Research Article

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The Effects of Lignin Peroxidase Enzyme and Feed Source on The Rumen Histology of Arabi Lambs

Jinan Abdul Aziz Bannai[1], Hannaa Ali Jabar Al-Galbi[2], Amera Katm Naser[2]

1- Department of Surgery and Obstetrics, College of Veterinary Medicine, University of Basrah. Basrah, Iraq.

2- Department of Animal Production, College of Agriculture, University of Basrah, Basrah, Iraq.

Corresponding Author Email Address: hanaa.jabar@uobasrah.edu.iq

ORCID ID: https://orcid.org/0000-0002-1568-4463

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Abstract

This study aimed to demonstrate the effect of using feed treated with enzymes on the rumen histology of lambs. This study was conducted at the animal field, College of Agriculture, and University of Basrah for 105 days. Sixteen Arabi male lambs aged 4-5 months and weighing 22.25 kg were used. Lambs were randomly distributed into four groups, with four lambs for each treatment. The concentrated diet mainly consisted of 54% barley, 30% bran, 10% ground yellow corn, 5% soybeans, and 1% vitamins and mineral salts. Lambs were fed 3% of their live body weight. The studied diets consist of 60% concentrated feed and 40% roughage without or with the addition of 10 gm of lignin peroxidase enzyme, or 40% of the concentrated feed and 60% of the roughage without or with the addition of the enzyme. The groups fed a diet supplemented with enzymes showed significantly higher papillary length, breadth, and surface area of the rumen ($P<0.05$) compared to the control group. The results also showed a significant effect ($P<0.05$) on the amounts of feed consumed and the weight gain in the groups fed a diet supplemented with enzymes compared to the control group. The group fed 60% concentrate showed a significant superiority in weight after a month of acclimatization and in final weight compared to the lambs fed 40% concentrate, which showed no significant effect. Adaptive responses to different structural parts of papillae can change how much concentrated feed is consumed and how well feed is converted between them. This shows how complicated rumen development is in response to changing nutritional inputs.

Keywords: Arabi lambs, enzymes, histology, rumen.

Introduction

Feeding practices influence both animal welfare and production (1). Farmers feed sheep and goats a diet that may jeopardize their well-being to meet the growing demand for lean meat (2). In many countries, mutton production has transitioned from a conventional approach to intensive rearing, where feeding depends on a highly concentrated diet to ensure rapid growth. Because lambs are fed a high-energy diet during the transition to weaning, significant problems, including rumen acidosis and aberrant anatomical and functional development of the rumen wall, have occurred (3, 4). Ligninolytic enzymes are different types of enzymes produced by fungi and bacteria that catalyze the breakdown of lignin (5). These enzymes are not hydrolytic but are oxidative (electron withdrawing) through their enzymatic mechanisms; chitinolytic enzymes include peroxidases, such as lignin peroxidase (6).

Bio-lignin removal from lignocellulosic materials has been considered an alternative approach (2). Microbial pretreatment is commonly used to improve the suitability of byproducts for enzymatic hydrolysis (7). Three groups of organisms capable of biodegrading quinine are white mold fungi, some soil microbes, and termites. Recently, attention has been paid to the increasing role of bacteria in the degradation of lignin and other agricultural by-products (8).

Lignin peroxidase (LiP) is an enzyme used to degrade lignin. It was first discovered in 1983. Fungi, also known as diarylpropyne peroxidase or ligninase (5), are the main producers. The method of using the lignin peroxidase enzyme involves directly introducing it into the feed as an additive to digest the lignin bound to cellulose and hemicellulose. These enzymes can settle on the base material and decompose complex plant carbohydrates, thereby improving the value of low-quality feed, increasing feed consumption, and improving digestion (9). There is evidence that adding enzymes to feed can make it more nutritious in the rumen by breaking down the cell walls and making the feed easier to digest (10). In this context, some research has been conducted to study the biological removal of lignin from the cell wall components of agricultural waste, especially cellulose and hemicellulose, using fungal and bacterial enzymes.

A lot of what controls the rumen mucosa in sheep is controlled by volatile fatty acids (VFAs), especially butyrate and propionate. These acids help the papillae grow. (11). In different regions of the world, different fattening strategies are very popular, with meadow, silage, and concentrate feed being the most common fattening systems (12). As a result of the significant increase in feed prices and to reduce rumen difficulties, breeders have tended to reduce concentrated feeds and switch to a roughage diet (12). Although it has been widely proven that a concentrated diet promotes higher gains (13). The objectives of this study were to demonstrate the effect of using feed treated with enzymes (lignin peroxidase) on some anatomical characteristics in rumen of lambs.

Materials and Methods

Experimental animals

This study was carried out in the animal field at the Department of Animal Production's, Microbiology Laboratory and the Agricultural Research Station/College of Agriculture/University of Basrah in Qarmat Ali. Sixteen male Arabi lambs were studied. The average weight of the lambs was 22.50±0.25 kg, and their age ranged from 4 to 5 months. The veterinarian in the animal field examined the lambs after they were bought from neighborhood markets to ensure they were healthy and disease-free.

Experimental design and feeding

The experimental animals were randomly divided into four groups (four animals for each). A semi-closed breeding system was adopted for the experiment. The concentrated diet was provided at a rate of 3% of the live body weight (14). Feed was provided in two meals, morning and evening. Mineral blocks were also provided to all, as shown in Table (1). The concentrated diet consists of 54% barley, 30% bran, 10% ground yellow corn, 5% soybeans meal, and 1% vitamins and mineral salts for lambs throughout the trial period to meet their needs for mineral nutrients (14).The amount of daily feed provided and remaining was calculated to calculate the feed consumed. The lambs were fed at the beginning of the experiment for a preliminary period of ten days and then weighed to record their initial weight. The diets that were looked at have 60% concentrated feed and 40% roughage (alfalfa hay), either with or without 10 gm of lignin peroxidase enzyme/kg of feed dry matter, or 40% of the concentrated feed and 60% of the roughage, with or without the enzyme.

Histological study

In accordance with (15), we collected rumen tissue samples for the analysis of papillae length (PL), papillae width (PW), and rumen wall thickness (RWT). Small biopsies (5x5 cm) were taken from the dorsal part of the rumen, at around 5 cm from the longitudinal pillar of each lamb. The papilla surface area was calculated as [2ϖx(W/2) x L], where W = papilla width and L = papilla length (16). Also, the muscular layer thickness was measured at four random locations in the rumen. Specimens were fixed in 10% phosphate-buffered formalin for 24 hours, then routinely processed, embedded in paraffin, sectioned at a 4 mm thickness, stained with Hematoxylin Eosin (H&E) (17), and viewed with a microscope attached to the camera (Olympus DP72 Microscope Digital Camera; Olympus NV, Aartselaar, Belgium).

Statistical analysis

The data were analyzed statistically using a Completely Randomized Design using the statistical program (18), version 26, following the mathematical model: $Y_{ijk} = \mu + A_{i.} + B_{.j} + Ab_{ij} + e_{ijk}$, Y_{ijk} = the studied characteristic related to observation k, $A_{i.}$ = effect of concentrate: roughage ratio i (1=60:40, 2=40:60), $B_{.j}$= effect of enzyme j (1= not added, 2= adding 10 gm/kg of lignin peroxidase enzyme), AB_{ij}= the effect of the interaction between concentrate: roughage ratio and enzyme, and E_{ijk} = effect of experimental error associated with observation k.

Table (1) Experimental nutritional groups .

Treatments	Concentrate feed %	Enzyme
1	60	Zero
2	60	Natozyme p50 (10 gm/kg dry matter)
3	40	Zero
4	40	Natozyme p50 (10 gm/kg dry matter)

Results and Discussion

Papillae length, width and surface area

Table (2) showed significant (P<0.05) differences in the histological changes in the treatment and control groups, as it was noted that the length of the rumen papillae was significantly affected (P<0.05) by both the level of the concentrated diet, the addition of the enzyme, and the interaction between them. The length of the rumen papillae of lambs fed 40% concentrate was greater than that of lambs fed 60% concentrate. While the lambs that had the enzyme added to their diet recorded a significant increase (P<0.05) in the average length of the rumen papillae compared to the lambs with no enzyme added to their diet, The lambs who received a 40% concentrated diet with the enzyme supplemented outperformed those who received a 60% concentrated diet with the same enzyme. The physical characteristics of feed have a significant (P<0.05) impact on the development of the size of the papillae and the muscle layers of the rumen (2).

The development of rumen papillae requires the establishment of viable microbial colonies and the formation of volatile fatty acids (VFA), mainly butyrate and propionate (19). This study agreed with the study conducted by (3), who noted that the papillae length increased when 50% of the estimated net energy in sheep was provided as short-chain fatty acids at physiological concentrations. The development of papillae depends largely on the synthesis of volatile fatty acids (VFAs) in ruminants, especially butyrate and propionate, which regulate the mucosa of the rumen in sheep (11, 20). It was also observed in this study that the width of rumen papillae was not affected by the level of the concentrated diet, whether the enzyme was added to it or not. Although there are computational increases when the enzyme is added. It was also noted that the number of papillae was not significantly affected by either the percentage of concentrated feed or the addition of the enzyme. However, there was a significant increase in the number of papillae when the enzyme was added compared to the papillae of lambs whose diets the enzyme was not added, which recorded a lower number**.** These results are consistent with those of (21)**.**

Table (2). Effect of different concentrate of roughage with different lignin peroxidase supplementation on the Papillae length, width and surface area of Arabi lamb's rumen (Mean +SE)

Concentrate: Roughage	Enzyme 0	Enzyme 10 gm/kg	Mean of diet
	Length (mm)		
40: 60	7.67±0.58	8.67±0.56	8.17±0.75
60: 40	8.33±0.56	9.33±0.58	8.83±0.75
Mean	8.50±0.55	8.50±0.56	LSD interaction=1.00
	Width (mm)		
40: 60	1.07±0.11	2.00±0.10	1.53b±0.12
60: 40	2.10±0.10	2.40±0.10	2.25a±0.19
Mean	1.58^{b}±0.17	2.20^{a}±0.23	LSD interaction=0.30
	Surface area (mm^2)		
40:60	29.12±4.45	48.19±3.63	38.65^{b}±5.15
60:40	52.38±3.62	58.65±3.62	55.52^{a}±4.73
Mean	40.75^{b}±3.24	53.43^{a}±6.59	LSD interaction=6.20
	Number of papillae/ cm^2		
40: 60	9.00±1.00	11.00±2.64	10.00±2.09
60:40	8.67±1.15	11.33±2.08	10.00±2.09
Mean	8.83±0.98	11.17±2.14	LSD interaction=NS

- **means of different traits with different superscripts in a column or row differ significantly at p≤ 0.05. LSD=Least Significant Different**

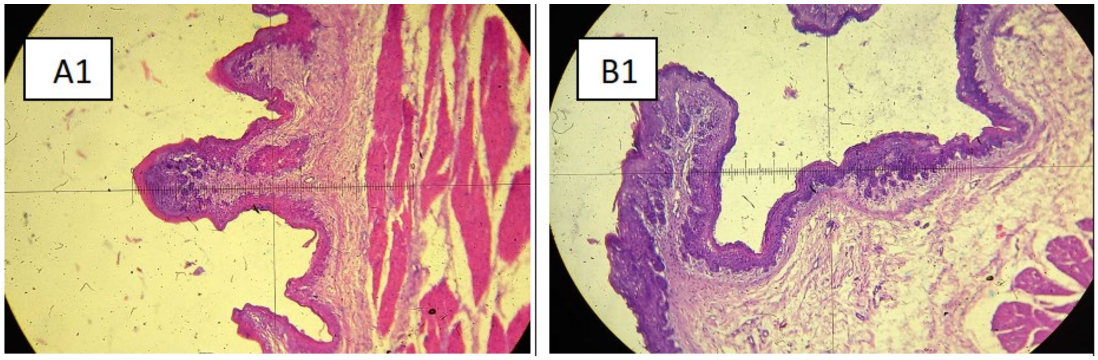
Mucosa, submucosa and Muscular externa

Table (3) and Figures (1-4) show the average mucosal thickness was significantly higher (15.83 µm) in the 60:40 concentrate-to-coarse ratio group compared to the 40:60 group (10.83 µm). Adding 10 g/kg of the enzyme to the diet increased average mucosal thickness from 11.00 µm to 19.33 µm, regardless of the concentration-to-bran ratio. The relationship between concentration-to-coarse ratio and enzyme supplementation was insignificant (LSD = NS interaction). The increased mucosal thickness, higher concentration-to-roughage ratio (60:40), and enzyme supplementation may be due to increased production of volatile fatty acids (VFA) and their absorption in the rumen. Increased VFA absorption is known to stimulate papillae growth and increase the mucosal surface area for nutrient absorption. Similar results have been reported in other studies examining the effects of diet composition on the morphology of rumen papillae in ruminants. The study (22) demonstrated on Naomi lambs that a high-concentrate diet resulted in increased rumen mucosa thickness compared to a forage-based diet. This suggests that increasing mucosal thickness in response to high-concentration diets is a consistent finding across different lamb breeds. (17) also reported that increasing the level of urea-treated straw in the diet resulted in a significant increase in rumen mucosa thickness. This indicates that the concentration-to-roughage ratio and the type of roughage (urea-treated straw) can affect the growth of rumen mucosa in Arabian lambs. However, (23) feeding lambs with different levels of granulated citrus pulp reported that as the level of citrus pulp increased (replacing barley grain), the thickness of the rumen mucosa decreased. This suggests that the type of concentrate (barley grain vs. citrus pulp) could have different effects on rumen mucosal development in lambs. The submucosal layer was statistically thicker (P<0.05) in the 60:40 (29.80 µm) concentration-to-coarse ratio group than in the 40:60 (28.50 µm) group, but the difference wasn't very important. The addition of 10 g/kg of enzyme to the diet increased the average submucosal thickness from 24.80 µm to 33.50 µm, regardless of the concentration-to-bran ratio. The relationship between concentration-to-coarse ratio and enzyme supplementation was not significant (LSD = NS interaction). The mean thickness of extracellular muscle was slightly higher in the 60:40 (150.00 µM) concentration-to-rough ratio group compared to the 40:60 (145.80 µM) group, but the difference was not statistically significant. Adding 10 g/kg of enzyme to the diet increased the average outer muscle thickness from 138.30 µm to 157.50 µm, regardless of the concentrate-to-bran ratio. The interaction between concentration-to-coarse ratio and enzyme supplementation was not significant (LSD = NS interaction). The lack of significant differences in submucosal thickness and extrinsic muscle thickness between dietary treatments may indicate that these structural components of the papillae are less responsive to the specific nutritional changes examined in this study

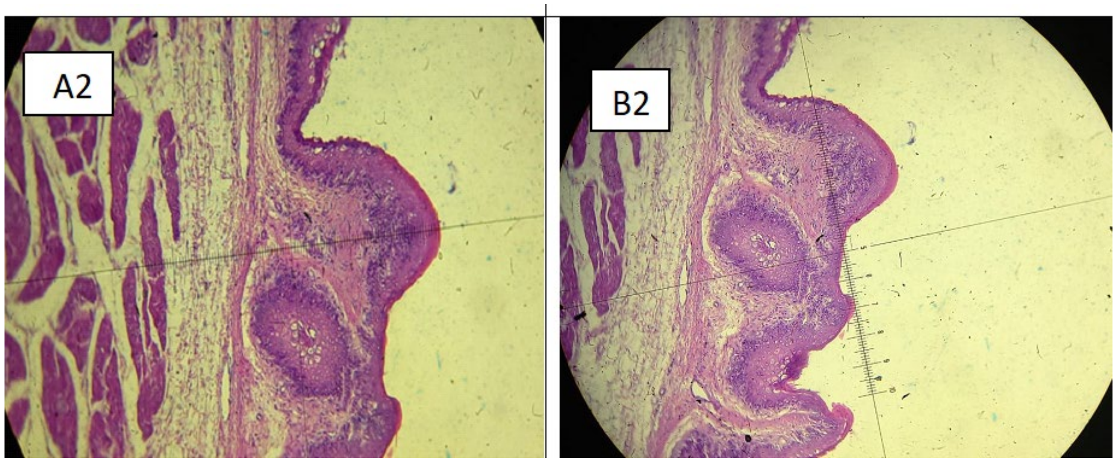
Table (3). Effect of different concentrate of roughage with different lignin peroxidase supplementation on the dimension of Mucosa, submucosa and Muscular externa of Arabi lamb's rumen (Mean +SE).

Concentrate: Roughage	Enzyme (gm/ kg) 0	10	Mean of diet
Mucosa (um)			
40: 60	9.67±0.58	12.00±0.56	10.83^{b}±0.75
60: 40	12.33±0.56	9.33±0.58	15.83^{a}±0.75
Mean	11.00^{b}±0.55	19.33^{a}±0.56	LSD interaction=NS
Submucosa (um)			
40: 60	24.70±0.11	32.30±0.10	28.50±0.12
60: 40	25.00±0.10	34.70±0.10	29.80±0.19
Mean	24.80±0.17	33.50±0.23	LSD interaction=NS
Muscular externa (um)			
40:60	140.00±4.45	151.70±3.63	145.80±5.15
60:40	136.70±3.62	163.30±3.62	150.00±4.73
Mean	138.30±3.24	157.50±6.59	LSD interaction=NS

Means with different superscript in each column differ significantly at P≤0.05

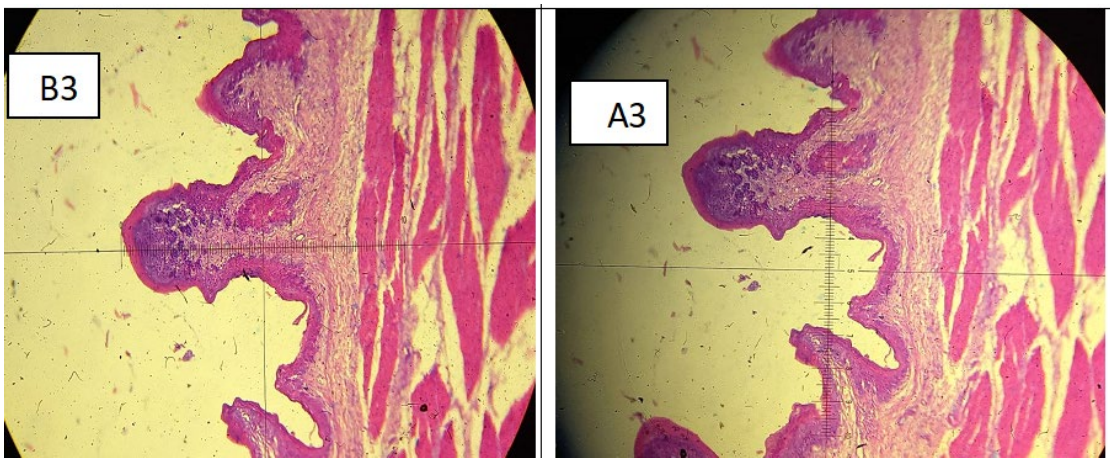


Figer (1): H&E (100x) of tissue section showing the length (A1) and width(B1) of the papillae in the rumen of sheep in the group treated with the Natozyme p50 enzyme+ 40% concentrated and 60% roughage feed.

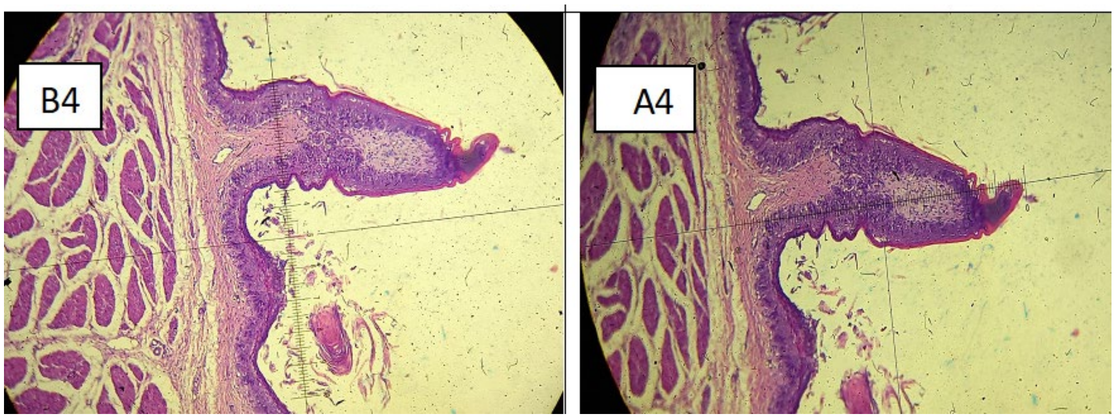


Figer (2): H&E (100x) of tissue section showing the length (A2) and width(B2) of the papillae in the rumen of sheep in the group untreated with the enzyme at concentrations of 40% concentrated and 60% roughage feed.

The development and growth of papillae involve complex interactions between various factors, and the submucosal and extramucous may be more influenced by other physiological or environmental factors. Similarly, the study (22) found that the thickness of the submucosa and extrinsic muscle did not differ significantly between forage-based diets and concentrate diets in Naemi lambs. This suggests that structural components of the papillae, other than the mucosa, may be less responsive. In the study by (17) on Arabi lambs, adding more urea-treated hay to their diet made both the submucosa and extrinsic muscles much thicker. This means that the type of roughage (urea-treated straw) might have a bigger impact on the rumen papillae's structural parts than the concentration-to-roughage ratio. On the other hand, research by (23) revealed a decrease in the thickness of the submucosa and exocrine layers as the level of granular citrus pulp increased. This suggests that the concentration type could influence the rumen papillae's structural components, similar to the effects observed on mucosal thickness.



Figer (3): H&E (100x) of tissue section of the papillae in the rumen of sheep in the group untreated with the enzyme+ 60% concentrated and 40% roughage feed showing the length (A3) and width(B3) of papillae



Figer (4): H&E (100x) of tissue section of the papillae in the rumen of sheep in the group treated with the enzyme+ 60% concentrated and 40% roughage feed showing the length (A3) and width(B3) of papillae.

Conclusion

The thickness of lambs' rumen papilla and mucosa is affected by diet type. There may be a complex interplay of trophic factors that affects the submucosa and muscular extra layer, as these responses vary. Their adaptations may not always match the changes seen in the mucosa. The adaptive responses of various structural components of the papillae can differ, underscoring the complexity of rumen development in response to changing nutritional inputs.

Conflicts of interest

The authors declare that there is no conflict of interest.

Ethical Clearance

This work is approved by The Research Ethical Committee.

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تأثير إنزيم بيروكسيديز اللجنين ومصدر العلف على أنسجة الكرش في الحملان العربية

جنان عبد العزيز بناي[1] ، هناء علي جبار الغالبي[2] , اميره كاظم ناصر[2] .

1-فرع الجراحة والتوليد، كليه الطب البيطري، جامعه البصرة، البصرة، العراق.

2- قسم الانتاج الحيواني، كلية الزراعة، جامعه البصرة، البصرة، العراق.

الخلاصة

هدفت الدراسة الحالية إلى بيان تأثير استخدام العلف المعامل بالإنزيمات في بعض أنسجة الكرش للحملان. أجريت هذه الدراسة في الحقل الحيواني/كلية الزراعة/جامعة البصرة ولمدة 105 يوماً. تم استخدام ستة عشر حملا من السلالة العرابية بعمر 4-5 أشهر وبوزن 22.25 كغم. تم توزيع الحملان عشوائيا على أربع مجاميع بواقع أربعة حملان لكل معاملة. تتكون العليقة المركزة بشكل أساسي من 54% شعير، 30% نخالة، 10% ذرة صفراء مطحونة، 5% فول الصويا، 1% فيتامينات وأملاح معدنية. تم تغذية الحملان بنسبة 3% من وزن جسمها الحي. تتكون العلائق المدروسة من 60% علف مركز و40% علف خشن بدون أو مع إضافة 10 غم أنزيم بيروكسيديز اللجنين أو 40% علف مركز و60% خشنة أيضاً بدون أو مع إضافة الأنزيم. أظهرت المجموعات التي غذائي بنظام غذائي مكمل بالإنزيمات ارتفاعًا ملحوظًا في طول وعرض والمساحة السطحية لحليمات الكرش ($P<0.05$) مقارنة بمجموعة السيطرة. كما أظهرت النتائج وجود تأثير معنوي ($P<0.05$) في كميات العلف المستهلكة والزيادة الوزنية في المجموعات التي تغذت علائق مدعمة بالإنزيمات مقارنة بمجموعة السيطرة. كما لوحظ تفوق معنوي للمجموعة المغذاة بتركيز 60% في الوزن بعد شهر من التأقلم وفي الوزن النهائي مقارنة بالحملان المغذاة عليقة مركزة بنسبة 40%، ولم يكن هناك تأثير معنوي في كمية العلف المركز المستهلك وكفاءة التحويل العلفي بينهما. يمكن أن تختلف الاستجابات للمكونات الهيكلية المختلفة للحليمات، مما يؤكد مدى تعقيد تطور الكرش استجابةً للمواد الغذائية المتغيرة.

الكلمات المفتاحية: الحملان العربية، الإنزيمات، انسجة الكرش.