Spirulina platensis as a feed additive: impact on growth, oxidative status, and gene expression in Awassi lambs

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Abstract

The purpose of this research was to investigate whether Spirulina platensis (SP) had an impact on the growth performance, blood metabolism, antioxidant and oxidative status, MDA, and GPX-3 genes of Awassi male lambs. A total of 24 Awassi lambs (3.5-4 months) with an average initial weight of 27kg were used in the study. The lambs were divided into three groups: the control group fed a basal diet without SP supplementation, while the lambs in T1 and T2 were fed a basal diet supplemented with 2 and 3% SP, respectively. Lambs administered with 2% SP enhancement growth performance and increased carcass weight. It seems that 2% Spirulina supplementation significantly surpasses ($P<0.05$) those lambs fed control and 3% SP in daily gain in weight and had a lower feed conversion ratio. The levels of malondialdehyde decreased numerically in the liver and considerably ($P<0.05$) in the serum by supplementing Spirulina in the diet. Additionally, spirulina powder increased antioxidant capacity, which raised the liver and serum levels of GPX. While controlling the proportionate transcription of antioxidant genes, spirulina powder enhanced the proportionate amount of expression of genes linked to GPX-3's antioxidant activity by 3.068 times. It can be concluded that the 2% spirulina has a substantial effect on daily gain, immunity, antioxidant status, and carcass characteristics.

Keywords: Daily Gain, Spirulina Platensis, Antioxidative Status, gene regulating.

Introduction

Spirulina platensis (SP) is a dietary supplement that is widely utilized in animal diets. Essential vitamins, minerals, and amino acids are all present in spirulina [1]. Some species of Spirulina are not harmful to the liver, kidneys, or reproductive system [2]. Nevertheless, Spirulina supplementation led to an increase in daily weight gain and live body weight of fattening lambs [3, 4, 5]. The immunomodulatory and antioxidant qualities of Spirulina in various animal species have been the subject of an increasing number of studies. Through scavenging free radicals, lowering DNA degradation, lowering lipid peroxidation, and increasing antioxidant enzyme activity,

Spirulina can enhance antioxidant activities [6]. Additionally, Spirulina might enhance immunological activity by stimulating T and B cells, enhancing the synthesis of cytokines and antibodies, and increasing macrophage phagocytic activity [7, 8, 9]. Higher colostrum concentrations of immunoglobulin (IgG) were observed in cows fed a diet supplemented with spirulina [10]. Moreover, fattening lambs can benefit from spirulina supplementation as an immunostimulant, growth promoter, and antioxidant in their feed [4].

Therefore, this investigation aimed to determine the effects of spirulina supplementation on growth, MDA oxidative stress, antioxidant status, meat quality, serum lipid metabolism, and gene expression related to the antioxidant GPX3 and oxidative marker-related gene (MDA) on Awassi male lambs.

Materials and Methods

Animal and Experimental Design

This experiment was carried out from April 2024 to June 2024 in a private farm in Bahrka, Erbil governorate-Iraq. Twenty-four Awassi male lambs (3.5-4 months) with an average initial weight of 27 kg were used in the present work. After [10] day period of adaptation, the lambs were weighed and divided into three equal groups [8 Lambs for each]: lambs in control group fed a basal diet [25% alfalfa hay and 75% concentrate ration] without spirulina supplementation (0% SP), while in treatments T1 and T2 fed control diet with 2 and 3% SP, respectively for 60 days. Before feed was offered in the morning (4% of live weight), each lamb was weighed once a week. The daily feed intake for each treatment was recorded after discarding the residue of the previous day. The percentage of crude protein of alfalfa hay was 13.02%, and [Table 1] represents the chemical composition of the concentrate ration.

Table (1): Ingredient and chemical composition of the ration

Feedstuff	%	Chemical composition*	%
Corn	14.39	Dry mater	94.23
Wheat bran	5.77	Ash	6.70
Barley	60.00	Crude fat	1.88
Soybean meal 48%	14.07	Crude protein	16.80
Salt	0.96	Energy [Mj/ kg DM]	2678
Limestone	4.56		
Vitamin+Mineral Premix	0.25		

*Determined according to AOAC [11].

Blood Sample and Antioxidant Status

At day 60 of the feeding experiment, blood samples were taken from the lamb's jugular vein, and the serum was separated by centrifuging the blood for 10 minutes at 3000 rpm. The serum was refrigerated at -20°C for later analyses. Lipid profile traits (Triglycerides, Total protein, Cholesterol, HDL, LDL) in the serum were analyzed by

an auto analyzer (Humalyzer-3000, USA). Antioxidant (SOD), Superoxide Dismutase Level SOD activity was recorded according to Marklund and Marklund [12], and Oxidative Stress, (MDA) Malondialdehyde was measured using a spectrophotometer and the Thiobarbituric Acid (TBA) test technique of Buege and Aust [10].

Slaughtering of the animals and carcass traits

As explained by Al-Sherwany and Alkass [13, 14], at the end of the experiment, all lambs were slaughtered in the slaughterhouse. Immediately after skinning was completed, the hot carcass was weighed, then chilled for 24 hours at 4^0C and an electric saw was used to divide the carcass along the vertebral column into two halves.

RNA isolation, cDNA synthesis, and qPCR

After slaughtering, until RNA extraction, liver samples were excised, snap-frozen in liquid nitrogen, and stored at −80 ºC. Bio-Rad, Fisher Scientific, Melford, or Sigma Aldrich supplied all chemical materials used for analytical grade. RNA and PCR products were estimated via agarose gel electrophoresis. Agarose 0.7% (w/v) was dissolved in 0.5X TBE buffer and dyed with GelRedTM for gel electrophoresis. 2ml of RNA or cDNA were loaded with 2ml of DNA loading dye and 6µl SDH_2O. GeneRulerTM 1kb DNA ladder was used as the DNA size marker. Samples were electrophoresed for around 90 min and visualized under a UV illuminator.

QRT-PCR thermo-cycle

A quantitative reverse transcription-polymerase chain reaction was performed on 48 samples. QRT-PCR, including both positive and negative controls. The process involved 30 thermal cycles with the following conditions: denaturation at 95°C for fifteen seconds, annealing of molecules at 57.5°C for sixty seconds, and extension at 72°C for fifteen seconds. Quant Fast SYBR Green PCR kit (Cat. no. 204054, Qiagen, Hilden, Germany) and optical grade plates were used in the QRT-PCR, which was carried out using a Bio-Rad CFX96 touch system (Bio-Rad Labs, Hercules, CA, USA).

By using the housekeeping gene β-actin as an internal control, the relative change in gene expression for QTR-PCR was analyzed using the 2-ΔΔCt method of Livak and Schmittgen [15]. Each sample was tested in triplicate, and the quantity of each measured sample was normalized to the β-actin housekeeping gene. Cycle threshold (CT) values were assigned based on the average of the triplicates. By deducting the experimental CT values for each sample from the CT values of the β-actin targets, the ΔCT values were calculated. For each amplified gene target, the reference point was the group with the greatest mean ΔCT value [which indicates the lowest gene expression], with its mean ΔCT value adjusted to zero. This calibrator [ΔΔCT] was then used to compare the mean ΔCT values of different groups. According to SAS

[16], the exponential nature of PCR was taken into consideration by converting the ΔΔCT data to fold changes using the formula 2^(-ΔΔCT) Table (2).

Table (2): Description of the investigation's primers

Gene	Primer F/R	Primer Seq 5′ → 3′	Primer Length	ORF Length	Primer TM/°C	
GPX3	GPX3-F	AGCCACCCTCAAGTATGTTC	20 nt.	451 bp	66	67 °C
	GPX3-R	AGGACAGGAGTTCTTCAGGA	19 nt.		66	
MDA	MDA_F	TCCTGGTGATGACTTCTGCT	22 nt.	1302 bp	63	53 °C
	MDA_R	TGCTGGTCTTCTGGAGTATCA	19 nt.		58	
ACTB	ACTB_F	GTCCGTGACATCAAGGAGAAG	21 nt.	375 bp	65	66 °C
	ATCB_R	AGGAAGGAAGGCTGGAAGAG	20 nt.		66	

GPX3= Glutathione peroxidase=, MDA= Malondialdehyde, ACTB= Housekeeping gene [beta actin]

Statistical analysis

Using SAS's general linear model (GLM) [16] to determine how diet supplementation with Spirulina affects biochemical parameters and growth performance. The mean effects of diets were then compared using the Duncan [17] (α=0.05).

Results and Discussion

Growth performance

Growth performance of Awassi male lambs fed on different levels of spirulina powder is presented in Table (3). Spirulina supplementation significantly influenced [P<0.05] average daily gain (ADG) (290 and 273g/d) for T1 and T2, respectively, in comparison to the control (265g/d). Feed conversion ratio was significantly [P<0.05] different between groups, and the best value was recorded in T1, which was 6.55kg/kg. Daily feed intake was significantly lower (P<0.05) in 2%spirulina, which was 1.91kg/d, compared to the control and 3% spirulina (2.1 and 2.0kg/d), respectively. Spirulina powder levels 2 and 3% significantly affected (P<0.05) hot carcass weight, compared to the control. The current findings were consistent with those reported by Peng et al. [18], who demonstrated that daily gain, carcass weight, and daily feed intake increased significantly (P<0.05) when Hu sheep's fed with spirulina supplementation for 60 days. Moreover, lambs administered spirulina-enriched feed had higher live weights and ADG than lambs that received no supplement [3, 4].

Table (3): Effect of spirulina supplementation on growth performance in Awassi male lambs

Treat.	IW Kg	FW Kg	ADWG g	TWG Kg	ADFI kg/d	FCR kg/kg	Hot Carcass weight kg
Control	26.8	42.7 b	265 b	15.9 a	2.1 a	7.5 a	20.4 b
T1	27.3	44.7 a	290a	17.4 a	1.91 b	6.55 b	23.8 a

T2	27.1	43.5 a	273 ab	16.4 a	2.0 a	7.3 a	21.6 ab
*** SEM**	0.308	0.086	0.308	0.098	0.086	0.098	0.615
P-value	NS	0.0001	0.006	0.0001	0.0001	0.0001	0.0001

* SEM: Standard Error Mean; Control 0% SP; T1= 2% Spirulina; T2= 3% Spirulina; IW: Initial Weight; FW: Final weight, ADWG: Average daily weight gain, TWG: Total weight gain. AD-FI=Average daily feed intake. FCR= Feed conversion ratio. a-b-c Various letters within a column indicate significant differences due to spirulina supplementation level [P<0.05]. NS: Non-significant [P>0.05].

Antioxidant activity and the Amount of Oxidation products

Effect of spirulina powder on oxidative stress and antioxidant activity, as shown in Table (4). Adding spirulina powder significantly (P<0.05) raised superoxide dismutase (SOD) levels for lambs in T2, which was 12.5u/ml compared to control 9.6u/ml in the liver. Similarly, it was noted [19] a rise in the activity of SOD in the blood plasma of ewes fed SP. Conversely, glutathione peroxidase (GPX) was considerably increased by 2% SP supplementation, detecting 68.1u/l in the liver and 152.7u/l in serum as opposed to 58.4 u/l in the liver and 118.7 u/l in the serum in the control. Total antioxidant capacity (T-AOC) was also strongly increased by 2% SP level, reporting 46.1 nmol/g in the liver and 642.8 nmol/l in the serum. On the other hand, malondialdehyde [MDA] in the serum was decreased significantly (P<0.05) in T1 and T2 (7.4 and 7.1nmol/m, respectively), in comparison to the control group [8.1nmol/m]. Likewise, Fouda et al. [20] reported that MDA concentration decreased in lambs fed SP compared to the control. It seems that oxidative stress causes MDA, a biomarker of lipid peroxidation [21]. Considering phycocyanin, polysaccharides, α-tocopherol, and β-carotene are effective antioxidants that can prevent reactive oxygen species-mediated lipid peroxidation, Spirulina has an antioxidant activity associated with these active ingredients [22].

Serum lipid metabolite

The impact of supplementing spirulina powder on serum lipid metabolites is presented in Table (5). Lambs fed a diet with 2% SP had significantly (P<0.05) lower serum cholesterol (0.8mmol/L). Conversely, serum triacylglycerol had dramatically dropped (0.3mmol/L) for both levels of spirulina supplementation, as compared to the control (0.4mmol/L). In addition, high-density lipoprotein (HDL) rose and low-density lipoprotein (LDL) dropped significantly (P<0.05) by supplementing Spirulina in the diet. The concentrations of total protein increased significantly (P<0.05), which were 46.3, 55.7, and 57.1g/L for control, T1, and T2, respectively. Similarly, other workers found that Spirulina treatments significantly increased serum total protein, albumin, and globulin concentrations, which was attributed to an increased protein content of Spirulina [23, 24, 25]. Also, in agreement with the present study [4] presented that Spirulina depressed the level of cholesterol in fattening lambs.

Furthermore, previously [20] demonstrated that serum triglycerides and cholesterol concentrations decreased significantly in the supplemented diet with SP compared

to the control. Spirulina is low in calories and high in protein, vitamins, chlorophyll, beta-carotene, gamma-linolenic acid, and other compounds that assist in regulating fat metabolism. In accordance with the findings of the study, spirulina supplementation could serve as an effective way of decreasing the risk of lipid metabolism in Awassi male lambs.

Table (4): Effect of spirulina supplementation on activities of antioxidants and oxidation products in serum and liver of Awassi male lambs

Item	Section	Control	T1	T2	P-values	*SEM
SOD U/mL	Liver Serum	9.6 b 10.2 b	12.2 a 12.6 a	12.5 a 10.3 b	0.0001 0.007	0.871 0.042
GPX U/L	Liver Serum	58.4 c 118.7 c	68.1 a 152.7 a	62.7 b 150.7 b	0.0001 0.042	0.136 0.111
MDA nmol/m	Liver Serum	6.8 a 8.1 a	5.3 a 7.4 b	5.6 a 7.1 b	0.0001 0.0001	0.043 0.943
T-AOC/liver [nmol/g] T-AOC/serum [nmol/l]	Liver Serum	34.6 b 445.4 b	46.1 a 642.8 a	42.2 a 625.1 ab	0.0001 0.0001	0.090 0.639

* SEM= Standard error of the mean; Control= 0% SP; T1= 2% Spirulina; T2= 3% Spirulina; SOD= Superoxide dismutase; GPX= Glutathione peroxidase; MDA= Malondialdehyde; T-AOC= Total antioxidant capacity. a-b-c Various letters within a row indicate significant differences due to spirulina supplementation level [P< 0.05]. NS: Not significant [P>0.05].

Table (5): Effect of spirulina supplementation on lipid profile and total protein of Awassi male lambs.

Treatments	Cholesterol, mmol/L	Triacylglycerol, mmol/L	LDL mmol/L	HDL mmol/L	Total protein, g/L
Control	1.7 a	0.4 a	1.4 a	0.6 b	46.3 b
T1	0.8 b	0.3 b	0.7 b	0.8 b	55.7 a
T2	1.1 ab	0.3 b	0.8 b	1.1 a	57.1 a
*** SEM**	0.067	0.014	0.102	0.036	0.776
P-value	0.005	0.003	0.009	0.001	0.0001

*SEM= Standard error of the mean; Control= 0% SP; T1= 2% Spirulina; T2= 3% Spirulina; LDL= Low-density lipoprotein; HDL= High-density lipoprotein. a-b-c Various letters within a column indicate significant differences due to spirulina supplementation level [P<0.05].

Gene expression pattern associated with antioxidants

The effect of spirulina powder supplements on the GPX-3, fold-change gene expression in Awassi lambs was shown in Figure (2). Supplementing Spirulina in the diet from 2 to 3% had positively affected the GPX-3 gene expression compared to the

internal reference ß-actin. It can be observed from Figures [1 and 2] that spirulina supplementation had a substantial impact on the mRNA expression patterns of anti-oxidant-related genes in the liver, such as GPX3 and MDA. Relative GPX3 mRNA expression levels in the liver were considerably elevated [P<0.05]. Lambs fed a supplement with 3% spirulina powder showed an overall increase in GPX3 expression (P<0.05) versus the control group.

In contrast, the oxidative marker MDA [Malondialdehyde] gene expression decreased with increasing spirulina powder levels in the diet. Furthermore, spirulina powder (2 and 3%) up-regulated GPX-3 gene expression change fold (2.078 and 3.068-fold), respectively, in comparison to the internal reference ß-actin gene (1.00-fold). Phycocyanin, polysaccharides, α-tocopherol, and β-carotene are all related to Spirulina's antioxidant activity as they may operate as powerful antioxidants that prevent reactive oxygen species from encouraging lipid peroxidation. Despite the present findings, it was discovered [26] that spirulina supplementation of crossbred bulls of Limousine ancestry did not influence the fundamental mRNA expression levels of pro-inflammatory and antioxidant genes in PBMCs throughout the experimental groups.

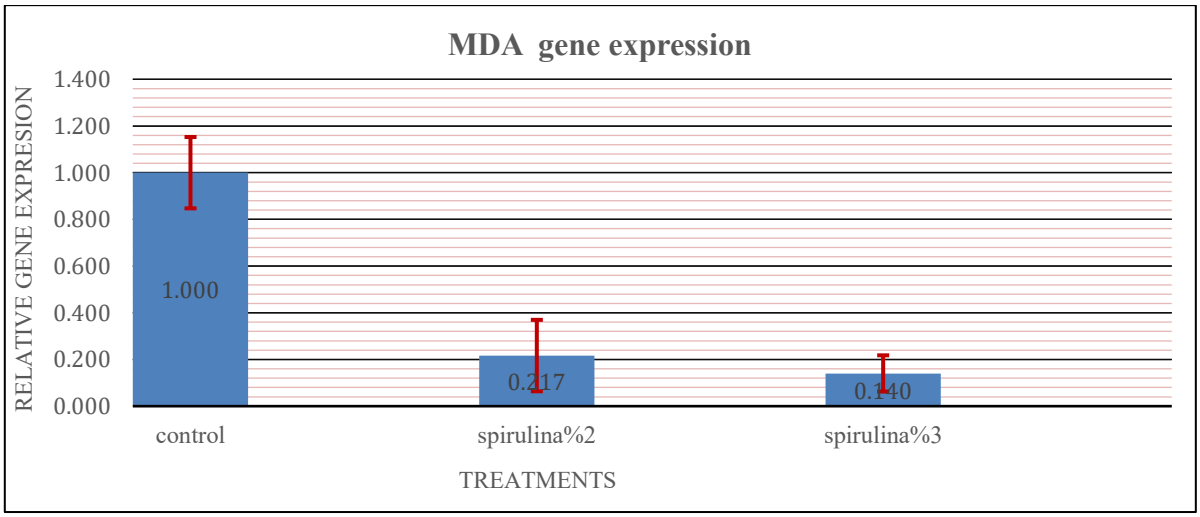

Figure (1): Expression pattern of antioxidant-related genes MDA= Malondialdehyde [A] in the liver of Awassi lambs

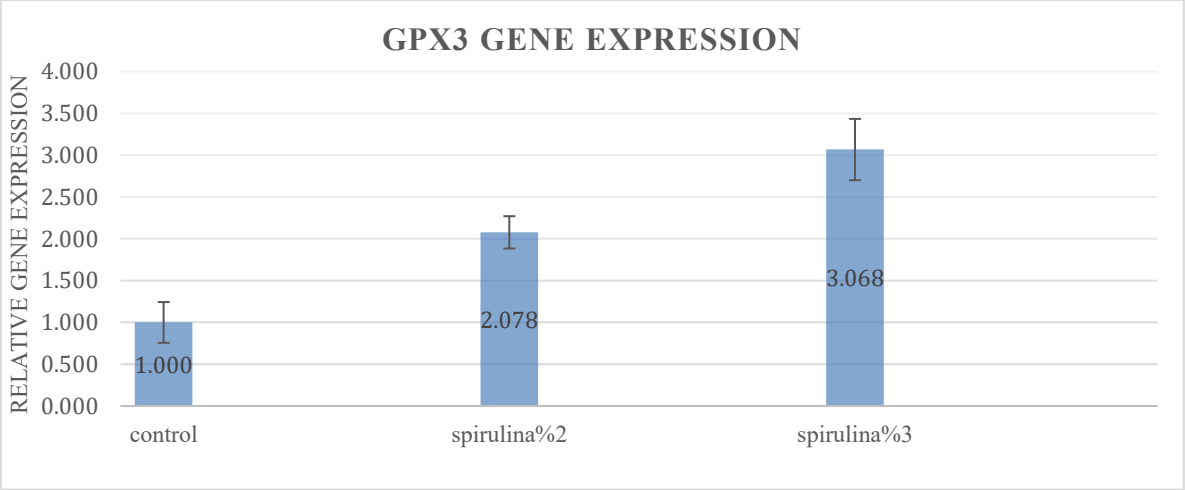

Figure (2): Expression pattern of antioxidant-related genes GPX3 = glutathione peroxidase. B, in the liver of Awassi lambs.

According to the results in the text, it can be concluded that supplementing 2% of Spirulina to the lamb's diet had the most significant impact on immunity, antioxidant status, and carcass characteristics. Furthermore, the SP could be administered to the diets of fattening lambs as a growth-promoting and antioxidant feed addition. Moreover, SP regulated the relative antioxidant ge
ne expression, and 3% spirulina supplementation up-regulated the relative gene expression of genes associated with antioxidant capacity GPX-3 by [3.068-fold] compared to the internal reference gene B-actin by [1.00-fold]. Also, down-regulating MDA relative gene expression, which is responsible for oxidative stress in the liver of Awassi male lambs fed with 2 and 3 percent spirulina, following 60 days.

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