Effect of Spirulina Supplementation and Feeding Restriction Periods on Selected Biochemical-hematological blood Parameters in Awassi Lambs

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Abstract

This study aimed to investigate the integrative effects of incorporating raw spirulina powder into the diets of Awassi lambs by evaluating its impact on key physiological and functional axes, including hematological parameters, oxidative balance, and immunological indicators, to clarify the potential role of this bio-additive in supporting the health and functional performance of the animals. A total of eighteen lambs, aged 3–4 months with an average initial body weight of 19.8 ± 0.9 kg, were randomly assigned to six equal experimental groups differing in diet composition and feed restriction duration. All animals received nutritionally balanced rations formulated to contain 11.53% crude protein and 2700 kcal/kg of metabolizable energy. The first and second groups were fed ad libitum, while the third and fourth groups were subjected to a feed restriction of 2.5% of body weight for 35 days. The fifth and sixth groups underwent the same restriction for 50 days. Spirulina powder was supplemented at a level of 10 g/kg in the diets of the second, fourth, and sixth groups. Statistical analyses revealed no significant differences in general physiological blood parameters among the treatment groups. However, notable variations were observed in blood biochemical markers. The second group recorded significantly higher values for cholesterol (224 mg/dL), high-, low-, and very-low-density lipoproteins (HDL, LDL, VLDL), triglycerides (237.81 mg/dL), total protein (7.03 g/dL), as well as urea (66.50 mg/dL) and creatinine (1.41 mg/dL). These findings highlight the metabolic influence of spirulina supplementation and its role in modulating renal and hepatic biochemical efficiency.

Keywords. Awassi lambs, Spirulina , Biochemical-hematological blood , Feeding regimes

Introduction

The integration of modern nutritional strategies in the livestock sector constitutes a strategic pillar for enhancing production efficiency, particularly in light of growing economic and environmental challenges. Among the most promising sustainable approaches is the use of bio-nutritional supplements. Spirulina, a unique natural source rich in proteins, vitamins, and essential micronutrients, has been shown to improve animal performance when incorporated into feed rations [1]. Farm animals are often subjected to health challenges such as oxidative stress, metabolic disorders, and compromised immunity, which negatively affect productivity and contribute to economic losses [2]. Consequently, global efforts are increasingly focused on identifying new, safe, and natural antioxidants with high bioavailability and protective efficacy, in

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order to reduce the widespread reliance on antibiotics in livestock production systems [3]. Researchers are also striving to develop nutritional supplements capable of mitigating metabolic and immune disorders and compensating for the effects of malnutrition [4]. Feed restriction is considered a critical factor that contributes to growth suppression and elevated biological stress [5]. In this context, Spirulina has demonstrated a remarkable adaptive capacity to counteract these challenges by enhancing metabolic activity and mitigating micronutrient deficiencies [6]. Its inclusion in the diet has been associated with improved feed utilization efficiency and increased resilience to environmental stressors [7]. Spirulina is classified as a filamentous blue-green microalga with a flexible nutritional profile. It is highly rich in proteins, essential fatty acids, vitamins, and possesses potent antioxidant and detoxifying properties [8]. Accordingly, this study aims to investigate the interactive effects of Spirulina supplementation and feed restriction on selected Biochemical-hematological blood parameters

Awassi lambs, oxidative stress markers, and vital organ function.

Materials and Methods

Statistical Analysis of the Data

The statistical analysis was performed using a completely randomized design (CRD) with a one-way layout. To determine the significant differences among treatments, Duncan's Multiple Range Test [9], was employed within the SAS statistical software package [10]. The data were analyzed according to the following mathematical model:

Yij=μ+Ti+eijY_{ij} = \mu + T_i + e_{ij}Yij=μ+Ti+eij

Where:

YijY_{ij}Yij = the observation value jjj for treatment iii,

μ\muμ = the overall mean of the studied trait,

TiT_iTi = the effect of treatment iii, where iii represents (control) and (spirulina,(

eije_{ij}eij = the experimental error, assumed to be normally and independently distributed with a mean of zero and a constant variance σ2\sigma^2σ2.

Experimental Site and Period:

This study was conducted at the Animal Production Field of the Department of Animal Production, College of Agriculture, University of Tikrit, over a period of 90 days, from September 29 to December 29, 2024. A total of 18 local Awassi lambs were used in the experiment. The lambs were purchased from local markets in Salah Al-Din Governorate, Iraq. Their ages ranged from 3 to 4 months, with an average initial body weight of 19.8 ± 0.9 kg. The animals were grouped based on body weight into six equal groups, each consisting of three lambs, and treatments were randomly assigned to the groups. All lambs were fed a diet containing 11.53% crude protein and 2700 kcal/kg of metabolizable energy. The first and second groups were fed ad libitum, while the third and fourth groups were subjected to a feed restriction at 2.5% of live body weight for 35 days. The fifth and sixth groups underwent the same restriction for 50 days. Spirulina powder was supplemented at a level of 10 g/kg of feed intake in the diets of the second, fourth, and sixth groups, As indicated in Table (1.(

Blood Sample Collection:

Blood samples were collected at the end of the experimental period after the animals had been fasted for 12 hours. Approximately 8 ml of blood was withdrawn from the jugular vein of

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each lamb using sterile syringes. The samples were distributed into two types of tubes: 2 mL were placed in glass tubes containing the anticoagulant EDTA for complete blood count (CBC) analysis. 6 mL were collected into plain tubes for serum separation. Serum was separated by centrifugation at 2900 rpm for 20 minutes using a centrifuge located in the Physiology Laboratory, Department of Animal Production. The obtained serum from each sample was aliquoted into 1 mL Eppendorf tubes, tightly sealed, and stored at −20°C until further biochemical analysis.

Hematological and Biochemical Analyses:

Biochemical analyses of the blood serum were performed, including measurements of triglycerides, cholesterol, high-density lipoprotein (HDL), total protein (TP), albumin, urea, and creatinine, using a specialized device designed for such tests. The low-density lipoprotein (LDL) concentration in the serum was calculated using the following equation:

LDL (mg/100 ml of blood) = Total cholesterol – HDL – (Triglycerides / 5) [11]. Very low-density lipoprotein (VLDL) concentration was estimated using the formula:

VLDL = Triglycerides / 5 [11]. Serum globulin concentration was calculated using the following formula:

Globulin (g/dL) = Total Protein (g/dL) – Albumin (g/dL) [12 .[

Table 1. Composition and Nutritional Profile of the Experimental Diet Used in the Study.

Feed Ingredients	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5	Treatment 6
Barley (%)	50	50	50	50	50	50
Wheat bran (%)	20	20	20	20	20	20
Corn (%)	19	19	19	19	19	19
Wheat (%)	9	9	9	9	9	9
Salt and Minerals (%)	2	2	2	2	2	2
Crude Protein (%)	11.53	11.53	11.46	11.53	11.46	11.53
Energy (kcal/kg)	2700	2700	2700	2700	2700	2700
Spirulina Addition (g/kg feed intake)	0	9	0	9	0	9

Results and Discussion

Serum Protein Concentrations

The results presented in the table indicate significant differences ($P \leq 0.05$) among the treatments in the concentrations of total protein (TP), albumin, and globulin in the

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blood serum. Treatment 2 (T2) showed a clear significant superiority in all studied parameters, recording the highest mean values for total protein (7.03±0.75 g/dL), albumin (4.82±0.47 g/dL), and globulin (2.20±0.81 g/dL). This may reflect enhanced physiological performance and improved nutritional or immune status resulting from the applied treatment. Treatment 3 (T3) ranked second in terms of total protein concentration (4.90±0.10 g/dL), with moderate levels of albumin (3.63±0.14 g/dL) and globulin (1.26±0.23 g/dL), indicating an intermediate physiological response compared to T2.

In contrast, Treatment 6 (T6) recorded the lowest significant values for all parameters: total protein (1.35±0.09 g/dL), albumin (1.03±0.03 g/dL), and globulin (0.32±0.12 g/dL). These values may indicate impaired protein synthesis or possible nutritional deficiency, oxidative stress, or liver dysfunction. Interestingly, Treatment 1 (T1) showed a relatively high globulin level (4.72±1.30 g/dL), despite recording lower values for albumin (2.21±1.79 g/dL) and total protein (2.75±0.06 g/dL). This may suggest an elevated immune response or abnormal stimulation of globulin production at the expense of other serum proteins. The variation in results reflects the differential effects of the treatments on metabolic and protein-related physiological functions, particularly those associated with plasma proteins, liver function, and immune response. Further investigations are recommended to clarify the physiological mechanisms behind each treatment effect .

Table (2). Effect of the experimental treatments on blood protein concentrations in local lambs (Mean ± Standard Error.(

Treatment	TP	ALBUMIN	GLOBULIN
T1	0.06 c±2.75	2.21 c±1.79	4.72 a±1.30
T2	7.03±0.75 a	4.82±0.47 a	2.20±0.81 a
T3	4.90±0.10 b	3.63±0.14 ab	1.26±0.23 ab
T4	3.05±0.21 c	1.67±0.62 c	1.37±0.41 ab
T5	3.39±0.27 c	2.37±0.70 bc	1.02±0.49 ab
T6	1.35±0.09 d	1.03±0.03 c	0.32±0.12 b

T_i: the effect of the i-th treatment, T1: ad libitum without Spirulina, T2: ad libitum with Spirulina, T3: restricted for 35 days without Spirulina, T4: restricted for 35 days with Spirulina, T5: restricted for 50 days without Spirulina, T6: restricted for 50 days with Spirulina ,

*Similar letters indicate no significant difference.

*Different letters indicate a significant difference at the 0.05 level (P < 0.05 .(

Serum Urea and Creatinine Concentrations

Table (3) indicates significant differences (P≤0.05) among all treatments in both blood urea and creatinine concentrations. Treatment 2 recorded the highest blood urea level (66.50 ± 3.20 mg/dL), while Treatment 6 recorded the lowest. The remaining treatments varied statistically among each other. This marked increase in urea concentration in Treatment 2 may be attributed to the free-choice feeding strategy, which likely provided lambs with a higher intake of dietary protein, in addition to the protein content of Spirulina, resulting in elevated blood urea levels. In contrast, the restricted feeding treatments

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showed lower values due to limited protein intake.
Similarly, Treatment 2 also exhibited a significantly higher (P≤0.05) creatinine concentration, further supporting the explanation of elevated protein intake compared to the other treatments, which showed no significant differences among them and had relatively close values.

Table (3). Effect of spirulina supplementation and feeding restriction period on serum urea and creatinine concentrations in Awassi lambs (Mean ± Standard Error.(

Treatment	Urea mg/dL	Creatinine mg/dL
T1	2.00 ±25.66 c	0.01 b±0.32
T2	66.50±3.20 a	2.00±0.33 a
T3	41.89±2.44 b	0.52±0.00 b
T4	24.40±1.39 c	0.35±0.00 b
T5	20.68±1.82 c	0.34±0.01 b
T6	11.59±0.53 d	0.37±0.02 b

T_i: the effect of the i-th treatment, T1: ad libitum without Spirulina, T2: ad libitum with Spirulina, T3: restricted for 35 days without Spirulina, T4: restricted for 35 days with Spirulina, T5: restricted for 50 days without Spirulina, T6: restricted for 50 days with Spirulina ,
*Similar letters indicate no significant difference.
*Different letters indicate a significant difference at the 0.05 level (P < 0.05 .(

The Effect of Experimental Treatments on Lipid Profile

Cholesterol Concentration

Table (4) shows highly significant differences (P≤0.05) among the treatments in mean serum cholesterol levels. Treatment 2 recorded the highest cholesterol concentration (224 mg/dL), significantly surpassing all other treatments. In contrast, Treatment 6 exhibited the lowest concentration (174 mg/dL.(
The elevated cholesterol levels in Treatment 2 may be attributed to an increase in the availability of free fatty acids in the free-choice feeding system, which could have enhanced lipid metabolism and cholesterol synthesis.
Lipoproteins

A significant effect (P≤0.05) was observed in Treatment 2, which involved free-choice feeding supplemented with Spirulina, and in Treatment 3, where feed intake was restricted for 35 days. Both treatments resulted in elevated concentrations of high-density lipoprotein (HDL), surpassing all other treatments, while Treatment 6 recorded the lowest HDL level. Regarding low-density lipoprotein (LDL), no significant differences (P≤0.05) were found among the first five treatments, all of which showed higher values than Treatment 6. Additionally, Treatment 2 exhibited a significant increase (P≤0.05) in very low-density lipoprotein (VLDL) concentration compared to the other treatments, while Treatment 6 again recorded the lowest value.
Triglyceride Concentration

Table (4) reveals significant differences (P≤0.05) in triglyceride concentrations among

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the treatments. Treatment 2 recorded the highest triglyceride level (237.81 mg/dL), significantly exceeding all other treatments, while Treatment 6 showed the lowest value (164 mg/dL). The elevated mean triglyceride concentration in Treatment 2 may be attributed to an increase in the level of free fatty acids associated with the free-choice feeding system, which likely enhanced triglyceride synthesis and accumulation in the bloodstream. lowest value.

The results of this study were consistent with those of [1]. and differed from the findings of [12.[

Table (4). Effect of spirulina supplementation and feeding restriction period on lipids profile concentrations in Awassi lambs(Mean ± Standard Error.(

Treatment	**CHOLE** mg/dL	**HDL** mg/dL	**LDL** mg/dL	**VLDL** mg/dL	**TRI** mg/dL
T1	188.71 ±2.55c	2.21 ±12,79 d	136.30 ±4.72a	28.67 ±1.14 c	2.85 c±198.05
T2	224.53±2.50 a	41.53±1.25 a	135.33±4.14 a	47.56± 0.67 a	237.81±3.36 a
T3	214.58±0.53 b	37.65±1.59 a	133.99±2.78 a	42.93±0.92 b	214.65±4.60 b
T4	196.24±2.58 c	28.34±5.26 b	129.16±3.63 ab	38.73±0.72 c	193.66±3.63 c
T5	194.90±2.30 c	22.33±1.45 bc	136.25±3.27 a	36.31±0.66 d	181.58±3.31 d
T6	174.00±3.00 d	19.56±2.35 cd	121.48±3.79 b	32.95±0.19 e	164.76±0.99 e

T_i: the effect of the i-th treatment, T1: ad libitum without Spirulina, T2: ad libitum with Spirulina, T3: restricted for 35 days without Spirulina, T4: restricted for 35 days with Spirulina, T5: restricted for 50 days without Spirulina, T6: restricted for 50 days with Spirulina, CHOL: Total Cholesterol, HDL: High-Density Lipoprotein (Good Cholesterol), LDL: Low-Density Lipoprotein (Bad Cholesterol), VLDL: Very Low-Density Lipoprotein, TRI: Triglycerides

*Similar letters indicate no significant difference.

*Different letters indicate a significant difference at the 0.05 level (P < 0.05 .(

Conclusions

The positive effects of Spirulina powder supplementation (10 g/kg dry matter feed) on most studied parameters indicate its safety for Awassi lambs. Furthermore, Spirulina supplementation under restricted feeding conditions did not exert any adverse effects; rather, it was among the most favorable treatments in terms of production performance and physiological indicators when compared to the control group and the standard feeding regimen.

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