AMERICAN JOURNAL OF BIOMEDICINE AND PHARMACY
https://biojournals.us/index.php/AJBP
ISSN: 2997-7177

Article

The Hepatoprotective and Antioxidant Benefits of Timely Dietary Supplementation with Green Tea Powder in Awassi Sheep

Salam Ketab Rebat[1]
[1] Department of Animal Production, College of Agriculture, University of Kirkuk, Iraq

Citation: Rebat S. K. The Hepatoprotective and Antioxidant Benefits of Timely Dietary Supplementation with Green Tea Powder in Awassi Sheep. American Journal of Biomedicine and Pharmacy 2026, 3(1), 138-142.

Received: 10th Sept 2025
Revised: 11th Nov 2025
Accepted: 24th Dec 2025
Published: 31th Jan 2026

Abstract: The aim of this investigation was to evaluate, over time, the effect of dietary supplementation with green tea (Camellia sinensis) powder on liver protection and antioxidant activity in Awassi sheep. The 12 clinically healthy adult Awassi sheep (3–4 years old) were randomly assigned to 3 groups of 4 sheep each. For 30 days, a standard feed was supplied to the control group (G1) and a standard feed supplemented with green tea powder at a rate of 150 g/10 kg of feed (G2) and 250 g/10 kg of feed (G3) respectively. On day 0, day 15 and day 30, blood samples were obtained from the same animals to determine the levels of serum liver enzymes (ALT, AST, ALP), markers of oxidative stress (MDA) and antioxidant capacity (GSH, TAC). All groups had similar baseline values at day 0. Early improvements were mainly seen in ALP, MDA and GSH levels by day 15 particularly in the higher dose group. The supplementation with green tea led to significant dose-dependent improvements by day 30 with decreased concentrations of ALT, AST, ALP and MDA and increased concentrations of GSH and TAC. The present results showed that treatment with green tea powder may improve the biochemical stability of the liver and strengthen the antioxidant defence mechanisms in Awassi sheep, depending on the dose and duration. Article title: Effect of green tea powder on the activities of liver enzymes in Awassi sheep.Article.

Keywords: Awassi sheep; Camellia sinensis; green tea powder; hepatic enzymes; oxidative stress; malondialdehyde (MDA); glutathione (GSH); total antioxidant capacity (TAC).

1. Introduction

Oxidative stress is the imbalance between the production of reactive oxygen and nitrogen species and the ability of the antioxidant system to remove these species. Oxidative stress in farm animals may be caused by a range of physiological and environmental stresses including, diet changes, heat stress, disease challenge, metabolic strain, and intensive production systems. When this balance is maintained for a prolonged time, it can cause damage to lipids, proteins and nucleic acids of cells and reduce the function of tissues, consequently affecting animal health [1,2,3,4].

The liver is important for nutrient metabolism, detoxification and redox reactions. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) are routinely used as biochemical markers of assessment of hepatocellular integrity and hepatobiliary

function.Changes in the levels of these enzymes reflect alterations in cell membrane permeability, tissue stress or the metabolic response to dietary manipulation [5,6,7].

Malondialdehyde (MDA) is a well-known lipid peroxidation marker that has a relationship with an increase in cell membrane oxidative damage. On the other hand, glutathione (GSH) is an important non-enzymatic antioxidant, which plays a role in detoxification and regulation of redox state of cells. Overall antioxidant capacity is indicated by the total antioxidant capacity (TAC). Therefore, simultaneous measurement of MDA, GSH, and TAC can be a useful tool for assessing the oxidative damage as well as the antioxidant defense mechanisms [8,9,10].

Green tea (Camellia sinensis) is abundantly rich in biologically active components, such as catechins, flavonoids, phenolic acids, amino acids and minerals. The catechin epigallocatechin-3-gallate (EGCG) is considered to be one of the most potent of these, with free-radical scavenging, metal-chelating, anti-inflammatory and antioxidant signaling properties [11,12,13,14].

Phytogenic feed additives have received a great deal of attention for their ability to promote health, increase antioxidant activity, modulate immunology and metabolism. However, many factors affect the biological activity of these plant-based additives including type of plant, dosage, length of feeding period, animal's physiological state and the base diet [15,16].

The Awassi breed is an important sheep in Iraq and the Middle East due to its adaptability and productivity. However, although highly adaptable, nutritional interventions that can boost antioxidant protection may be beneficial in addressing their health in hand-held applications. This study is aimed at evaluating the time-dependent effects on liver enzymes and oxidative stress markers in Awassi sheep supplemented with 30 days green tea powder in their diet.

2. Materials and Methods

2.1 Animals and Experimental Design

The present study was carried out at the animal production department, College of Agriculture, Kirkuk University, from 1st of February 2025 till 3rd of March 2025. A total of 12 clinically healthy Awassi sheep aged 3-4 years was used in the experiment. Sheep were managed in a uniform manner and fed identically and separated randomly into three experimental groups, with four sheep per group. The experimental design was completely randomized with the feeding trial conducted for 30 consecutive days.

2.2 Dietary Treatments

The dietary treatments were structured as follows: Group 1 (G1) served as the control group and was fed a basal diet without the addition of green tea powder; Group 2 (G2) received the basal diet supplemented with 150 g of green tea powder per 10 kg of feed; and Group 3 (G3) was given the basal diet with a higher supplementation of 250 g of green tea powder per 10 kg of feed. The green tea powder was thoroughly mixed with the daily feed to ensure even distribution and minimize selective feeding.

Table 1. Experimental Design and Dietary Treatments.

Group	Treatment	Supplementation level
G1	Control	Basal diet without green tea powder
G2	Low dose	Basal diet + 150 g green tea powder/10 kg feed
G3	High dose	Basal diet + 250 g green tea powder/10 kg feed

The feeding trial lasted for 30 days, with each group comprising four Awassi sheep

2.3 Blood Sampling and Serum Preparation

At three-point sample time, before supplementation (day 0), middle (day 15), and end of experimental period (day 30), blood samples were taken from the same experimental animals. Jugular vein samples were collected in plain tubes with no anticoagulants. The serum was then separated after the collection and used to determine liver enzymes (ALT, AST, ALP), oxidative stress (MDA) and antioxidants (GSH, TAC) levels. This method of repeated sampling facilitated the assessment of the effects of treatment, time, and the interaction between treatment and time over the course of the supplementation period.

2.4 Biochemical Measurements

Serum ALT, AST and ALP activities were measured by conventional biochemical techniques and results expressed in units/liter (U/L). Oxidative stress and antioxidant status were determined using the markers of lipid peroxidation (MDA), primary endogenous antioxidant (GSH) and comprehensive measure of serum antioxidant capacity (TAC). The measurement of MDA was in nanomoles per milliliter (nmol/mL); GSH was in micromoles per liter (μmol/L) and TAC was in millimoles per liter (mmol/L)..

2.5 Statistical Analysis

The collected data for days 0, 15 and 30 were analyzed using a two-way repeated measures ANOVA. Between-subject factor was dietary treatment and within-subject factor was sampling time. To determine whether the effect of green tea powder supplementation changed during the course of the study, the interaction between treatment and time was assessed in the statistical model. If there were significant main or interaction effects they were separated using Duncan's multiple range test. Differences were deemed statistically significant at a P-value of 0.05 or less. The results are reported as mean ± standard error (SE).

3. Results

3.1 Temporal Variations in Liver Enzyme Activity

All experimental groups had comparable baseline hepatic biochemical profile values on day 0, indicating similar baseline levels of ALT, AST and ALP before supplementation. A decrease in these enzyme levels was observed for all supplemented groups by day 15, especially in the high dose group. On day 30, the serum ALT and AST levels were significantly decreased in a dose-dependent manner by the supplementation of green tea powder and ALP in the high-dose group was significantly decreased. When compared to day 0, ALT levels had decreased by about 19.3% in group G2 and 33.2% in group G3 by day 30. The approximate reduction in AST levels were 17.5% in G2 and 28.1% in G3 and ALP levels reduced about 11.6% and 23.5% respectively.

Table 2. presents the effects over time of dietary green tea powder supplementation on serum liver enzyme activity in Awassi sheep.

Parameter	Day	G1 Control	G2 Low dose	G3 High dose
ALT (U/L)	0	48.0 ± 3.2^{a}	49.2 ± 2.3^{a}	48.5 ± 1.1^{a}
ALT (U/L)	15	47.3 ± 8.2^{a}	43.3 ± 2.1^{a}	41.6 ± 2.4^{a}
ALT (U/L)	30	48.3 ± 2.1^{a}	39.7 ± 1.8^{b}	32.4 ± 1.5^{c}
AST (U/L)	0	52.3 ± 2.1^{a}	53.6 ± 4.2^{a}	51.2 ± 5.6^{a}
AST (U/L)	15	52.7 ± 1.8^{a}	48.2 ± 4.0^{a}	44.5 ± 4.9^{a}
AST (U/L)	30	52.6 ± 2.4^{a}	44.2 ± 2.0^{b}	36.8 ± 1.7^{c}
ALP (U/L)	0	148.3 ± 2.1^{a}	145.3 ± 2.3^{a}	144.2 ± 4.6^{a}
ALP (U/L)	15	146.7 ± 1.8^{a}	140.2 ± 2.0^{ab}	130.5 ± 4.1^{b}
ALP (U/L)	30	143.2 ± 5.6^{a}	128.5 ± 4.9^{a}	110.3 ± 4.2^{b}

Values are presented as mean ± SE. Within the same row, different superscript letters denote statistically significant differences among groups at $P \leq 0.05$.

3.2 Time-Course Changes in Oxidative Stress and Antioxidant Biomarkers

On day 0, the TAC, MDA, and GSH levels were similar across all groups. By day 15, the high-dose group exhibited early improvements, notably through decreased MDA levels and increased GSH. By day 30, both supplemented groups showed significant increases in TAC and GSH, while MDA levels decreased in a dose-dependent manner. Compared to day 0, MDA levels dropped by approximately 27.7% in group G2 and 45.9% in group G3 by day 30. Conversely, GSH levels rose by approximately 31.5% in G2 and 67.2% in G3, while TAC levels increased by about 23.1% and 60.3%, respectively.

Table 3. Time-course effect of dietary green tea powder supplementation on oxidative stress and antioxidant biomarkers in Awassi sheep.

Parameter	Day	G1 Control	G2 Low dose	G3 High dose
TAC (mmol/L)	0	0.75 ± 0.04^{a}	0.78 ± 0.02^{a}	0.78 ± 0.05^{a}

TAC (mmol/L)	15	0.77 ± 0.06^{a}	0.84 ± 0.05^{a}	0.98 ± 0.07^{a}
TAC (mmol/L)	30	0.78 ± 0.03^{c}	0.96 ± 0.04^{b}	1.25 ± 0.05^{a}
MDA (nmol/mL)	0	5.85 ± 0.25^{a}	5.82 ± 0.20^{a}	5.82 ± 0.21^{a}
MDA (nmol/mL)	15	5.83 ± 0.32^{a}	5.12 ± 0.25^{a}	4.11 ± 0.20^{b}
MDA (nmol/mL)	30	5.82 ± 0.21^{a}	4.21 ± 0.18^{b}	3.15 ± 0.14^{c}
GSH (μmol/L)	0	3.02 ± 0.10^{a}	3.08 ± 0.10^{a}	3.11 ± 0.14^{a}
GSH (μmol/L)	15	3.04 ± 0.15^{c}	3.55 ± 0.10^{b}	4.15 ± 0.10^{a}
GSH (μmol/L)	30	3.10 ± 0.12^{c}	4.05 ± 0.15^{b}	5.20 ± 0.17^{a}

Values are presented as mean ± SE. Different superscript letters within the same row denote significant differences among groups at $P \leq 0.05$

4. Discussion

This study showed that feeding Awassi sheep with green tea powder (GTP) for the long term resulted in improvements in liver enzyme activity and antioxidant biomarkers in the sheep. Day 0 values are not significantly different among the groups, suggesting that experimental animals were a homogeneous group prior to supplementation. This initial similarity further supports the finding that the changes found on days 15 and 30 were the result of the dietary treatment and not pre-existing differences between the groups.

This attests to increased stability of the hepatocellular membrane or decreased metabolic stress as can be seen by the reduction of ALT and AST within the supplemented groups at day 30. Generally, these enzymes are considered indicators of liver cell integrity, and reduced activity in the serum may indicate reduced leakage from liver cells. The results were better in the high dose group, suggesting a dose-dependent effect of green tea powder.

This decline of ALP was observed earlier than the decline of ALT and AST particularly in high dose group on day 15. This could be the first sign of the positive impact green tea powder has on hepatobiliary and/or metabolic function. However, care must be taken when assessing ALP results as it is produced by many tissues. The simultaneous decrease of all three enzymes (ALT, AST, and MDA) is indicative of a good biochemical response and not an isolated fluctuation in one enzyme [5,6,7].

One should not only interpret a favourable biochemical response but also not interpret isolated changes in enzymes as a biochemical response [5,6,7].

The results on oxidative stress provide very strong biological proof for the observations made in liver enzymes. MDA concentration continued to decrease over time in the supplemented groups, indicating a decrease in lipid peroxidation. MDA is one of the byproducts of lipid oxidation in cellular membranes, which means that the consumption of green tea powder also helped protect the cellular membranes against the free-radical damage [8,11,12].

The observed rise in GSH and TAC is indicative of an increased antioxidant defense. GSH is one of the most important endogenous non-enzymatic antioxidants, and the total antioxidant capacity (TAC) of serum is a measure of all these antioxidants. The simultaneous reduction of MDA and increase of GSH and TAC indicated the effectiveness of green tea powder to reduce oxidative damage and enhance antioxidant defense [8,10].

The beneficial properties of green tea powder may be due to its catechins and other phenolics. Tea polyphenols are capable of scavenging reactive species, chelating metal ions, inhibiting lipid peroxidation, and activating the body's own antioxidant defense mechanisms. These processes could account for the observed liver function and oxidative stress markers improvements [11,12,13,14].

One strength of the current study is that it is a time-course study. The results showed that the effects of green tea powder did not just occur at the final sampling time. Rather, by day 15, early changes were seen and by day 30, more pronounced changes were seen especially in group G3. This pattern indicates a treatment-by-time interaction and indicates that the biological effects of green tea powder are gradually built over the course of the supplementation.

5. Conclusion

The supplementation of Awassi sheep with green tea powder for 30 days increased the activity of liver enzymes as well as the antioxidant levels, depending on the time and amount of the supplement. The values of the control group remained unchanged during the study, whereas those of

the supplementation groups gradually decreased in ALT, AST, ALP and MDA levels, while GSH and TAC levels increased. The highest dose (250 g/10 kg of feed) had the most noticeable results. Based on these findings, it can be concluded that green tea powder may be a natural phytogenic feed additive to maintain the biochemical stability and antioxidant defenses of the liver in Awassi sheep.

REFERENCES

[1] H. Sies, "Oxidative stress: A concept in redox biology and medicine," *Redox Biology*, vol. 4, pp. 180–183, 2015.

[2] B. Halliwell and J. M. C. Gutteridge, *Free Radicals in Biology and Medicine*, 5th ed. Oxford, U.K.: Oxford University Press, 2015.

[3] E. N. Ponnampalam, A. Kiani, S. Santhiravel, *et al.*, "The importance of dietary antioxidants on oxidative stress, meat and milk production, and their preservative aspects in farm animals," *Animals*, vol. 12, no. 23, p. 3279, 2022.

[4] M. Piao, Y. Tu, N. Zhang, Q. Diao, and Y. Bi, "Advances in the application of phytogenic extracts as antioxidants and their potential mechanisms in ruminants," *Antioxidants*, vol. 12, no. 4, p. 879, 2023.

[5] J. J. Kaneko, J. W. Harvey, and M. L. Bruss, *Clinical Biochemistry of Domestic Animals*, 6th ed. Academic Press, 2008.

[6] C. A. Burtis, E. R. Ashwood, and D. E. Bruns, *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics*, 5th ed. Elsevier, 2012.

[7] M. A. Thrall, G. Weiser, R. W. Allison, and T. W. Campbell, *Veterinary Hematology and Clinical Chemistry*, 2nd ed. Wiley-Blackwell, 2012.

[8] M. Valko, D. Leibfritz, J. Moncol, *et al.*, "Free radicals and antioxidants in normal physiological functions and human disease," *International Journal of Biochemistry & Cell Biology*, vol. 39, pp. 44–84, 2007.

[9] A. Meister, "Glutathione metabolism," *Methods in Enzymology*, vol. 251, pp. 3–7, 1995.

[10] D. Koracevic, G. Koracevic, V. Djordjevic, S. Andrejevic, and V. Cosic, "Method for the measurement of antioxidant activity in human fluids," *Journal of Clinical Pathology*, vol. 54, pp. 356–361, 2001.

[11] M. Radeva-Ilieva, S. Stoeva, N. Hvarchanova, and K. D. Georgiev, "Green tea: Current knowledge and issues," *Foods*, vol. 14, no. 5, p. 745, 2025.

[12] Z. Yan, Y. Zhong, Y. Duan, Q. Chen, and F. Li, "Antioxidant mechanism of tea polyphenols and its impact on health benefits," *Animal Nutrition*, vol. 6, pp. 115–123, 2020.

[13] L. C. Bawono, M. A. Khairinisa, S. Jiranusornkul, and J. Levita, "The role of catechins of *Camellia sinensis* leaves in modulating antioxidant enzymes," *Journal of Applied Pharmaceutical Science*, vol. 13, no. 12, pp. 52–65, 2023.

[14] V. L. Truong, M. Jun, and W. S. Jeong, "Cellular defensive mechanisms of tea polyphenols," *International Journal of Molecular Sciences*, vol. 22, no. 17, p. 9109, 2021.

[15] N. A. Nastoh, M. Waqas, A. A. Çınar, and M. Salman, "The impact of phytogenic feed additives on ruminant production: A review," *Journal of Animal and Feed Sciences*, vol. 33, no. 4, pp. 431–453, 2024.

[16] J. Wang, L. Deng, M. Chen, Y. Che, L. Li, L. Zhu, G. Chen, and T. Feng, "Phytogenic feed additives as natural antibiotic alternatives in animal health and production," *Animal Nutrition*, vol. 17, pp. 244–264, 2024.