

EFFECT OF DIETARY LEMONGRASS (*Cymbopogon citratus*) AND ROSELLE (*Hibiscus sabdariffa*) TO SAIDI EWES ON: (A) MILK PRODUCTION, RUMEN FERMENTATION AND LAMB'S PERFORMANCE

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SUMMARY

The experiment was carried out to study the influence of Lemongrass and Roselle supplementation in the diet of Saidi ewes. Twenty-eight ewes in the last month of pregnancy with an average body weight of 45 ± 2 kg were assigned to 4 treatment groups (7 ewes in each group) for 75 days (15 before parturition and 60 days after parturition) using completely randomized block design. The treatments included: (1) control group (CON): received a ration consisted of concentrate mixture and wheat straw as 60:40 % on dry matter (DM) bases; (2) LG treatment group: the ewes were fed the control ration plus 4g dried Lemongrass /kg of concentrate mixture; (3) RO treatment group: the ewes were fed the control ration plus 4g dried Roselle/kg of concentrate mixture; and (4) LGRO treatment group: the ewes were fed the control ration plus 2g dried Lemongrass and 2 g dried Roselle /kg of concentrate mixture. Milk yield was recorded biweekly and milk samples were taken to analyze its chemical composition. Rumen liquor samples were collected to measure the pH, total volatile fatty acids (VFA's) concentration and protozoal count. Lambs were weighed at birth and then once a week during four weeks of the experiment period and the daily weight gain (DWG) was calculated.

Results revealed that milk efficiency as fat corrected milk (FCM) and milk yield/DM intake were not significantly affected by Lemongrass or Roselle supplementation. Milk yield tended to increase ($P > 0.05$) in treated ewes. Milk fat increased ($P < 0.05$) in milk of ewes fed lemongrass diet compared to those fed the other diets. In rumen samples, the protozoal count was significantly higher due to Lemongrass and Roselle supplementation together compared to the control and LGRO diets. Also, there was a highly significant increase in total VFA's concentration in LGRO and RO compared to the CON group. Also, there was a significant ($P < 0.05$) increase in milk total solids, solids not fat, protein and lactose in ewes fed lemongrass and Roselle diets compared with those fed control diet. In addition, milk density percent was higher ($P < 0.05$) in milk of treated ewes compared to untreated ones, whereas minerals content did not differ among groups. Both body weight of lambs and daily gain of treated groups were not significantly affected by the experimental additives.

Finally, the findings of this study revealed that the addition of lemongrass and Roselle herbs improved milk composition and rumen fermentation patterns without any harmful effect on animal performance.

Keywords: Lemongrass, Roselle, Lactating ewes, Milk composition, Rumen fermentation

INTRODUCTION

Herbs and their extracts have been evaluated for their ability to alter ruminal fermentation and improve nutrient utilization in ruminants (Korošec *et al.*, 2009). Phenolic compounds are one of the most important phytochemicals in plant extracts. The main effect is to inhibit the oxidative reaction caused by oxidative stress and mitigate its consequences (Shahidi, 2006).

Lemongrass is a herb of interest, and it is widely used in human food in tropical countries, especially in Southeast Asia. The principal constituent of lemongrass extract is citral which is the essential oil for vitamin A synthesis (Kholif *et al.*, 2017). Lemongrass herb has been reported to have antibacterial, antioxidant and anti-hyper ammonia-producing ruminal bacterial activities (Wanapat *et al.*, 2008).

Roselle is a species of Hibiscus native to the old world tropics (Idris *et al.*, 2016). The extract of Roselle was reported as an antibacterial, antifungal, diuretic, uricosuric, and mild laxative substance (Da-Costa-Rocha *et al.*, 2014). In addition, the polyphenols components of *Hibiscus sabdariffa* extract exhibit anti-inflammatory potency (KAO *et al.*, 2009), hypoglycemic, hypolipidemic, antioxidant effects, and induce tumor cell apoptosis (Chiu *et al.*, 2015). Few studies have been conducted to study the effect of Roselle and Lemongrass herbs inclusion on milk composition, rumen fermentation and lambs growth rate. The objective of this experiment was to study the effect of dried roselle and lemongrass powder supplementation on dry matter intake, lamb's growth performance, rumen ecology and milk production of Saidi ewes.

MATERIALS AND METHODS

Experimental location, duration, and animals:

The experiment was carried out at the Research Farm of Animal Production Department, Faculty of Agriculture, Assuit University, Assuit, Egypt. Twenty-eight pregnant Saïdi ewes (at the 3rd or 4th lactating seasons and weighing an average 45.2±2kg) were used. The experiment lasted for 75 days starting from 15 days before parturition and 60 days after parturition.

Diet and experimental design:

Dry Lemongrass (*Cymbopogon citratus*) and Roselle (*Hibiscus sabdariffa*) were obtained from a local supplier in Assiut, Egypt. Ewes were divided into four groups (seven each group) and allocated randomly to one of four dietary treatments using completely randomized block design. The dietary treatment groups were as follows; (1) control group (CON): received a ration consisted of concentrate mixture and wheat straw as 60:40 %, as a dry matter

(DM) basis; (2) LG treatment group: the ewes were fed the control ration plus 4g dried Lemongrass /kg of concentrate mixture; (3) RO treatment group: the ewes were fed the control ration plus 4g dried Roselle/kg of concentrate mixture; and (4) LGRO treatment group: the ewes were fed the control ration plus 2g dried Lemongrass and 2 g dried Roselle /kg of concentrate mixture. Tested feed additives were homogeneously mixed with the concentrate mixture. The offered feeds were assessed to cover the maintenance and production requirements for each animal (NRC, 1985). The concentrate mixture was offered for each animal individually once daily at 9.00 am, while wheat straw was offered at 12.00 hr. Fresh water was provided *ad libitum*.

Samples of feed ingredients were chemically analyzed for dry matter, ash, crude protein and ether extract according to methods of (AOAC, 2012). The neutral detergent fiber (NDF) and acid detergent fiber (ADF) were identified according to (Van Soest *et al.*, 2010). The chemical composition of the ingredients is shown in Table 1.

Table 1. The ingredients and chemical composition of the experimental diets

Item	(g/kg control ration) *
Wheat Straw	400
Maize Grain	300
Soybean Meal	90
Sunflower Meal	72
Wheat Bran	120
Limestone	12
Sodium chloride	3
Trace mineral and vitamin premix [‡]	3
Total	1000.00
Chemical composition (g/kg DM basis)	
OM	822.69
CP	116.95
EE	28.46
NDF	424.23
ADF	235.71

* CON: Control ration consisted of concentrate mixture and wheat straw (60:40% as DM basis).

[‡]The premix comprised (per kg) 20,000,000 IU vitamin A, 200,000 IU vitamin D3, 10,000 mg vitamin E, 10,000 mg Fe, 2500 mg Cu, 20,000 mg Mn, 100 mg Mo, 100 mg Co, 800 mg I, 20,000 mg Zn and 100 mg Se.

Voluntary feed intake and lambs' growth performance:

Feed intake (g/ewe/day) was daily recorded by weighing the offered diets and subtracting the refusal obtained the following day during the experimental period. Ewes were weighed at the beginning of the experiment and over two weeks to adjust the feed requirements. Lambs were weighed at birth and then once a week during four weeks of the experimental period and daily weight gain (DWG) was calculated.

Sampling and analysis of rumen liquor:

Rumen liquor samples were collected from 4 animals in each group by using a stomach tube at the end of the trial Sampling was performed 3-4 hrs

after offering the dietary concentrate ration. Rumen liquor samples were divided into two parts, the first part was filtrated through one layer of cheese-cloth, and was used to measure the protozoal count, while the second part was filtrated through four layers of cheese-cloth and the pH of filtrate portion was immediately measured using a pH meter. Few drops of a saturated solution of mercuric chloride were added to the filtrates to stop the microbial activity, and then the samples were stored at -20°C to determine total volatile fatty acids (total VFA's) utilizing the procedures of (Warner, 1964).

Milk yield and its composition:

After 15 days of lambing and by using lambs suckling technique, the daily milk yield was measured as reported by (Ashmawy, 1980). Then the milk was mixed thoroughly to get a homogeneous sample per animal and the daily milk yield was recorded. For the analysis of milk composition, a sample of 100 ml per animal per day was collected individually from all animals and every two weeks during the experiment lactating period (60 days). Milk samples were analysed for total solids, fat, protein, lactose, density and minerals using an ultrasonic milk analyzer (Lactoscan MCCWS, Milkotronic Ltd, Bulgaria).

Experimental design and statistical analysis :

Data of dry matter intake, milk yield and chemical composition of milk samples from ewes were subjected to repeated measurement analysis of variance (ANOVA) with sampling period as the repeated factor, experimental diets as fixed factor and ewes as a random factor.

The model used was: $Y_{ijk} = \mu + \alpha_i + e_{ij} + \delta_{ijk}$, where: Y_{ijk} = The ijk^{th} observation, μ = The general mean, α_i = The effect of the i^{th} treatment ($i = C, LG, RO, LGRO$), e_{ij} = The experimental error and δ_{ijk} = is the sampling error.

The data of the rumen parameters, the body weight and changes of body weight of ewes and the daily weight gain of lambs were evaluated using the General Linear Models (GLM) procedure for analysis of variance. The model used was: $Y_{ijk} = \mu + \alpha_i + e_{ij}$, where Y_{ijk} = The ijk^{th} observation, μ = The overall mean, α_i = The effect of the i^{th} treatment ($i = C, LG, RO, LGRO$) and e_{ij} = The experimental error.

Duncan's multiple range tests were used to detect the differences among the treatments using the SPSS

statistical package 22 (SPSS Institute, Chigaco, IL, USA). The data are presented as means \pm SE. Probability values less than 0.05 ($P < 0.05$) was considered significant.

RESULTS AND DISCUSSION**Dry matter intake and milk yield:**

Dry matter intake (g/d) was not significantly affected by dietary treatment of either lemongrass or roseolle (Table 2). Similarly, Kholif *et al.* (2017) and (Wanapat *et al.*, 2008) found no significant effect of feeding rosemary and lemongrass on DM intake by Damascus goats and cows, respectively.

The average daily milk yield, fat corrected milk and milk yield/DMI were not significantly affected by dietary lemongrass and roselle (Table 2). Milk yield recorded in the present trial is comparable with previous work in Egypt for ewes (654.3 g/day) rearing single lambs (Abd Allah *et al.*, 2011). However, Kholif *et al.* (2017) found that an increase of milk yield in lactating Damascus goats fed rosemary and lemongrass diets. In addition, dietary lemongrass residue as feed for cows increased 23% of the milk production as reported by (Manurung *et al.*, 2015). This difference in the results could be attributed to the difference of the animal type. Enhanced ruminal fermentation with feeding lemongrass is likely the main reason for higher milk production (Kholif *et al.*, 2017). In addition, elevating milk yield reported in the present study may have been influenced by the phenolic compounds found in lemongrass. Ruminal fermentation processes could be enhanced by phenolic compounds which create a strictly anaerobic environment in the rumen (Salem *et al.*, 2014b).

Table 2. Effect of treatments on dry matter intake and milk performance of Saidi ewes

Item	Treatment*			
	CON	LG	RO	LGRO
Live body weight, kg	45.14 \pm 3.81	44.87 \pm 2.70	45.28 \pm 2.81	45.14 \pm 2.83
Dry matter intake, g/d	1205	1192	1232	1253
Milk yield, g/d	648.06 \pm 6.41	660 \pm 8.66	670.60 \pm 5.29	682.03 \pm 5.79
Fat corrected milk, 4%	804.95 \pm 22.16	741.37 \pm 20.74	759.79 \pm 19.55	793.20 \pm 17.32
Milk yield/DMI	0.54 \pm 0.01	0.55 \pm 0.01	0.54 \pm 0.001	0.54 \pm 0.001

*CON: Control ration consisted of concentrate mixture and wheat straw as 60:40 %, as a dry matter (DM) basis; LG treatment group: the ewes fed control ration plus 4g dried Lemongrass /kg of concentrate mixture; RO treatment group: the ewes fed control ration plus 4g dried Roselle/kg of concentrate mixture; and LGRO treatment group: the ewes fed control ration plus 2g dried Lemongrass and 2 g dried Roselle /kg of concentrate mixture.

Rumen Liquor Parameters:

The effect of the experimental additives on rumen parameters are shown in Table 3. Ruminal pH values were not significantly affected by the experimental additives. The pH was within the range considered optimal for microbial digestion of fiber and protein (Ryle and Ørskov, 2011). This result agrees with Kholif *et al.* (2017) who reported that the rumen pH values in lactating Barki goats were not changed between control and goats treated with lemongrass.

There was a higher significant ($P < 0.05$) increase in concentration of total VFA's in the rumen fluid of ewes fed RO and LGRO diets compared to those received LG or CON diets (Table 3). However, feeding ewes diets with LG and RO together decreased ($P < 0.05$) total protozoal count compared to feeding LG or RO separately. Similar results were reported by Kholif *et al.* (2017) and Nanon *et al.* (2014) who found that dietary LG had effect on the concentration of rumen total VFA's. The higher concentration of total VFA's for RO and LGRO

groups cleared that adding these herbs to the ruminant diet could change the microbial population and rumen fermentation (Nikaido, 1994). These results indicate that the herb additives stimulate rumen protozoal activity and enhance digestibility which is in accordance with those noted by (Busquet

et al., 2005; Castillejos *et al.*, 2010 and Kongmun *et al.*, 2010). Volatile fatty acids are the end products of rumen microbial fermentation and represent the main supply of metabolizable energy for ruminants (Van Soest, 1994).

Table 3 Effect of treatments on rumen parameters of Saidi ewes

Item	Treatments*			
	CON	LG	RO	LGRO
pH value	6.00±0.11	5.90±0.07	6.08±0.09	6.05±0.05
Total VFA's, m-equiv/ 100 mL	7.00 ^c ±0.58	7.75 ^{bc} ±0.25	9.75 ^a ±0.75	9.25 ^{ab} ±0.75
Protozoa count, 10 ⁵	9.38 ^b ±0.22	20.10 ^a ±0.37	13.05 ^a ±0.66	9.29 ^b ±0.83

Values are mean of 7 samples ± SE. Samples were taken 3hrs. after feeding .

a, b, c, means at the same row with different superscript are significantly different (P<0.05).

*CON: Control ration consisted of concentrate mixture and wheat straw as 60:40 %, as a dry matter (DM) basis; LG treatment group: the ewes fed control ration plus 4g dried Lemongrass /kg of concentrate mixture; RO treatment group: the ewes fed control ration plus 4g dried Roselle/kg of concentrate mixture; and LGRO treatment group: the ewes fed control ration plus 2g dried Lemongrass and 2 g dried Roselle /kg of concentrate mixture.

The protozoal count was significantly high in the rumen liquor of ewes fed LG (Fig. 1b) and RO (Fig. 1c) diets compared to those fed LGRO and CON diet (Fig. 1 a and Fig. 1d, respectively). These results agree with those reported by Khattab *et al.* (2017) who found that dietary Lemongrass significantly increased protozoa count in the rumen

of lactating Baladi goats. In this field, the presence of secondary metabolites in the herbs may have optimized the availability and activity of the rumen microflora and other ruminal function resulting in improved nutrient digestion by goats (Wanapat *et al.*, 2008).

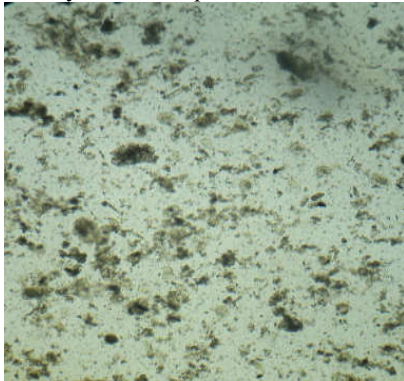


Fig. 1a : CON group

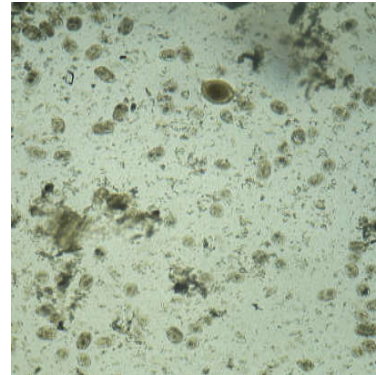


Fig. 1b: LG group (Marked increase)

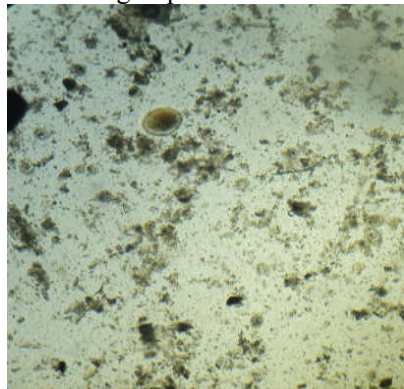


Fig. 1c: RO group (slight increase)

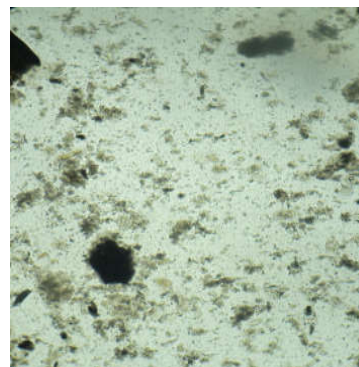


Fig. 1d: LGRO group(unmarked increase)

Figure 1 Effect of dietary lemongrass and Roselle on protozoal count of Saidi ewes

Milk composition:

The effect of feeding lemongrass and Roselle on milk composition of Saidi ewes is presented in Table (4). Data showed that milk concentrations of fat, total

solids, solids not fat, protein and lactose were increased (P<0.05) with lemongrass and Roselle supplementation. In addition, density percent was higher (P<0.05) in treated ewes compared to

untreated ewes, whereas mineral content did not differ among groups. Kholif *et al.* (2017) found in lactating Damascus goats that feeding rosemary and lemongrass diets have increased milk constituents compared to the control diet. The increase of milk fat content with feeding lemongrass in the present study (Table 4) may be due to increased acetate production in the rumen as reported by Kholif *et al.* (2017). They added that milk fat content and milk fatty acid composition depend mainly on dietary feed, animal and environmental factors, with the major effect being feed. Moreover, ruminal acetate/propionate ratio plays a fundamental role in the synthesis of milk

fat. Inclusion of lemongrass and Roselle produced milk with higher lactose content (Table 4). Propionate is the precursor for gluconeogenesis and lactose synthesis, and increasing glucogenic precursors has a favorable effect on milk lactose content. In addition, a high milk constituents (fat, SNF, TS, protein and lactose contents) reported in the present study, may have been influenced by the phenolic compounds found in Lemongrass and Roselle which could improve ruminal fermentation processes by creating a strictly anaerobic environment (Bodas *et al.*, 2012; Salem *et al.*, 2014a).

Table 4. Effect of treatments on milk composition of Saidi ewes

Item	Treatments*			
	CON	LG	RO	LGRO
Fat, %	4.85 ^b ±0.22	5.61 ^a ±0.19	4.89 ^b ±0.19	5.09 ^{ab} ±0.19
SNF, %	8.36 ^b ±0.52	9.51 ^{ab} ±0.52	9.75 ^{ab} ±0.52	10.05 ^a ±0.52
Total solids, %	13.31 ^b ±0.72	15.12 ^a ±0.61	14.64 ^{ab} ±0.61	15.14 ^a ±0.61
Protein, %	3.02 ^b ±0.11	3.51 ^a ±0.11	3.49 ^a ±0.11	3.54 ^a ±0.11
Lactose, %	4.56 ^b ±0.16	5.27 ^a ±0.19	5.23 ^a ±0.16	5.33 ^a ±0.16
Density, %	29.67 ^b ±0.98	33.83 ^a ±0.98	34.23 ^a ±0.98	34.26 ^a ±0.98
Minerals, %	0.85±0.04	0.83±0.05	0.76±0.04	0.81±0.04

Each value represents an average of twenty-one samples.

a, b means at the same row with different superscript are significantly (P<0.05) different.

*CON: Control ration consisted of concentrate mixture and wheat straw as 60:40 %, as a dry matter (DM) basis; LG treatment group: the ewes fed control ration plus 4g dried Lemongrass /kg of concentrate mixture; RO treatment group: the ewes fed control ration plus 4g dried Roselle/kg of concentrate mixture; and LGRO treatment group: the ewes fed control ration plus 2g dried Lemongrass and 2g dried Roselle /kg of concentrate mixture.

Lamb's growth performance:

The effects of the experimental additives on average body weight and daily gain of lambs are shown in (Table 5). Both body weight and daily gain of treated lambs were not significantly affected by the experimental additives. These results are in agreement with those reported by (Beshir and Babikers, 2009) who found that dietary treatments with RO levels (10 and 20%) in the diets of male lambs of Sudan desert sheep ecotype Kabashi had no significant effect on average daily gain and final body

weight. However, (Sariözkan *et al.*, 2016) observed that the quail diet supplemented with 3% LG leaf meal showed a significantly lower final body weight compared to the control (P<0.05). This finding was similar to those of Tiwari *et al.*, (2018) who reported that reduced body weight in broilers fed with 2% LG leaf supplemented diet. However, this result was contrary to those of (Mmereole, 2010; Mukhtar *et al.*, 2012) who reported that supplementation of LG leaf meal or oil resulted in significantly higher body weight in broilers.

Table 5. Effect treatments on lamb's growth performance

Period	Treatment*			
	CON (n=7)	LG (n=7)	RO (n=8)	LGRO (n=9)
1 st WK	3.51±0.19	3.43±0.41	3.42±0.41	3.00±0.30
2 nd WK	6.61±0.56	6.44±0.62	6.12±0.62	6.15±0.58
3 rd WK	7.67±0.58	7.87±0.83	6.95±0.83	7.08±0.71
4 th WK	9.37±0.81	9.65±1.15	8.36±1.15	8.55±0.98
Daily weight gain (g/day)	180±0.30	200±0.40	140±0.40	160±0.45

*CON: Control ration consisted of concentrate mixture and wheat straw as 60:40 %, as a dry matter (DM) basis; LG treatment group: the ewes fed control ration plus 4g dried Lemongrass /kg of concentrate mixture; RO treatment group: the ewes fed control ration plus 4g dried Roselle/kg of concentrate mixture; and LGRO treatment group: the ewes fed control ration plus 2g dried Lemongrass and 2g dried Roselle /kg of concentrate mixture.

CONCLUSION

Finally, from the present findings, it could be concluded that the addition of lemongrass and Roselle herbs can be used in dairy ewes without any

harmful effect on animal performance. These kinds of herbs and at this level (4 g/kg of concentrate mixture), succeed to improve milk composition and rumen fermentation in treatment ewes but this effect did not pass to their lambs.

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تأثير إضافة حشيشة الليمون والكرديه في عليقة النعاج الصعيدي على: (أ) إنتاج اللبن وتخمرات الكرش وعلى أداء الحملان

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أجريت هذه التجربة بهدف دراسة تأثير إضافة حشيشة الليمون والكرديه في عليقة النعاج الصعيدي على إنتاج وتركيب اللبن وتخمرات الكرش ونمو مواليدها. تم استخدام ٢٨ نعجة في نهاية مرحلة الحمل بمتوسط وزن 45 ± 2 كجم. قسمت النعاج الى أربعة مجاميع (عدد ٧ نعجة في المجموعة) لمدة ٧٥ يوم (١٥ يوم قبل الولادة و ٦٠ يوم بعد الولادة) وكانت العليقة المقدمة لهذه المجموعات كالتالى: مجموعة الكنترول (المجموعة الاولى) عبارة عن ٦٠% المخلوط المركز و ٤٠% تبن القمح (على أساس المادة الجافة). مجموعة حشيشة الليمون (المجموعة الثانية): عليقة المجموعة الكنترول+ ٤ جرام حشيشة الليمون/كجم من المخلوط المركز. مجموعة الكركديه (المجموع الثالثة): عليقة المجموعة الكنترول+ ٤ جرام بتلات زهرة الكركديه/كجم من المخلوط المركز. مجموعة خليط حشيشة الليمون والكرديه (المجموعة الثالثة): عليقة المجموعة الكنترول + ٢ جرام حشيشة الليمون+ ٢ جرام بتلات زهرة الكركديه/كجم من المخلوط المركز.

تم تغذية الحيوانات بشكل فردى ومتابعة وزن النعاج كل ١٥ يوم لتحديد المقررات الغذائية حسب حالة الحيوان. وتم متابعة وزن الحملان المولودة كل اسبوع ولمدة شهر وتم حساب معدل الزيادة اليومية. تم تسجيل المأكول وإنتاج اللبن وتم أخذ عينة لبن كل اسبوعين للتحليلات الكيميائية. وفي نهاية التجربة تم تجميع عينات من سائل الكرش وتم تقدير درجة الحموضة والأحماض الدهنية الطيارة الكلية وأعداد البروتوزوا. والنتائج تشير الى الأتى: لم يتأثر إنتاج اللبن أو اللبن المعدل بالمعاملات المختلفة. هناك زيادة معنوية في مستوى نسبة الدهن في لبن مجموعة حشيشة الليمون مقارنة بالمجموعات الأخرى. هناك زيادة معنوية في مستوى نسبة الجوامد الصلبة والجوامد غير الدهنية والبروتين واللاكتوز في لبن نعاج مجموعتي حشيشة الليمون والكرديه مقارنة بمجموعة الكنترول. هناك زيادة معنوية في مستوى كثافة اللبن للنعاج بمجموعتي حشيشة الليمون والكرديه مقارنة بمجموعة الكنترول. هناك زيادة في أعداد البروتوزوا الكرش و الأحماض الدهنية الطيارة الكلية في المجموعة التى تم فيها مزج كلا من حشيشة الليمون و بتلات زهرة الكركديه. لم تتأثر الوزن عند الميلاد ومعدل نمو الحملان معنوياً بالمعاملات المختلفة. نستنتج من النتائج السابقة أنه يمكن استخدام كلا من حشيشة الليمون و بتلات زهرة الكركديه بمستوى ٤ جرام/كجم مخلوط مركز لكل منهما مما أدى الى تحسين مكونات اللبن و تخمرات الكرش في الاغنام ولكن لم يظهر أى تأثير معنوى على معدلات نمو المواليد.