The Effect of Exogenous Enzyme Supplementation on Digestibility, Feed Conversion Efficiency, and Weight Gain in Awassi Lambs

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Abstract
This study was conducted to investigate the effect of exogenous enzymes on nutrient digestibility and productive traits of local Awassi lambs, and to evaluate the possibility of utilizing these indicators for enhancing weight gain under field conditions at the Animal Farm, College of Agriculture, Tikrit University, during the period from October 7, 2024, to January 5, 2025.A total of 16 local Awassi lambs were used in this experiment and randomly allocated into four equal groups, with four lambs in each group. The first group served as the control, while the second group received 3 g of enzyme mixture in their diet. The third group was supplemented with 6 g of enzyme mixture, and the fourth group received 9 g of enzyme mixture in the ration.The results revealed significant differences ($P \leq 0.05$) in nutrient digestibility, feed intake, and feed conversion efficiency. A significant decrease was observed in ruminal fluid pH, accompanied by a significant increase in ruminal ammonia concentration (N-NH3) and volatile fatty acid (VFA) percentages. Moreover, exogenous enzyme supplementation significantly ($P \leq 0.05$) improved daily and total weight gain, as well as growth rate.

Keywords: Awassi lambs, Exogenous enzymes, Nutrient digestibility,Feed conversion efficiency, Rumen fermentation, Volatile fatty acids (VFA), Growth performance, Weight gain.

Introduction
Fibrolytic enzymes are commonly used to enhance the nutritional value of animal feeds. Therefore, researchers in the field of animal nutrition have focused on improving feed efficiency through the addition of enzymes that facilitate digestion. Supplementation with these enzymes has been reported to improve the digestibility of nutrients in ruminants [1]. Sheep diets typically contain high levels of fiber, which can be partially degraded by ruminal microorganisms and their associated enzymes. However, ruminal digestion is not fully efficient, and a considerable proportion of plant fiber remains undigested and is excreted without being utilized [2]. Ruminants rely on their symbiotic microbial populations, as they are unable to produce sufficient amounts of fibrolytic enzymes required for cellulose degradation. Recently, the incorporation of feed additives such as fibrolytic enzymes, probiotics, and yeasts into ruminant diets has attracted the attention of animal nutritionists due to their potential to enhance ruminal fermentation and consequently improve animal performance, particularly in young ruminants [5,9]. Fibrolytic enzymes can be categorized according to their specific activity, such as cellulases, which hydrolyze plant cell wall fibers into glucose, playing a vital role in the hydrolytic breakdown of fibrous feed components. The use of enzyme-based products with fibrolytic activity has been shown to increase feed intake efficiency and enhance fiber digestibility in the rumen of ruminant animals [6,12]. The inclusion of enzymes in calf diets has been shown to reduce nitrogen excretion through feces and urine [7]. Several studies

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have indicated that the increase in feed intake observed with fibrolytic enzyme supplementation may be partially attributed to improved palatability, resulting from the release of soluble sugars during fiber hydrolysis prior to ingestion, as well as to the post-ingestive effects of enzymes, such as enhanced efficiency and digestibility levels [10]. Supplementation with exogenous enzymes has also been reported to promote better utilization of nutrients, with higher digestibility of dry matter, fiber, and crude protein [13]. According to [12], the addition of a commercial enzyme product to agricultural residues, along with a locally produced enzyme extracted from white-rot fungi, improved the digestibility of organic matter, dry matter, and fiber. This, in turn, increased feed intake, enhanced feed conversion efficiency, and daily weight gain, ultimately lowering the economic cost of feeding. Similarly, [17] demonstrated that fibrolytic enzyme supplementation facilitated the degradation of plant cell walls, leading to the release of fermentable soluble sugars that could be utilized by bacteria to reduce silage acidity. Furthermore, previous studies have confirmed that locally produced enzymes possess the ability to degrade or cleave complex linkages within plant cell walls, thereby increasing the surface area available for ruminal microorganisms to access nutrients, improving the conversion of low-digestible feed into higher-quality rations [12]. Exogenous enzyme supplementation has also been shown to enhance fiber digestion by releasing sugars during hydrolysis, thereby stimulating microbial activity in the rumen [14]. According to [19], a significant improvement in weight gain and feed conversion efficiency was observed in male goats fed a free-choice diet supplemented with enzymes extracted from Aspergillus spp. and Trichoderma, compared with the enzyme-free diet. Similarly, a significant increase in final body weight and total weight gain of Awassi lambs was reported when Safizym enzyme was added to the concentrate ration [8]. A subsequent study demonstrated significant enhancements in daily weight gain, total volatile fatty acids (VFAs), and the proportions of butyrate and propionate, along with a decrease in ruminal pH value and a significant increase in ruminal ammonia nitrogen ($N\text{-}NH_3$) concentrations when enzymes were incorporated into sheep diets [14,17]. In another experiment [3], ammonia ($N\text{-}NH_3$) concentrations reached their peak three hours after fermentation when wheat bran was biologically treated and enzymes were added to rice straw, maize stover, olive cake, and date pits.

Material and Methods

This study was conducted at the Animal Farm, College of Agriculture, Tikrit University, during the period from October 7, 2024, to January 5, 2025. A preliminary adaptation period of 10 days was implemented to allow the lambs to acclimate to the experimental diet and the enzyme supplementation, which was introduced gradually prior to the start of the trial. A total of sixteen (16) local Awassi lambs, aged between 4 and 5 months with an average body weight of 19.4 ± 0.5 kg, were purchased from the local market in Salahuddin Province. The animals were randomly allocated into four groups, with each group comprising four lambs per treatment. The lambs were subjected to a comprehensive health and preventive care program as follows:

1. Vaccination against enterotoxemia at a dose of 2 mL per animal.
2. Vaccination against internal and external parasites using ivermectin at a concentration of 20% (5 mL per animal).

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3. Vaccination against liver flukes with closantel at a concentration of 5% (5 mL per animal).
4. Vaccination against general infections with oxyvaccine at a concentration of 20% (3 mL per animal).
5. Vaccination against foot-and-mouth disease (FMD) at a dose of 1 mL per animal. Diet and Feeding Regimen.

Diet and Feeding Regimen

The experimental diet, with its composition detailed in Table 1, consisted of barley, wheat bran, maize, soybean meal, wheat straw, and limestone. Animals were fed individually with two daily meals: the first at 08:00 h and the second at 14:00 h, with ad libitum access to clean water at all times. Feed was offered at a rate of 3.5% of live body weight, according to the experimental plan, and the amount of feed provided was adjusted weekly based on the weight gain of each animal.

Studied Traits

1. Digestibility Coefficient

Samples were collected during the digestibility trial in the last three days of the experiment as follows:[4].

. Feed Samples

A representative sample was taken from each diet, finely ground, and stored until chemical analysis was performed.

. Fecal Samples

Fecal samples from the digestibility trial were collected during the last three days of the experiment. For each animal, feces were collected for three consecutive mornings prior to feed distribution. The daily feces were weighed, and a subsample representing 10% of the total weight was placed in polyethylene bags and refrigerated. At the end of the collection period, the three daily fecal samples from each animal were pooled, homogenized, and a subsample representing 20% was taken. This composite sample was oven-dried at 65 °C for 24 hours, ground thoroughly, and stored in sealed bags for subsequent chemical analysis.

.Determination of Digestibility Coefficient

The digestibility coefficient was estimated as follows:

Digestibility % = { (Intake – Fecal Output) / Intake}×100

.Determination of Moisture Content

Moisture was determined by placing a weighed sample in a pre-dried porcelain crucible. The crucible with sample was weighed, then placed in an oven at 105 °C for 16 hours. Afterwards, it was transferred to a desiccator to cool to room temperature and weighed again. Moisture content was calculated using the following equation:

Moisture%={(InitialWeight-Dried Weight) /InitialWeight} ×100

.Determination of Ash Content

Ash content was determined by weighing 1 g of the sample into a pre-weighed porcelain crucible. The sample was placed in a muffle furnace at 600 °C for 3 hours, then cooled in a desiccator and reweighed. Ash percentage was calculated as:

Ash% = (Weight after Ashing /Initial SampleWeigh)×100

.Determination of Crude Protein

Total nitrogen content was measured using the Kjeldahl method. The sample was digested with concentrated sulfuric acid at 350–450 °C for 30 minutes until the solution became colorless. After cooling, the digested sample was diluted with distilled water and distilled using a Kjeldahl apparatus. Sodium hydroxide was added to convert ammonium salts to ammonia (NH_3), which was absorbed in

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boric acid containing an indicator. The distillate was titrated against 0.01 N hydrochloric acid, and nitrogen content was determined based on the volume of acid used. Crude protein was calculated as follows:

Nitrogen% = {(Volume of Acid \times ×Normality ×1.401)/Sample Weight}×100

Crude Protein% = Nitrogen % × 6.25

.Determination of Crude Fiber

Crude fiber was determined using the Weende method. A 1.5 g sample was mixed with 200 mL of 1.25% H_2SO_4 and boiled for 30 minutes with continuous stirring. The mixture was filtered through muslin cloth and washed thoroughly with hot water. The residue was then treated with 1.25% NaOH on a heating source to remove fats, filtered again, and oven-dried at 120 °C for 3 hours. After drying, the residue was incinerated in a muffle furnace at 550 °C for 60 minutes, cooled, and weighed to determine crude fiber content according to the following equation:

Crude Fiber% = {(Weight of crucible with residue after ashing - Weight of crucible with residue after drying)/ Initial Sample Weight}×100

.Determination of Crude Fat (Ether Extract)

Crude fat was determined using a Soxhlet apparatus. The sample was placed in a water bath with an organic solvent until boiling occurred. The solvent evaporated and condensed back in the condenser, allowing gradual extraction and dissolution of crude fat. The extraction process continued for 8 hours. Afterwards, the solvent was separated from the crude fat by evaporation in the Soxhlet unit. The residue was then oven-dried at 105 °C for 30 minutes in a ventilated oven, weighed, and crude fat percentage was calculated as follows:

Crude Fat % ={Weight of crude fat after extraction / Initial SampleWeight}×100

2. Rumen Fluid Sampling

Rumen fluid was collected before the morning feeding and 4 hours post-feeding using a suction pump. Approximately 200 mL of rumen fluid was obtained, and the pH was immediately measured using a digital pH meter. The fluid was filtered through medical gauze, and aliquots were transferred into special bottles and stored at −20 °C until further analysis.

.Determination of Ammonia

Five milliliters of rumen fluid were mixed with 45 mL of tungstic acid solution. The tungstic acid reagent was prepared by mixing 10 mL of 1 N sulfuric acid, 10 mL of 10% sodium tungstate solution, and 0.1 mL of orthophosphoric acid, diluted to 800 mL with distilled water. The mixture was preserved in sealed bottles and stored at −20 °C until analysis, following the method of [4]

. Volatile Fatty Acids (VFAs)

Volatile fatty acids were extracted by weighing 5 g of the sample and placing it in a dark 50 mL bottle. Twenty-five milliliters of ether were added, and the bottle was shaken for 5 hours. The mixture was then filtered, and the extract was stored in a refrigerator until analysis.

The determination of VFA was conducted at the Environment and Water Research Center, Directorate of Scientific Research, using a Shimadzu 2010 gas chromatograph (Japan) equipped with a flame ionization detector (FID). A capillary column (DB-1; 30 m × 0.25 μm × 0.25 mm) was used. The injector and detector temperatures were set at 280 °C and 330 °C, respectively. The column oven temperature was programmed from 90 °C to 150 °C at a rate of 5 °C/min.

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Nitrogen gas was used as the carrier gas at a flow rate of 105 KPa [16].

3. Animal Weight

• Total Weight Gain: Calculated as the difference between final and initial body weights.

• Daily Weight Gain: Calculated by dividing total weight gain by the number of experimental days.

• Growth Rate: Relative growth rate was calculated using the formula:

Growth Rate (%)=Total Weight Gain\Initial Weight× 100

4. Feed Conversion Efficiency

Feed conversion efficiency was calculated using the following formula:

Feed Conversion Efficiency = Feed Intake (kg)\Weight Gain (kg).

Table 1. Ingredients and Chemical Composition of the Experimental Diet forLambs

Ingredient	Inclusion (%)	DM%	CP%	CF%	EE %	ASH %
Barley	50	89	12	5	2.1	3
Yellow corn	20	88	8	2	6.9	2
Soybean meal	5	91	49	6	1.6	7
Wheat bran	20	89	17	11	4.5	7
Wheat straw	4	91	3	43	1.8	8
Limestone	1	88	0	0	0	98
Total	%100	89	13.6	7.12	3.48	4.95

Results and Discussion

The analysis results(Table 2) showed no significant effect of enzyme supplementation on dry matter and ash digestibility. The experiment results, however, indicated a significant increase ($P \leq 0.05$) in protein digestibility, while no significant effect was observed on crude fiber digestibility. Regarding ether extract digestibility, the results demonstrated a significant increase with enzyme addition. The analysis results (Table 3) indicated a significant decrease ($P \leq 0.05$) in ruminal pH for the third treatment before feeding on the enzyme-supplemented diet during the first period. However, no significant difference in pH was observed three hours after feeding. Moreover, the results showed a significant increase in ammonia nitrogen for the third treatment at both rumen fluid sampling periods. The results (Table 4) showed a significant superiority ($P \leq 0.05$) of the third and fourth treatments over the second treatment and the control in both rumen fluid sampling periods regarding the total volatile fatty acids (VFA) percentage. Moreover, the third and fourth treatments showed a significant increase ($P \leq 0.05$) in acetic acid percentage compared to the second treatment and the control during both sampling periods. The third treatment was significantly higher ($P \leq 0.05$) in butyric acid during both the first and second sampling periods compared to the other treatments, and a similar trend was observed for propionic acid. On the other hand, the third, second, and fourth treatments showed a significant decrease ($P \leq 0.05$) in the acetate-to-propionate ratio during the first sampling period, while no significant difference was observed during the second sampling period. The study results (Table 5) showed no significant effect of initial body weight. However, the

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third treatment exhibited a significant increase ($P \leq 0.05$) in final body weight compared to the fourth, second, and control treatments. Additionally, the third treatment showed a significant increase ($P \leq 0.05$) in total and daily weight gain as well as in growth rate compared to the fourth, second, and control treatments. The study results (Table 6) showed a significant superiority ($P \leq 0.05$) of the third treatment in daily and total feed intake compared to the other treatments. However, the third treatment exhibited a significant decrease in daily feed conversion efficiency.

Table 2. Effect of Enzyme Supplementation on Digestibility Coefficient

Treatments	DM%	ASH%	CP%	CF%	EE%
T1	63.64 ± 0.995 a	33.45 ± 8.66 a	76.08 ± 1.97 c	56.66 ± 7.06 a	56.11 ± 0.200 c
T2	62.93 ± 2.985 a	35.87 ± 9.645 a	84.81 ± 0.220 b	63.39 ± 9.325 a	58.91 ± 3.17 c
T3	65.63 ± 0.170 a	61.83 ± 7.845 a	92.72 ± 0.810 a	76.53 ± 3.905 a	77.29 ± 0.025 a
T4	64 ± 1.570 a	41.01130. ± a	87.29 ± 0.260 b	54.71 ± 1.695 a	65.95 ± 0.365 b

The letters within the same column differ significantly at the 0.05 level.

Table 3. Effect of Enzyme Supplementation on pH and Ammonia Nitrogen

Treatments	T1	T2	T3	T4
Traits				
Before feeding				
pH	6.5 ± 0.50 ab	7 ± 0.5 a	5.25 ± 0.250 b	6.25 ± 0.250 ab
N-NH3 mg/100ml	7.675 ±2.175 b	11.59 ± 1.83 ab	14.225 ± 0.275 a	9.90 ± 1.40 ab
Three hours after feeding				
pH	6.25 ± 0.75 a	5.75 ± 0.250 a	4.75 ± 0.250 a	5.75 ± 0.750 a
N-NH3 mg/100ml	11.6 ± 2.00 ab	9.16 ± 0.340 b	15.02 ± 0.50 a	11.24 ± 2.01 ab

The letters within the same row differ significantly at the 0.05 level.

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Table 4. Effect of Enzyme Supplementation on Volatile Fatty Acids

Before feeding

Treatments	Total VFA	Acetic acid%	Butyric acid%	Propanoic acid%	AC/PR
T1	46.575±4.275 c	35.45 ± 2.45 c	8.485 ± 1.235 b	2.640 ± 0.59 b	13.915±2.185 a
T2	57.265±5.745 bc	37.2 ± 2.40 c	13.655 ±1.455 ab	6.41 ± 1.89 ab	6.235 ± 1.465 b
T3	88.355±3.375 a	56.5 ± 1.7 a	22.99 ± 0.890 a	8.865 ± 0.785 a	6.405 ± 0.375 b
T4	69.14 ± 1.84 b	45.75 ± 1.55 b	17.65 ± 4.15 ab	5.74 ± 0.760 ab	8.08 ± 0.800 b

Three hours after feeding

Treatments	Total VFA	Acetic acid%	Butyric acid%	Propanoic acid%	AC/PR
T1	58.235±7.485 c	42.05 ± 2.85 b	10.775 ±3.225 c	5.41 ± 1.41 b	8.19 ± 1.610 a
T2	65.94 ± 3.43 bc	44.55 ± 2.25 b	14.275 ±0.945 bc	7.115 ± 0.235 ab	6.260 ± 0.110 a
T3	97.22 ± 1.84 a	63.6 ± 0.40 a	22.94 ± 0.620 a	10.68 ± 0.820 a	± 5.990.42 a
T4	82.5 ± 2.98 ab	54.4 ± 3.20 a	18.57 ± 1.33 ab	9.53 ± 1.550 ab	5.92 ± 1.30 a

The letters within the same column differ significantly at the 0.05 level.

Table 5. Effect of Enzyme Supplementation on Weekly Weight Gain

Treatments	Initial weight kg	Final weight kg	Weight gain kg	Daily weight gain/ gm	Growth rate
T1	19.29±0.357 a	27.225 ± 0.415 c	7.937 ± 0.199 c	88.177 ±2.219 c	41.195±1.258 c
T2	19.5 ±0.430 a	33.875 ± 0.748 b	14.375 ±0.899 b	159.7 ± 9.99 b	73.99 ± 5.827 b
T3	19.35±0.573 a	37.375 ± 0.542 a	18.025 ±0.103 a	200.27±1.145 a	93.415 ± 2.967 a
T4	19.45±0.688 a	33.7371.173 ± b	14.287 ±0.981 b	158.74±10.91 b	73.78 ± 5.747 b

The letters within the same column differ significantly at the 0.05 level

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Table 6. Effect of Enzyme Supplementation on Feed Intake and Feed Conversion Efficiency

Treatments	Feed intake rate gm/ day	Total feed intake kg	Feed conversion efficiency
T1	0.847 ± 0.0145 c	76.252 ± 1.311 c	9.617 ± 0.248 a
T2	0.945 ± 0.0186 b	85.927 ± 1.682 b	6.032 ± 0.317 b
T3	1.033 ± 0.0212 a	93.015 ± 1.912 a	5.157 ± 0.123 c
T4	0.967 ± 0.0323 ab	86.28 ± 3.169 b	6.092 ± 0.350 b

The letters within the same column differ significantly at the 0.05 level.

Conclusion

The use of enzymes led to improved digestibility coefficients, increased palatability of the feed, and enhanced utilization of feed components. Additionally, it caused changes in rumen acidity due to elevated levels of volatile fatty acids (VFA) and increased ammonia nitrogen (NH_3-N), resulting from protein degradation, deamination, and amino acid breakdown. This process converts insoluble protein into NH_3-N. The presence of exogenous enzymes also enhanced the production of VFA through the breakdown of plant fibers and improved the digestion of feed components by various microorganisms in the rumen, which ferment the feed and increase nutrient digestibility and feed efficiency. Enzymes act by breaking chemical bonds within plant cell walls, thereby optimizing the use of natural resources and reducing environmental impacts in animal production. They also enhance feed intake due to improved palatability, leading to better digestion and increased production. Furthermore, these enzymes function in the rumen shortly after feeding and before the onset of microbial enzyme activity, allowing synergistic interactions between exogenous and endogenous enzymes. This enhances fiber degradation and substrate breakdown during the early stages of digestion.

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