

Dietary Silymarin (*Silybum marianum*) Supplementation Modulates the Adrenal, Thyroid, and Metabolic Hormone Axes in Growing Awassi Lambs: A 90-Day Feeding Trial

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KEYWORDS

Milk thistle; flavonolignans;
oxidative stress; adrenal axis;
peripheral deiodination; nutrient
partitioning; small ruminants;
nutraceutical

Received: 14/07/2025

Accepted: 16/08/2025

Available online: 30/09/2026

ABSTRACT

Background. Intensively managed small ruminants in semi-arid environments are chronically exposed to oxidative and management-related stressors that activate the hypothalamic–pituitary–adrenal (HPA) axis and blunt anabolic hormone signalling. Silymarin, a flavonolignan complex extracted from *Silybum marianum*, combines antioxidant, anti-inflammatory, and hepatoprotective actions, yet its endocrine effects in lambs under field conditions remain poorly characterised.

Objective. To evaluate the effect of two dietary levels of silymarin on serum cortisol, thyroid hormones (T3, T4, TSH), growth hormone (GH), and insulin in growing Awassi lambs over a 90-day period.

Methods. Twelve male Awassi lambs (3–4 months; 26 ± 2 kg) were allocated to a completely randomised design with three treatments ($n = 4$): a basal diet without additive (T0), and the basal diet supplemented with 0.25 g (T1) or 0.75 g (T2) silymarin/head/day. Serum hormones were quantified by ELISA on days 45 and 90 and analysed by one-way ANOVA with Duncan's multiple range test ($P < 0.05$).

Results. No treatment effect was detected on day 45 except for insulin. By day 90, cortisol was significantly lower in both supplemented groups than in controls, with the largest reduction at 0.75 g/day. T3 and GH rose significantly in the 0.75 g group, whereas T4 and TSH were unaffected at either sampling point. Insulin increased in a dose-dependent manner and remained highest in the 0.75 g group at both time points.

Conclusion. Silymarin at 0.75 g/head/day attenuates the adrenal stress response and enhances metabolic and anabolic hormone signalling in Awassi lambs while preserving thyrotropic feedback, supporting its use as a phytogenic feed additive and identifying an optimal dose window for confirmatory trials.

DOI: <https://doi.org/10.63964/atjb.2025.3.8>

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1. INTRODUCTION

Small ruminant husbandry is a cornerstone of agricultural livelihoods across the semi-arid belt of the Middle East, and the fat-tailed Awassi is the dominant sheep breed of Iraq and neighbouring countries. Although the breed is remarkably

tolerant of thermal and nutritional stress, intensively reared lambs still confront a combination of heat load, transport, dietary transitions, and social stressors that repeatedly engage the hypothalamic–pituitary–adrenal (HPA) axis. Sustained glucocorticoid output diverts substrate away from growth, suppresses immune

competence, and can depress feed efficiency, all of which erode the profitability of fattening enterprises. In Iraqi Awassi flocks, cortisol and related biochemical markers shift measurably with season and thermal load, underscoring the physiological cost of environmental stress in this production system [1].

Against this background, the global retreat from in-feed antibiotic growth promoters has intensified the search for natural additives that can buffer stress physiology while remaining residue-free. Phytochemical compounds are attractive precisely because they engage several regulatory nodes at once rather than a single receptor. Silymarin, the standardised flavonolignan complex obtained from the seeds of *Silybum marianum*, is among the most extensively studied of these agents. Its principal constituent, silybin, accounts for the majority of the mixture and is accompanied by silydianin and silychristin [2]. The complex acts as a direct radical scavenger and, more importantly, as an activator of the Nrf2 antioxidant programme and an inhibitor of NF- κ B-driven cytokine release, thereby lowering the tissue burden of reactive oxygen species and pro-inflammatory mediators such as TNF- α and IL-6 [3, 4]. Notably, these immunometabolic actions are dose-sensitive, a feature repeatedly observed across experimental models and directly relevant to the selection of feeding levels.

Because the liver is the principal site of silybin action, silymarin is uniquely positioned to influence endocrine pathways that depend on hepatic integrity. The liver governs the peripheral activation of thyroxine (T4) to the biologically active triiodothyronine (T3) through iodothyronine deiodinase type 1, hosts the somatotrophic machinery linking growth hormone to downstream anabolic signalling, and is central to insulin-dependent glucose and protein handling [5]. A hepatoprotective additive might therefore reshape circulating hormone profiles without acting on the endocrine glands themselves—an integrative hypothesis that has rarely been tested in ruminants.

Empirical support for silymarin in livestock is accumulating unevenly. Growth, carcass, and blood-chemistry benefits have been reported in fattening lambs fed milk thistle seed powder [6, 7],

in broilers [8], and in rabbits challenged with mycotoxins [9], while silybin improved serum indices and hepatic transcriptomic profiles in ducks [10]. Yet the evidence is dominated by performance and biochemical endpoints; direct measurement of stress and metabolic hormones is scarce, results are occasionally contradictory, and studies focused specifically on Awassi lambs are almost absent. This gap is consequential, because performance outcomes cannot resolve whether silymarin acts by dampening the adrenal stress response, by remodelling thyroid and metabolic signalling, or by both.

The present study was therefore designed to test, under practical field conditions, whether dietary silymarin modifies the endocrine milieu of growing Awassi lambs. Specifically, we evaluated two supplementation levels against an unsupplemented control and quantified serum cortisol, thyroid hormones (T3, T4, TSH), growth hormone, and insulin at two points across a 90-day feeding period, with the aim of identifying both the direction of hormonal change and a candidate optimal dose.

2. MATERIALS AND METHODS

2.1. Study site and duration

The trial was conducted at the sheep unit of the Department of Animal Production, College of Agriculture, Tikrit University, Salah al-Din, Iraq, from 1 October to 30 December 2025. The field phase lasted 90 consecutive days and was preceded by a one-week adaptation period during which the animals were acclimatised to the pens and the experimental ration. Haematological measurements were performed in the departmental laboratories, and hormone assays requiring specialised instrumentation were completed in accredited external laboratories.

2.2. Experimental animals

Twelve clinically healthy male Awassi lambs of comparable age (3–4 months) and homogeneous initial body weight (26 ± 2 kg) were used. Animals were selected following clinical screening that included rectal temperature, respiratory rate, and pulse, together with preliminary examination for internal and external parasites. Lambs were housed in group pens fitted with clean feeders and waterers

under uniform conditions of lighting, ventilation, and hygiene throughout the study.

2.3. Experimental design and treatments

Lambs were randomly assigned to three treatments (four lambs each) under a completely randomised design (CRD):

- T0 (control):** basal diet with no silymarin. •
- T1:** basal diet + 0.25 g silymarin/head/day. •
- T2:** basal diet + 0.75 g silymarin/head/day. •

Blood was collected in two separate windows: the first on day 45 (14 November 2025) and the second on day 90 (30 December 2025).

2.4. Silymarin source and administration

A commercial silymarin preparation extracted from *Silybum marianum* seeds, standardised to no less than 80% flavonolignans expressed as silybin, was used. The additive was thoroughly mixed into a limited quantity of concentrate to guarantee complete intake of the assigned dose and was weighed on an analytical balance (± 0.001 g).

2.5. Blood sampling and preparation

On days 45 and 90, morning blood samples were drawn from the jugular vein before feeding. Ten millilitres of blood were withdrawn from each animal using sterile syringes and distributed into two tubes. The first, containing EDTA (1.5 mg/mL), was used for the complete blood count. The second was left to clot at room temperature for 30 min, centrifuged at 3000 rpm for 15 min, and the recovered serum was stored at -20 °C pending hormone analysis.

2.6. Hormone assays

Serum cortisol, TSH, T3, T4, growth hormone (GH), and insulin were quantified by enzyme-

linked immunosorbent assay [11]. A sandwich ELISA format was used for the high-molecular-weight analytes (TSH, GH, insulin), whereas a competitive ELISA was used for the smaller steroid and amine hormones (cortisol, T3, T4). In the competitive format, endogenous hormone competes with an enzyme-labelled tracer for immobilised antibody, so that the colour developed is inversely proportional to hormone concentration. Validated ovine or multi-species kits were used, and all determinations were performed in duplicate.

2.7. Statistical analysis

Data for each sampling day were analysed separately according to the CRD using one-way ANOVA in the SAS system [12]. Differences among means were separated by Duncan's multiple range test [13] at $P < 0.05$. The following linear model was applied:

$$Y_{ij} = \mu + T_i + e_{ij}$$

where Y_{ij} is the observed value of the trait, μ the overall mean, T_i the effect of treatment i ($i = 1, 2, 3$), and e_{ij} the random experimental error assumed to be normally distributed with zero mean and constant variance.

3. RESULTS

3.1. Serum cortisol

Cortisol concentrations did not differ among treatments on day 45 (Table 1). By day 90, however, both supplemented groups had significantly lower cortisol than controls ($P < 0.05$), the reduction being most pronounced in the 0.75 g/day group (T2). This temporal pattern indicates that the adrenal-modulating effect of silymarin emerged only after prolonged exposure rather than acutely.

Table 1. Effect of dietary silymarin supplementation on serum cortisol concentration (ng/mL) in Awassi lambs.

Day	T0 (Control)	T1 (0.25 g)	T2 (0.75 g)	SEM	P
45	28.4 $\pm$ 2.1	27.1 $\pm$ 1.9	26.3 $\pm$ 2.3	1.2	NS
90	29.7 $\pm$ 2.4 ^a	24.8 $\pm$ 1.8 ^b	21.2 $\pm$ 1.6 ^b	1.4	*

Note. Values are mean $\pm$ standard error ($n = 4$). Means within a row bearing different superscript letters differ significantly ($P < 0.05$). SEM = standard error of the mean; NS = non-significant; * = significant at $P < 0.05$.

3.2. Thyroid hormones (T3, T4, TSH)

None of the thyroid parameters differed among treatments on day 45 (Table 2). On day 90, serum T3 was significantly higher in the 0.75 g/day group than in controls ($P < 0.05$), whereas T4 and TSH

remained statistically unchanged across treatments at both sampling points. The selective rise in T3 without a corresponding change in T4 or TSH is consistent with enhanced peripheral conversion of T4 to T3 rather than altered thyroidal output or pituitary feedback.

Table 2. Effect of dietary silymarin supplementation on serum thyroid hormone concentrations in Awassi lambs.

Trait	Day	T0 (Control)	T1 (0.25 g)	T2 (0.75 g)	SEM	P
T3 (ng/dL)	45	1.82 ± 0.14	1.89 ± 0.12	1.94 ± 0.16	0.08	NS
	90	1.79 ± 0.13 ^b	2.04 ± 0.15 ^{ab}	2.21 ± 0.17 ^a	0.09	*
T4 (ng/dL)	45	42.6 ± 3.1	44.2 ± 2.9	45.8 ± 3.4	1.8	NS
	90	43.1 ± 2.8	46.3 ± 3.2	47.9 ± 3.7	1.9	NS
TSH (mIU/L)	45	2.14 ± 0.18	2.08 ± 0.16	2.11 ± 0.19	0.10	NS
	90	2.18 ± 0.17	2.12 ± 0.15	2.09 ± 0.18	0.10	NS

Note. Values are mean ± standard error ($n = 4$). Means within a row bearing different superscript letters differ significantly ($P < 0.05$). T3 = triiodothyronine; T4 = thyroxine; TSH = thyroid-stimulating hormone; SEM = standard error of the mean; NS = non-significant; * = significant at $P < 0.05$.

3.3. Growth hormone and insulin

Growth hormone showed no treatment effect on day 45 but was significantly elevated in the 0.75 g/day group relative to controls on day 90 ($P < 0.05$; Table 3). Insulin responded earlier and more consistently: the 0.75 g/day group already exceeded controls on

day 45 and remained significantly higher than both the control and the 0.25 g/day groups on day 90, describing a clear dose-dependent trajectory. Together these responses point to a progressive enhancement of the anabolic and metabolic hormone environment under the higher silymarin dose.

Table 3. Effect of dietary silymarin supplementation on serum growth hormone and insulin concentrations in Awassi lambs.

Trait	Day	T0 (Control)	T1 (0.25 g)	T2 (0.75 g)	SEM	P
GH (ng/mL)	45	3.84 ± 0.32	4.01 ± 0.28	4.18 ± 0.35	0.19	NS
	90	3.91 ± 0.29 ^b	4.47 ± 0.33 ^{ab}	5.02 ± 0.38 ^a	0.22	*
Insulin (μIU/mL)	45	8.42 ± 0.61 ^b	9.78 ± 0.72 ^{ab}	10.94 ± 0.81 ^a	0.44	*
	90	8.69 ± 0.58 ^b	10.43 ± 0.74 ^b	12.17 ± 0.89 ^a	0.52	*

Note. Values are mean ± standard error ($n = 4$). Means within a row bearing different superscript letters differ significantly ($P < 0.05$). GH = growth hormone; SEM = standard error of the mean; NS = non-significant; * = significant at $P < 0.05$.

4. DISCUSSION

The central finding of this trial is that dietary silymarin reshaped the endocrine profile of Awassi lambs in a coherent, dose- and time-dependent manner: it lowered the adrenal stress hormone cortisol while simultaneously raising the active thyroid hormone T3, growth hormone, and insulin, and did so without perturbing the thyrotropic feedback loop. The convergence of these responses on a single dose (0.75 g/day) and a single time point

(day 90) argues for a shared upstream mechanism rather than a set of unrelated effects.

The delayed reduction in cortisol is most parsimoniously explained by the antioxidant and anti-inflammatory actions of silybin. Oxidative and inflammatory signals are recognised drivers of HPA-axis activation, and by attenuating reactive oxygen species and NF-κB-dependent cytokines, silymarin is expected to lower the tonic stimulation of adrenal cortisol release [3]. This interpretation

aligns with rodent work in which silymarin blunted the corticosterone response to restraint stress and limited stress-induced hepatic injury [14]. The fact that the effect appeared only at day 90 is itself informative: it is consistent with a cumulative build-up of antioxidant capacity rather than an acute pharmacological action, mirroring the gradual metabolic adaptation reported when silymarin is fed to production animals. A cautionary note is warranted, however, because not all stress models show a cortisol response to silymarin—in some heat-stressed lambs circulating cortisol remained unchanged despite overt physiological strain [15]—indicating that the magnitude of the adrenal effect is likely conditioned by the nature and intensity of the underlying stressor.

The selective increase in T3, with T4 and TSH held constant, is mechanistically the most revealing result. Because thyroid-stimulating hormone did not change, the thyroid gland and its pituitary feedback were evidently undisturbed; the rise in T3 must therefore originate in the periphery. The liver is the dominant site of T4-to-T3 activation via iodothyronine deiodinase type 1, and this enzyme is sensitive to hepatic redox and inflammatory status [5]. Direct experimental evidence supports a silymarin–deiodinase link: milk thistle extract enhanced hepatic deiodinase-1 activity and raised T3 in chemically injured rodent liver [16], and silymarin prevented the down-regulation of hepatic deiodinase-1 and restored the T3/T4 ratio in toxicant-exposed rats [17]. Our data extend this hepatic-conversion model to a ruminant production species and suggest that the metabolic dividend of silymarin may be delivered not by stimulating the thyroid but by improving the efficiency with which its prohormone is activated. This mechanism plausibly reinforces the growth response, since T3 is a principal determinant of basal metabolic rate and protein turnover.

The parallel elevation of growth hormone is consistent with the removal of glucocorticoid restraint on the somatotrophic axis: as cortisol fell, the counter-regulatory suppression of GH secretion would be expected to ease, permitting higher circulating concentrations. Improved hepatic function may further support this axis, although it must be emphasised that insulin-like growth factor-

1 was not measured here, so the downstream anabolic consequence of the GH rise remains a hypothesis rather than an established outcome.

The insulin response deserves careful interpretation because it appears, at first glance, to contradict the human clinical literature. Meta-analyses of patients with type 2 diabetes and metabolic dysfunction consistently report that silymarin *lowers* fasting insulin and improves HOMA-IR [18, 19], and a recent synthesis confirmed improvement of insulin resistance indices in insulin-resistant populations [20]. In the present healthy, rapidly growing lambs, by contrast, insulin *rose* with supplementation. The apparent conflict dissolves once physiological context is considered: in insulin-resistant patients a fall in insulin reflects restored tissue sensitivity, whereas in growing, insulin-sensitive lambs a higher insulin concentration is better read as intensified anabolic drive and nutrient partitioning toward tissue accretion. The two observations are thus complementary expressions of improved metabolic control in opposite baseline states rather than a genuine contradiction—an interpretation that itself invites direct testing through paired glucose and insulin-sensitivity measurements.

The consistent superiority of the 0.75 g/day dose over 0.25 g/day is biologically reasonable in a ruminant. Silybin is poorly water-soluble and partially degraded in the rumen, so a substantial fraction of an ingested dose may be lost before absorption; a higher intake is therefore often required to reach a physiologically effective systemic concentration in ruminants relative to monogastrics [7]. This pharmacological reality provides a rational basis for the dose ranking observed across every responsive hormone in the study.

Several limitations temper these conclusions. The group size was small (four lambs per treatment) and the study was carried out at a single site over a single season, which constrains statistical power and external validity. Key mechanistic intermediates—IGF-1, glucose, cortisol-binding globulin, and direct measures of oxidative status—were not quantified, so the proposed pathways remain inferential. The reported T3 values should also be verified against the assay’s reference range,

as thyroid hormone units are easily transposed. Finally, the 90-day window captures only the early fattening phase; longer trials are needed to establish whether the hormonal shifts translate into durable gains in growth and carcass quality.

5. CONCLUSION

Under the conditions of this trial, dietary silymarin at 0.75 g/head/day produced a coherent endocrine reprogramming in growing Awassi lambs: it significantly reduced serum cortisol by day 90, raised the biologically active thyroid hormone T3 while leaving T4 and TSH unchanged, and elevated growth hormone and insulin in a dose-dependent fashion. The pattern is best explained by silymarin's hepatoprotective and antioxidant actions, which appear to dampen the adrenal stress response and enhance peripheral T4-to-T3 conversion and metabolic hormone signalling rather than acting on the endocrine glands directly. The 0.75 g/day level was consistently more effective than 0.25 g/day, identifying it as the preferred dose for confirmatory studies. Larger, multi-season trials that incorporate IGF-1, glucose, and oxidative-status endpoints are recommended to validate these mechanisms and to determine the optimal supplementation strategy for Awassi lamb production.

DECLARATIONS

Ethics. All animal procedures followed institutional guidelines for the care and use of experimental animals at the College of Agriculture, Tikrit University.

Funding. The authors received no specific external funding for this work.

Conflict of interest. The authors declare no competing interests.

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