Effects of Dietary SupplementationwithMarjoramand Rosemary Powders and TheirMixture on SerumAST, ALT, and Glutathione Levels in Awassi Lambs

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Abstract

This study was conducted for a period of 70 days, preceded by a 15-day preliminary period to adapt the lambs to the concentrate diet. The experiment was divided into two periods (days 0–35 and days 36–70, respectively). Sixteen (16) Awassi lambs aged 4–5 months, with an average initial weight of 26.062 ± 2.25 kg, were used in the trial. The lambs were divided into four groups (four lambs per group), and the treatments were randomly assigned as follows:

- T1: Control diet
- T2: Addition of 2.5% dried marjoram per 100 kg
- T3: Addition of 1% dried rosemary per 100 kg
- T4: Addition of a mixture of 1.25% dried marjoram and 0.5% dried rosemary per 100 kg

The lambs in the above treatments were fed a concentrate feed at 3% of live body weight on a dry matter basis, along with 150 g/day/lamb of wheat straw. Animals were weighed weekly to adjust the provided feed quantity according to the new weight.

Treatments T2 and T4 recorded a significant decrease ($P \leq 0.05$) in AST enzyme activity on day 35, after which their activity rose significantly ($P \leq 0.05$) to lead the results on day 70. No significant differences ($P \leq 0.05$) were observed in ALT enzyme activity on day 35, whereas T4 and T2 significantly excelled ($P \leq 0.05$) on day 70, with T3 exhibiting the significantly lowest activity ($P \leq 0.05$). Treatments showed no significant differences in glutathione (GSH) concentration on day 35, while T3 recorded the significantly highest concentration ($P \leq 0.05$) on day 70.

Keywords: Marjoram and Rosemary, Enzyme Activity, Awassi Lambs.

Introduction

The livestock sector represents a fundamental pillar and an indispensable backbone for achieving global food security, as it contributes approximately 40% to the GDP of the agricultural sector globally [11] Sheep hold a prominent status, particularly in the Middle East and North Africa[17,2,]

This has led to a broad wave of research aimed at developing natural feed additives capable of filling the gap left by antibiotic growth promoters. Researchers and animal nutrition specialists have increasingly turned toward exploring the

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potential of phytogenic feed additives. Among these plant materials are marjoram (Origanum majorana) and rosemary (Rosmarinus officinalis), which are among the most widely used medicinal plants in animal studies due to their active compounds such as thymol, carvacrol, rosmarinic acid, carnosol, and other compounds possessing diverse antioxidant, antimicrobial, and anti-inflammatory properties[21 ،14 ، 6].

Several experiments have incorporated natural additives and herbs in the form of powder [8،13] or essential oils[23]. into ruminant feed due to their bioactive components, such as carvacrol in marjoram[24]. and their positive role in rumen fermentation and nutrient digestibility. However, these outcomes depend on the plant species, inclusion level, and method of addition—whether as a whole plant, powder, or oil extract[18]. This study aimed to detect the role of supplementation with Marjoram and Rosemary Powders and Their Mixture on Serum AST, ALT, and Glutathione Levels.

Materials and Methods

Location and Time of Experiment

This study was conducted in the sheep farm belonging to the Department of Animal Production / College of Agriculture – University of Tikrit from November 5, 2025, to January 15, 2026 (70 days), preceded by a 15-day preliminary adaptation period for the lambs to acclimate to the concentrate diet. The experiment was divided into two periods (days 0–35 and days 36–70, respectively).Management of Experimental Animals Sixteen (16) Awassi lambs with an average weight of 26.062 ± 2.25 kg were used, purchased from local markets in Saladin Governorate, ranging in age from 4 to 5 months. The lambs were weighed in the morning following the end of the adaptation period after withholding feed for 12 hours to establish the initial body weight using an electronic field scale. They were randomly assigned into four groups (4 animals/group) with close initial average weights (26 ± 2.25, 25.75 ± 1.35, 26 ± 1.3, and 26.5 ± 1.1 kg for groups 1, 2, 3, and 4, respectively).

Experimental Design

The experimental treatments were divided into four groups:

- T1: Control treatment
- T2: Addition of 2.5% dried marjoram
- T3: Addition of 1% dried rosemary
- T4: Addition of a mixture of 1.25% marjoram and 0.5% rosemary

Note: Both marjoram and rosemary were obtained from local markets in Samarra city.

All biosecurity and procedures for the herd were followed before starting, including preparing and disinfecting the barn and administering vaccinations.

Feeding System

Animals were fed throughout the 70-day trial in two daily meals (morning and evening) at a rate of 3% of live body weight to cover their nutritional requirements and achieve a targeted weight gain of 150 g/day/animal.[19]. Dietary ingredients were mixed thoroughly and stored in 50 kg plastic bags. The quantity of concentrate feed was adjusted based on weekly live body weight changes. Feed was offered twice daily at 08:00 AM and 04:00 PM. The inclusion percentages and chemical composition of the concentrate diet were as follows[20].

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Feedstuff	% in Diet	Chemical Component	%
Ground Barley	60	Dry Matter (DM)	89.90
Wheat Bran	26	Organic Matter (OM)	85.45
Soybean Meal	12	Crude Protein (CP)	15.60
Common Salt	1	Crude Fiber (CF)	7.40
Limestone	0.5	Ether Extract (EE)	2.19
Vitamin & Mineral Premix	0.5	Nitrogen-Free Extract (NFE)*	60.35
		Ash	4.36
		Digestible Protein (DP)	12.17
		Total Digestible Nutrients (TDN)	67.24
		Metabolizable Energy (ME, MJ/kg DM)	11.476

**Nitrogen-Free Extract (NFE) = Organic Matter - (Crude Protein + Crude Fiber + Ether Extract)*

Animal Weights

At the start of the experiment, lambs were weighed after 12 hours of feed deprivation prior to the morning feeding to record the initial body weight. Subsequently, lambs were randomly distributed into individual/group pens, and weighing continued weekly until the end of the trial. Refused feed was weighed daily to determine actual feed intake per treatment.

Blood Sampling

Blood samples (10 mL) were collected from the jugular vein of animals on days 35 and 70 using disposable syringes. Two milliliters (2 mL) were placed in tubes containing Ethylenediaminetetraacetic acid (EDTA) anticoagulant, while the remaining portion (8 mL) was placed in anticoagulant-free test tubes and left at room temperature (25 °C) for 15 minutes. Serum was separated from the clot and plasma by centrifugation at 3000 rpm for 15 minutes. The serum was aliquoted into clean 1.5 mL Eppendorf tubes and stored at -18 °C to preserve enzymatic activity until laboratory analysis.

Liver Function Measurement

Liver function was evaluated by analyzing serum samples for a group of enzymes and proteins reflecting liver cell integrity and functional capacity. Key indicators included Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), and Glutathione (GSH).

Note: Liver enzymes were measured using Enzyme-Linked Immunosorbent Assay (ELISA) kits supplied by the Chinese manufacturer Sunlong (SL000445p for GSH , SLD00144Sp for AST , SL00046Sp for ALT).

Statistical Analysis

Data were statistically analyzed using SPSS version 27 according to a 4 × 2 factorial arrangement in a completely randomized two-way design to evaluate the effects of treatments and periods on studied parameters. Differences among means were tested using Duncan's Multiple Range Test according to the following [6] mathematical model:

$$Y_{ijk} = \mu + T_i + S_j + TS_{ij} + e_{ijk}$$

Where:

Y_{ijk}: Observation value k belonging to treatment i and period j.

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μ: Overall mean.

T_i: Effect of treatment i.

S_j: Effect of period j.

TS_ij: Interaction effect between treatment i and period j.

e_ijk: Random error, normally distributed with mean zero and variance σ^2_e.

Results and Discussion

Effect of Supplementing Marjoram and Rosemary Powders and Their Mixture on Enzyme Activity

AST Enzyme

Figure (1) indicates significant effects ($P \leq 0.05$) of additive type and trial period on serum AST activity (IU/L) in Awassi lambs. On day 35, the marjoram treatment (T2) and the mixture treatment (T4) recorded a noticeable drop in AST activity compared to the control (T1) and rosemary treatment (T3), which recorded the highest significant values. As the experiment progressed to day 70, a significant shift occurred: AST activity increased in T2 and T4 to record the highest levels, whereas T1 and T3 experienced a significant decrease in comparison.

The fluctuation of AST activity within normal physiological limits is attributed to active hepatic metabolic responses to bioactive compounds present in marjoram and rosemary. The significant elevation in T2 and T4 on day 70 does not indicate a pathological state, but rather an elevated metabolic rate and enhanced muscle protein synthesis in line with advancing animal age and physiological demand. These results confirm that the plant supplements maintained liver and muscle tissue integrity and that phytogenic stimulants enhance metabolic efficiency without causing organic stress[10]. Essential oils in marjoram and rosemary exert hepatoprotective properties and balance blood enzymes, contributing to improved feed conversion efficiency and biological safety in small ruminant nutrition[9].

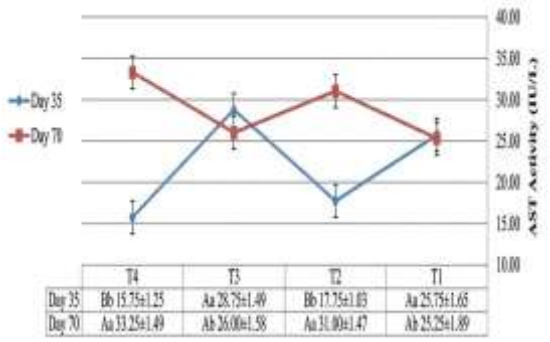


Figure (1) indicates significant effects ($P \leq 0.05$) of additive type and trial period on serum AST activity (IU/L) in Awassi lambs.

ALT Enzyme

On day 35, Figure (2) shows no significant differences in serum ALT activity (IU/L) among all experimental groups. This initial stability reflects homogeneous hepatic functional health prior to cumulative phytogenic effects. By day 70, a clear significant variance emerged: T4 and T2 recorded the highest enzyme activity levels compared to control. Conversely, T3 exhibited the lowest significant activity. Over time, T3 and T4 maintained statistically stable enzyme levels between both periods, whereas T2 and T1 experienced a significant increase at day 70 compared to day 35.

The significant reduction in ALT activity in the rosemary treatment (T3) is attributed to essential oils like carnosol and rosmarinic acid, which protect hepatocyte membranes by acting as natural antioxidants, preventing enzyme leakage into the bloodstream [16]. The observed physiological elevation in other treatments within normal limits reflects increased protein metabolism and intensive nutrient conversion to support rapid growth and

weight gain during the late fattening phase [19]. In the study of mention it found that the type of food alter the levels of liver enzymes toward decrement about 16% in summer[1]

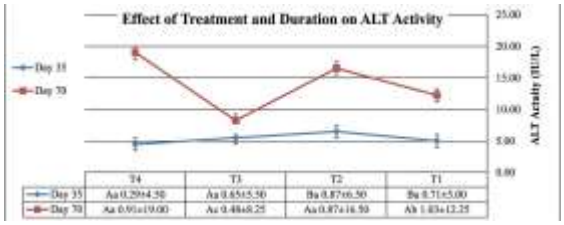


Figure (2) shows no significant differences in serum ALT activity (IU/L) among all experimental groups.

Glutathione (GSH) Concentration

Figure (3) demonstrates a significant effect ($P \leq 0.05$) of dietary supplementation on serum GSH concentration (µmol/L). At day 35, no significant differences were detected among treatments (T1, T2, T3, and T4). By day 70, T3 recorded the significantly highest GSH concentration (39.62 µmol/L), followed by T2 and T4, while T1 recorded the lowest (30.52 µmol/L).

Comparing day 35 to day 70, T1 and T4 maintained steady GSH levels, while T2 and T3 exhibited a significant increase. This superiority is attributed to the rich phenolic and volatile oil profile of rosemary and marjoram (e.g., rosmarinic acid, carnosol, thymol), which stimulate endogenous antioxidant production and activate the glutathione system, protecting cellular membranes against oxidative stress [7 , 15].

The differences observed when comparing these results with other studies on sheep may be attributed to individual variation or genetic factors [3] This aligns with the findings of study (24), which demonstrated that ALT and AST enzyme activities can serve as reliable indicators for assessing liver health in sheep [5] Furthermore, the results of study (25) indicated that elevated liver enzyme levels were linked to increased biological activity in Awassi rams during the breeding season; specifically, rams treated with Gonadotropin-Releasing Hormone (GnRH) exhibited higher levels of ALT, AST, and ALP compared to control groups, both during and outside the breeding season [4]

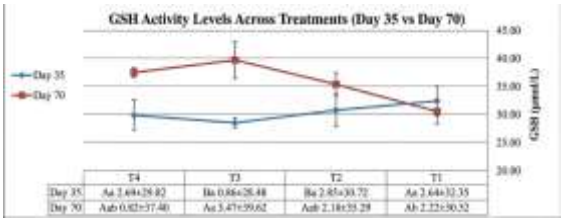


Figure (3) demonstrates a significant effect ($P \leq 0.05$) of dietary supplementation on serum GSH concentration (µmol/L).

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