestibility of dry matter and organic matter in lambs fed SLP5 and SLP10 compared with those fed SLP15 and SLP0 may be due to improved rumen fermentation. Herbs can stimulate the function of proteases, amylases, and lipases in the pancreas by increasing the activity of digestive enzymes in ruminant<sup>s7</sup>. Furthermore, some active ingredients with lower antimicrobial activity, like monoterpenoids with alcohol and hydrocarbon structures, may be degraded in the rumen and used as a carbon source by microorganisms in rumen<sup>s3,38</sup>. Similar results were reported in lambs<sup>s27,28</sup> and<sup>s29</sup> when fed animals on rosemary leaves (*Lamiaceae* family).

Regarding rumen fermentation variables, with no significant changes in ruminal pH with feeding sage leaves, the values were within the reference range for optimum microbial function<sup>s39</sup>. Several *in vitro* studies reported<sup>s2,16</sup> that increasing levels of sage essential oils do not affect ruminal pH. A similar observation was reported by<sup>s27</sup>, where no significant differences were observed in rumen pH when 0.2 g rosemary leaves /kg live body weight/ day were added to the diet of Barki lambs. Similar findings were reported by<sup>s28</sup>, who found that feeding 0.5%, 1% of rosemary and laurel leaves to crossbred Rahmani lambs did not affect ruminal pH values. However,<sup>s17</sup> reported increased rumen pH with sage leaves in an *in vitro* study. The effects of rumen pH may differ due to the main chemical constituent's dependent on the concentrations of the essential oils or compounds used in the diets<sup>s5,40</sup>. In the current study, there was no significant occurrence in the concentration of ammonia nitrogen (NH<sub>3</sub>-N) in the rumen fluid. However, a numerical decrease occurred with the increase in the level of addition to a limit of SLP10. This may be attributed to the regulatory action of sage leaves, which regulates the uptake and release of NH<sub>3</sub>-N in the rumen. These results agreed with the results of<sup>s26,27</sup>. Antibacterial activity has been demonstrated in different environments<sup>s10</sup>.

Furthermore, there is a difference between *in vitro* and *in vivo* rumen systems that have greater buffering capacity against environmental stresses and are more likely to adapt and recover from intense inhibitors such as compounds found in essential oils<sup>s37,40</sup>. The primary nitrogen source in the rumen is NH<sub>3</sub>-N, and its decrease from the standard range leads to a decrease in microbial protein production within the normal range<sup>s19,29</sup>. Similar results reported that NH<sub>3</sub>-N concentrations were not affected by increasing levels of sage essential oils<sup>s15,16</sup>. Contrary to our results,<sup>s3</sup> reported a quadratic increase in NH<sub>3</sub>-N concentrations with increasing levels of sage oil in an *in vitro* study using rumen fluid from rams, regarding significant increases in TVFA concentrations in lambs fed low-level (SLP5) and medium-level (SLP10). This may be due to the significant improvement in the current study's dry matter and organic matter digestion treatments in those lambs. This result agreed with those reported by<sup>s2,17</sup> when using sage leaves or sage essential oil in a laboratory experiment but disagreed with those reported by<sup>s3,15</sup>. This is attributed to the effect of phenolic compounds in sage on gram-positive bacteria.

On the other hand, the effect of flavonoids in sage will stimulate the fermentation activity in the rumen<sup>s16</sup>. The results of the current study were similar to those of studies conducted on animals fed on medicinal plants of the family or their participation in the same active compounds. It agreed with the results in lambs<sup>s27,28</sup> and goats<sup>s29</sup> with an increase in TVFA's concentrations when incorporating rosemary leaves in the diet of lambs.

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## CONCLUSIONS

The results of this study suggest that feeding sage leaves to Awassi lambs can improve feed intake, growth performance, nutrient digestibility, and rumen fermentation. The positive effects of sage leaves on rumen fermentation were likely due to the phenolic compounds and flavonoids in sage. These compounds can stimulate the activity of bacteria in the rumen and increase the production of TVFAs. The positive effects of sage leaves on growth performance were likely due to sage's antioxidants and essential oils. These compounds can scavenge free radicals and improve the health status of the lambs. The results of this study suggest that sage leaves could be used as a feed additive to enhance the performance of ruminants. Sage leaves are a good source of antioxidants, essential oils, and phenolic compounds, which can benefit rumen fermentation and growth performance. Further research is needed to investigate the long-term effects of feeding sage leaves to

ruminants. More research is also necessary to determine the optimal dosage of sage leaves for different types of ruminants.

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