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Effects of Moringa oleifera Leaf Supplementation on Growth Performance, Biochemical Parameters, and Antioxidant Gene Expression in Awassi Lambs

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Abstract

Because they contain bioactive chemicals that promote growth and enhance health, plant-based feed additives such as Moringa oleifera have recently attracted increased amounts of attention. Lambs in dry locations suffer from oxidative stress and nutritional deficits that impact their productivity. The objective of this study was to assess the effects of varying amounts of Moringa oleifera leaf powder (1.5%, 3.0%, and 4.5%) on growth performance, specific blood biochemical parameters, oxidative stress indices, and antioxidant enzyme-encoding gene expression in Awassi lamb feeds. In this study, 24 Awassi lambs (22±3 kg) were randomly divided into four groups (6 per group) for 90 days. Measurements included body weight, feed consumption, serum analysis (glucose, total protein, cholesterol, urea, total antioxidant capacity (TAC), and malondialdehyde (MDA)), and gene expression analysis of the SOD1, GPX1, CAT, and Nrf2 genes using PCR technology. The results showed that the addition of moringa to lamb feeds led to a significant improvement in production performance, with the 4.5% group outperforming the others in terms of final weight.

(36.89 kg), average daily gain (187 g/day) and best feed conversion ratio (6.60). The total protein concentration (72.9 g/L), total antioxidant capacity (0.99 µmol), cholesterol concentration (2.25 mmol/L) and malondialdehyde concentration (3.11 µmol) also significantly increased. At the molecular level, the gene expression of all the studied genes (SOD1, GPX1, CAT, and Nrf2) significantly increased, especially in the 4.5% group. The results suggest that up to 4.5% moringa leaf powder could be considered a natural addition to lamb feeds to improve growth and enhance antioxidant defense in dry areas.

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Introduction

Small ruminants in arid zones are afflicted with heat stress, nutritional deficiencies, and exposure to pathogens (Berihulay et al., 2019; Kareem et al., 2024). Phytogenic feed additives have recently attracted much attention because of their bioactive components, which increase metabolism, improve rumen fermentation, and modulate the antioxidant defense system while reducing the dependence on synthetic growth promoters (Tsiplakou et al., 2021; Egash & Teyene, 2024; Gessner et al., 2017). Moringa oleifera has been termed a “miracle tree" because it is among the most nutritionally valuable fodder plants because of its high crude protein content (23–30%), a profile rich in essential amino acids, and high amounts of vitamins A, C, and E plus minerals such as potassium, calcium, and iron (Rizwan et al., 2024; Abbas et al., 2018; Anwar et al., 2007). The phytochemical compounds in Moringa leaves are also effective at fighting bacteria, reducing cholesterol, and possessing high antioxidant value (Maizuwo et al., 2017; Sreelatha & Padma, 2009). The bioactive compounds in ingestible supplements increase the potential for microbial action in the rumen, increase the digestibility of nutrients, and counteract oxidative stress, which results in a higher ruminant productivity rate (Samal et al., 2015; Al-Shaar et al., 2023; Wafa et al., 2017). In addition, other studies have shown that supplementation with Moringa oleifera to sheep and goats increases their antioxidant potential and feed efficiency for weight gain (Belhi et al., 2018; Kholif et al., 2015). Most of the published studies related to growth and biochemical reactions do not offer much insight into the molecular aspects of antioxidants, such as how the Nrf2 pathway works. Awassi lambs are a major type of sheep that are ideal for rearing in the Middle East; hence, this study used Awassi lambs (Saha et al., 2020; Mustafa et al., 2023). The goal of the current research was to evaluate the effects of varying amounts of Moringa oleifera leaf powder on growth performance, specific blood biochemical markers, oxidative stress biomarkers, and the expression of antioxidant-related genes in Awassi lambs. It was predicted that by upregulating important antioxidant genes in a dose-dependent manner, moringa supplementation would enhance growth performance and antioxidant status to determine whether moringa supplementation may be used as a form of sustainable feed addition in small ruminant production systems in dry environments.

Materials and Methods

Experimental Site and Ethical Approval:
Under carefully monitored housing and management circumstances, this research was carried out in the spring of 2025 at the Animal Research Station, the Division of Animal Production Technologies, Technical Agricultural College, Northern Technical University, Mosul, Iraq. All animal-related operations were carried out in accordance with the rules set out by Northern Technical University's Animal Welfare Committee (Approval ID: NTU-AWC-2024-MOL-015).

Animals, Experimental Design, and Diets
Twenty-four clinically healthy Awassi male lambs (10–12 months old; initial body weight 22 ± 3 kg) were randomly assigned to a completely randomized design (CRD) consisting of four dietary treatments (n = 6/group):

- T1: Control diet (0% Moringa oleifera leaf powder)
- T2: Diet containing 1.5% Moringa oleifera leaf powder
- T3: Diet containing 3.0% Moringa oleifera leaf powder
- T4: Diet containing 4.5% Moringa oleifera leaf powder

Diets were formulated to meet or exceed the NRC (2007) nutrient requirements for growing lambs (Table 1). Wheat straw was freely available, and a pelleted concentrate combination was served twice a day. After being shade-dried and powdered into 1–2 mm particles, the concentrate was completely combined with Moringa oleifera leaves (Sarwatt et al., 2004). Body weights were monitored every two weeks, and food consumption was recorded every day. To ensure that any changes could be attributable to the bioactive components in Moringa rather than variations in crude protein or calorie consumption, the experimental diets were designed to be roughly isonitrogenous and isoenergetic by varying the inclusion rate of soybean meal.

Table 1: Ingredients and chemical composition of the experimental concentrate diets (%, on a dry matter basis).

Ingredients (%)	**Control (T1)**	**1.5% Moringa (T2)**	**3.0% Moringa (T3)**	**4.5% Moringa (T4)**
Crushed barley	66.5	66.0	65.5	65.0
Wheat bran	19.0	19.0	19.0	19.0
Moringa oleifera leaf powder	0.0	1.5	3.0	4.5
Soybean meal	11.0	10.0	9.0	8.0
Urea	0.75	0.75	0.75	0.75
Salt	1.0	1.0	1.0	1.0
Limestone	1.0	1.0	1.0	1.0
Sodium bicarbonate	0.75	0.75	0.75	0.75
Chemical composition (% of DM)				
Dry matter	92.35	92.43	92.38	92.40
Crude protein	16.42	16.32	16.22	16.10
Ether extract	2.85	2.90	2.95	3.00
Crude fibre	8.50	8.60	8.70	8.80
Ash	6.80	6.90	7.00	7.10
Nitrogen-free extract	57.78	57.71	57.51	57.40
Metabolizable energy (kcal/kg)	2524	2512	2505	2495

Chemical analysis of the components of the concentrated feed was performed according to (AOAC, 2000), and the energy value of the concentrated feed was calculated according to (MAFF 1975).

Growth Performance Evaluation

During the 90-day feeding study, the average daily gain (ADG) was computed using the starting and end weight data. Daily dry matter intake (DMI) divided by ADG was used to calculate the feed conversion ratio (FCR). Notably, wheat straw was provided collectively on a per-group basis; therefore, the DMI and FCR data presented in this study refer exclusively to the intake of concentrated feed.

Blood Sampling and Biochemical Analysis

On day 90, blood samples were collected from the jugular vein into clot-activator tubes. Serum was separated by centrifugation (3000 rpm for 10 min). Glucose, total protein, urea, cholesterol, total antioxidant capacity (TAC), and malondialdehyde (MDA) were analysed using commercial ovine (sheep)-specific diagnostic kits (BioSystems SA, Spain; catalogue numbers: GLUC, TP-200, UREA, CHOL, TAC-520, MDA-510) using a spectrophotometer and by wavelength indicated in the manufacturer's instructions.

Antioxidant Gene Expression

A spin-column isolation kit (RNeasy Plus Mini Kit, Qiagen, Germany) was used to extract total leukocyte RNA, which was then quantified using a NanoDrop spectrophotometer. A RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA) was used to synthesize complementary DNA (cDNA). SYBR Green Master Mix was used for quantitative PCR using a Step One Plus™ Real-Time PCR System (Applied Biosystems, USA). β-actin served as the housekeeping gene, and the target genes were SOD1, GPX1, CAT, and Nrf2. The specific primer sequences that were employed are listed in Table 2. The $2^{-\Delta\Delta Ct}$ approach was validated by calculating the amplification efficiency for each primer pair using standard curves, which varied from 95% to 103%. The reference gene (β-actin) was found to be stable since there was no significant difference in its cycle threshold (Ct) values across treatment groups ($P > 0.05$). The $2^{-\Delta\Delta Ct}$ technique was used to determine the relative fold change (Livak & Schmittgen, 2001). White blood cells (leukocytes) were chosen for this analysis because their primary function is to defend the body. As a result, their oxidative status is always vigilant and unstable since they are biologically engineered to generate copious quantities of reactive oxygen species (ROS) as part of their method for eliminating microorganisms. This means that, even if the condition of metabolic tissues such as the liver or muscle is not accurately understood, monitoring their antioxidant gene expression is highly sensitive to the body’s ability to fight oxidative stress.

Table 2. Primer sequences, amplicon size, accession numbers, and amplification efficiency used for quantitative real-time PCR analysis of antioxidant-related genes.

Gene	**Primer Sequence (5' → 3')**	**Amplicon Size (bp)**	**Accession No.**	**Efficiency (%)**
SOD1	F: CATTCCATCATTGGCCGTAC R: GCCATGCTTCCCACACAT	120	NM_001145185	98.5
GPX1	F: CAACCAGTTTGGGCATCAG R: TCTCGGCATTTCGATCTCC	135	NM_001114188	97.2
CAT	F: CATGGTCTGGGACTTCTGG R: CAAGTTTTTGATGCCCTGGT	145	NM_001035266	95.8
Nrf2	F: AGCCCAGTCTTCATTGCAG R: TGGGCTGGAATAATGGTGA	150	XM_027968690	101.0
β-actin	F: TGGACTTCGAGCAGGAGAT R: GGATGTCGACGTCACACTTC	110	NM_001009784	99.1

F: Forward primer; **R:** Reverse primer.

Primer sequences were designed using the Primer-BLAST tool on the basis of gene sequences obtained from the National Center for Biotechnology Information database

Chemical Analysis of Moringa oleifera Leaves

Three replicates of Moringa oleifera powder were taken, and using accepted techniques, the approximate composition of Moringa oleifera leaf powder was determined (AOAC, 2005). The Kjeldahl technique (N × 6.25) was used to estimate crude protein, Soxhlet extraction was used to identify crude fat, the Van Soest method was used to determine crude fibre, and cremation at 550°C was used to determine ash. The Folin–Ciocalteu technique (Singleton et al., 1999) was used to calculate the total phenolic content. The values are expressed in mg of gallic acid equivalent per gram (mg GAE/g). We calculated the total flavonoid content using the aluminum chloride colorimetric method described by Zhishen et al. (1999). We determined the total amount of flavonoids in milligrams of quercetin per gram of sample. The 2,6-dichlorophenolindophenol titration method was used to determine how much vitamin C was present in the sample, while the high-performance liquid chromatography method described in Desai (1984) was used to determine how much vitamin E was present in the sample.

Statistical Analysis

We employed SPSS version 26.0 for data analysis. This study employed a one-way ANOVA with a completely randomized design. The significance of differences in means was set to a probability of less than 0.05. For gene expression data, statistical analysis was performed on the ΔCt values to ensure normality of distribution before the data were converted to fold-change ($2^{-\Delta\Delta Ct}$) for presentation in tables. Duncan's multiple range test was used for mean separations, as it is a commonly accepted post hoc test in agricultural and livestock research.

Results

Chemical Composition of Moringa oleifera Leaves

The results of the analysis of the Moringa oleifera leaf sample used in this research revealed high nutrient value (Table 3). They are rich in nutrients, with total phenolics of 48.2 mg GAE/g, total flavonoids of 28.7 mg QE/g, vitamin C of 245 mg per 100 g, and vitamin E of 45 mg per 100 g. In terms of nutrition, they contain 26.5% crude protein and 5.8% crude fat. The values are presented as the mean ± standard deviation (SD) of three independent replicate analyses (n=3).

Table 3: Chemical composition and bioactive compounds of the *Moringa oleifera* leaves used in the study (means ± SDs, n=3).

Component	**Value**	**Unit**
Moisture	8.2 ± 0.3	%
Crude protein	26.5 ± 1.2	%
Crude fat	5.8 ± 0.4	%
Crude fibre	12.3 ± 0.8	%
Ash	9.1 ± 0.5	%
Total Phenolics	48.2 ± 2.1	mg GAE/g
Total Flavonoids	28.7 ± 1.5	mg QE/g
Vitamin C	245 ± 12	mg/100 g
Vitamin E	45 ± 3	mg/100 g

GAE: Gallic Acid Equivalent; QE: Quercetin Equivalent.

Growth Performance

Moringa oleifera supplementation in their diet significantly affected lamb performance ($p < 0.05$) (Table 4). The final body weight, total weight gain, and ADG increased with increasing dosage, with the 4.5% group having the greatest increase. On the other hand, the FCR improved greatly with increasing amounts of Moringa supplementation, indicating improved efficiency. The dry matter intake in this study did not differ significantly among the treatments.

Table 4: Effect of *Moringa oleifera* leaf powder supplementation on the growth performance of Awassi lambs (mean ± SEM, n=6).

Parameter	**Control (T1)**	**1.5% Moringa (T2)**	**3.0% Moringa (T3)**	**4.5% Moringa (T4)**	**p value**
Initial weight (kg)	20.11 ± 0.90	20.05 ± 1.00	20.13 ± 0.80	20.08 ± 1.10	0.943
Final weight (kg)	32.45 ± 1.30	34.00 ± 1.40	35.65 ± 1.60	36.89 ± 1.50	**0.021**
Total weight gain (kg)	12.34 ± 0.70	13.95 ± 0.80	15.52 ± 0.90	16.81 ± 0.80	**0.014**
Average daily gain (kg/day)	0.137 ± 0.010	0.155 ± 0.010	0.172 ± 0.010	0.187 ± 0.010	**0.011**
Dry matter intake (kg/day)	1.19 ± 0.04	1.20 ± 0.05	1.22 ± 0.05	1.23 ± 0.05	0.317
Feed conversion ratio	8.70 ± 0.50	7.80 ± 0.40	7.10 ± 0.30	6.60 ± 0.30	**0.009**

*Different superscript letters within the same row indicate significant differences ($p < 0.05$) according to Duncan's multiple range test. DMI and FCR data refer to the intake of the concentrated feed only, as wheat straw was provided collectively. FCR = Dry matter intake (kg/day)/Average daily gain (kg/day).

Blood Biochemical and Oxidative Status

Higher concentrations of Moringa oleifera in the diet significantly ($p < 0.05$) increased total protein and TAC while lowering cholesterol and MDA (Table 5). The glucose concentration slightly but significantly increased, whereas the urea concentration remained within the normal physiological range in all the groups.

Table 5: Effect of *Moringa oleifera* leaf powder supplementation on blood biochemical and antioxidant marker levels in Awassi lambs (mean ± SEM, n=6).

Parameter	**Control (T1)**	**1.5% Moringa (T2)**	**3.0% Moringa (T3)**	**4.5% Moringa (T4)**	**p value**
Glucose (mmol/L)	3.49 ± 0.15	3.56 ± 0.14	3.61 ± 0.12	3.69 ± 0.11	**0.049**
Total protein (g/L)	68.5 ± 1.8	71.5 ± 1.6	72.1 ± 1.5	72.9 ± 1.3	**0.031**
Urea (mmol/L)	4.45 ± 0.20	4.31 ± 0.18	4.28 ± 0.15	4.24 ± 0.14	0.265
Cholesterol (mmol/L)	2.91 ± 0.10	2.61 ± 0.09	2.43 ± 0.08	2.25 ± 0.07	**0.013**

Parameter	Control (T1)	1.5% Moringa (T2)	3.0% Moringa (T3)	4.5% Moringa (T4)	p value
Total antioxidant capacity (μM)	0.95 ± 0.05	0.97 ± 0.04	0.98 ± 0.04	0.99 ± 0.03	**0.028**
Malondialdehyde (μM)	3.48 ± 0.16	3.35 ± 0.15	3.22 ± 0.13	3.11 ± 0.10	**0.019**

Different superscripted letters within the same row indicate significant differences (p < 0.05) according to Duncan's multiple range test.

Antioxidant Gene Expression

SOD1, GPX1, CAT, and Nrf2 expression was significantly greater in treated lambs than in control lambs ($p < 0.05$) (Table 6). The 4.5% Moringa group showed the greatest change, indicating a dose–response relationship.

Table 6: Relative expression of antioxidant-related genes in the leukocytes of Awassi lambs supplemented with *Moringa oleifera* leaf powder (mean ± SEM, n=6).

Gene	Control (T1)	1.5% Moringa (T2)	3.0% Moringa (T3)	4.5% Moringa (T4)	p value
SOD1	1.00 ± 0.05	1.28 ± 0.07	1.41 ± 0.06	1.59 ± 0.08	**0.008**
GPX1	1.00 ± 0.06	1.22 ± 0.05	1.36 ± 0.05	1.52 ± 0.07	**0.011**
CAT	1.00 ± 0.07	1.19 ± 0.06	1.29 ± 0.05	1.37 ± 0.06	**0.017**
Nrf2	1.00 ± 0.04	1.16 ± 0.05	1.27 ± 0.05	1.35 ± 0.05	**0.031**

Different superscript letters within the same row indicate significant differences ($p < 0.05$). Relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method, with β-actin used as the housekeeping gene. Statistical analysis was performed on the ΔCt values to ensure normality. The n=6 represents six independent biological replicates (individual lambs). *

Discussion

Moringa oleifera leaf powder supplementation increased the rate of gain, improved the antioxidant index, and modulated gene expression. The results of the biochemical analysis validated the substantial nutritional values of the leaves employed in this experiment, which included a substantial amount of crude protein (26.5%), GAE/g total phenolics (48.2 mg), QE/g total flavonoids (28.7 mg QE/g total flavonoids), and high amounts of antioxidant vitamins C and E (28.7 mg/g) (Table 3). The substantial amount of important phytochemical constituents explains a plausible scientific reason for the outcomes of this study. The dose-dependent increase in average daily gain along with a corresponding improvement in the feed conversion ratio indicate that supplementation with moringa has a positive role in enhancing nutrient bioefficacy. The substantial amount of protein, especially a well-balanced amino acid profile, in moringa leaf supplementation possibly explains their role in protein and muscle development, as evidenced by improved blood total protein. The improvement in FCR without a corresponding increase in dry matter intake indicates enhanced nutrient utilization, as also reported by Son et al. (2024). Notably, the diets were formulated to be approximately isonitrogenous, which supports the interpretation that the growth benefits are due to the phytochemicals in Moringa rather than just higher protein intake. These findings are consistent with past research showing that feeding Moringa to small ruminants improved their weight growth and feed efficiency. This can be explained by the high-quality protein, necessary amino acids, and easily fermentable elements of the plant that promote rumen microbial protein production (Belhi et al., 2018; Samal et al., 2015). Improved energy metabolism and nitrogen retention as a result of better digestibility and effective rumen fermentation may account for the observed increases in blood glucose and total protein levels. Despite higher growth performance, the steady urea levels across all treatments resulted in enhanced nitrogen consumption efficiency in lambs supplemented with moringa, which is consistent with the results of Berjawi (2025) for dairy cattle. The reduction in serum cholesterol with increasing Moringa inclusion supports the hypolipidemic effects of flavonoids and isothiocyanates, which modulate cholesterol synthesis and improve hepatic lipid clearance, which is consistent with the findings of Kumar et al. (2025), Saadi et al. (2024), and Nejad et al. (2020). Oxidative stress markers also responded significantly to supplementation. The notable increases in TAC and decreases in MDA observed might provide strong evidence that moringa enhances systemic antioxidant defense by scavenging free radicals through high concentrations of total phenolics (48.2 mg GAE/g), flavonoids (28.7 mg QE/g), vitamin C (245 mg/100 g), and vitamin E (45 mg/100 g), thereby preventing lipid peroxidation. These results support previous reports that Moringa leaves are a source of antioxidant compounds that reduce oxidative damage to livestock under metabolic and environmental stresses (Wafa et al., 2017; Mahmoud et al., 2019). At the molecular level, the upregulation of the SOD1, GPX1, CAT, and Nrf2 genes proves that Moringa is an activator of endogenous antioxidant pathways. The high phenolic and flavonoid contents in the Moringa leaves likely stimulated the Nrf2/ARE signalling pathway to increase the transcription of antioxidant enzymes. Nrf2 is a master transcription factor that regulates responses to antioxidants, and its increased expression indicates that phytochemicals from Moringa stimulate the Nrf2/ARE axis, thereby enhancing the transcription of major antioxidant enzymes (Afolabi et al., 2026; Manjunath et al., 2023). The physiological roles of moringa in preserving redox equilibrium and shielding young lambs from oxidative stress shock are reinforced by this

kind of molecular knowledge. Moringa oleifera may work through both cellular and nutritional channels, according to the biochemical and genetic reactions linked to increased growth efficiency. This thorough examination of Moringa leaf biochemistry provides a solid foundation for understanding dose-related gains in growth performance, antioxidant status, and genetic expression. Specifically, even at 4.5% integration, there were no toxicological effects, indicating that Awassi lambs can withstand the inclusion of moringa leaves without experiencing any negative consequences, confirming its usage as recommended by Dare (2022). Although these findings are encouraging, there are several limitations to this study. Although the sample size of six lambs per group is adequate for a controlled experiment, further extensive field research would be helpful to show that these results can be applied to commercial herd circumstances. Furthermore, the assessment of gene expression in leukocytes may not accurately reflect the antioxidant state in main metabolic organs such as the liver or muscle but may be a sensitive predictor of oxidative stress reactivity. The results concerning improved antioxidant defense might be strengthened by additional research using protein expression analysis or enzyme activity testing. These results are crucial for understanding sustainable lamb production, particularly in dry environments where oxidative stress and feed quality are key issues.

Conclusion

Awassi lambs' growth performance, biochemical conditions, and antioxidant gene expression significantly improved when up to 4.5% Moringa oleifera leaf powder was added to their meals. Moringa leaves are rich in antioxidant-producing vitamins, protein, polyphenols, and flavonoids. The inclusion of moringa leaves improved the antioxidant status at the molecular and biochemical levels and accelerated development. These results suggest that moringa may be used in sheep raising as a natural growth stimulant and antioxidant, providing a possible method to increase ruminant productivity in dry regions. For developing lambs, we thus advise the addition of Moringa oleifera leaves to livestock feed up to a level of 4.5%. However, as their physiological reactions can differ, the relevance of this study to other animal groups (such as ewes, lactating animals, or younger weaned lambs) needs more research.

Ethical and Environmental Considerations
Under carefully monitored housing and management circumstances, this research was carried out in the spring of 2025 at the Animal Research Station, the Division of Animal Production Technologies, Technical Agricultural College, Northern Technical University, Mosul, Iraq. All animal-related operations were carried out in accordance with the rules set out by Northern Technical University's Animal Welfare Committee (Approval ID: NTU-AWC-2024-MOL-015).

Author Contributions
All the authors contributed equally.

Conflict of Interest
The authors declare that there are no conflicts of interest.

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