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Research Article Effect of Substitution Soybean by Blue Green Alga *Spirulina platensis* on Performance and Meat Quality of Growing Rabbits

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Abstract

Background: *Spirulina platensis* plays an important role as a source of protein for animal feed, due to its high protein content, fast growth and simplified production. Little studies examined the effect of soybean substitution by *Spirulina* in rabbit feed on performance and meat quality. **Methodology:** In addition to control rabbit's diet, soybean was replaced by *Spirulina* in diets with percentage of 20, 40 and 60%. A total of 36 weaned New Zealand white rabbits (NZW) were divided into 4 experimental groups, 9 replicates each. With exception of digestion trials (3 replicates each group), feed intake and body weight gain, chemical composition, blood parameters and immunoglobulin titer were statistically calculated based on 9 replicates each group. **Results:** There were no significant differences between various treatments and control in live body and empty carcass weights. Whearas, significant increase in meat quality between the treament of 60% replacement and other treatments were observed, especially in meat contents of polyunsaturated fatty acid, essential and non essential amino acids. Also, no significant differences were found among the different treatments and control in kidney function (ALT, AST) and immune response (IgG, IgM). Generally, all parameters were within normal range of rabbits. **Conclusion:** The substitution 60% was recommended to apply within an industerial scale as the best diet for rabbit.

Key words: Spirulina platensis, growing rabbits, performance, meat quality, blood parameters

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Soybean meal, fishmeal and groundnut meal are the main protein supplement choice for rabbits. However, due to the scarcity and increasing costs of these meals, searching for alternative protein sources is gaining importance¹. Microalgae play an important role as a source of protein for animal feed, due to its high protein content, fast growth and simplified production. Among these microalgae species, *Spirulina platensis* is an excellent source of protein, essential polyunsaturated fatty acids, minerals, vitamins, carbohydrates, sterols and easily digestible²⁻⁵. Also, *Spirulina* rich in photosynthetic pigments, chlorophyll, carotenoids and phycobiliproteins (phycocyanin, allophycocyanin and phycoerythrin), which are mainly responsible for antioxidant activities^{6,7}.

Spirulina is a photosynthetic, filamentous, spiral, non-heterocysts, multicellular blue green algae which grows in wide range fresh, marine and brackish water. It grows well in a highly alkaline environment⁸ of pH 10-12. *Spirulina platensis* is reported to be easy in culturing, harvesting and drying process. It becomes the most common popular species in microalgal biotechnology studies⁹. The biochemical composition depends upon the *Spirulina* source, culture conditions and season of production^{4,10}.

Spirulina has been trialed in the feed rations of commercially farmed meat rabbits. Rabbits receiving dietary Spirulina have an increased total feed consumption compared to those receiving no Spirulina¹. Dietary Spirulina levels of 1% of total dry matter was found to improve crude protein digestibility in rabbits fed diets compared to those receiving no Spirulina¹¹. Rabbit meat quality has been improved when rabbits received dietary Spirulina. Meineri *et al.*¹² identified dietary Spirulina as a causal factor for increasing γ -linolenic acid and n-6/n-3 poly-unsaturated fatty acids ratios within rabbit muscle lipid contents.

Although, the Egyptian aquatic environment is a rich media with nutrients for *Spirulina* production, there are no available studies used *Spirulina* as a supplement in rabbit feed. So, the objective of this study is to evaluate the effect of substitution soybean by blue green alga *Spirulina platensis* on performance, blood parameters and meat quality of growing rabbits.

MATERIALS AND METHODS

The experimental protocol used in this study was approved by the Animal Care and Use Committee of Cairo University, Faculty of Agriculture, Egypt. **Microorganism:** *Spirulina platensis* strain was isolated from Al-Khadra Lake, Wadi Al-Natroon, El-Baheira governorate, Egypt. Isolation and purification of *S. platensis* was performed by streaking plate method of Stein¹³ using BG-11 agar medium¹⁴. Morphological identification of *S. platensis* was carried out using a phase contrast microscope (Carl Zeiss, Jena, Germany) according to Desikachary¹⁵ and Prescott¹⁶.

Cultivation and production of *S. platensis*: Using modified BG-11 medium¹⁷, outdoor mass production of *S. platensis* was done in Algal Biotechnology Unit, NRC, Egypt. Outdoor production scale was achieved within two ponds with a final capacity 30 m³ of net cultivation volume, 0.3 m depth with plastic sheet cover. Sub culturing was performed within sequences and gradual volumes till 1200 L plate photobioreactor. Harvesting method was done using continuous separating centrifuge apparatus (Westevalia Separator centrifuge 5000 L h⁻¹) and drain water was recycled to the ponds. Biomass was overnight dried in oven at 50°C.

Experimental diet: Three levels of *Spirulina* were used as a soybean meal replacement (Table 1). The experimental diets were formulated to cover the nutrients requirements of growing rabbits as recommended by NRC¹⁸. All diets were pelleted and stored in darkness to avoid auto-oxidation of the lipid sources. Simultaneously, the percentage of yellow corn was increased by increasing *Spirulina* in diets to equilibrate the high percentage of protein in *Spirulina*.

Experimental animals and management: A total number of 36 weaned New Zealand white rabbits (NZW) at 6 weeks of age (about 540 g as a general average of body weight) were individually weighed and randomly assigned into 4 experimental groups, 9 each. All animals were kept under the same management and hygienic conditions and were housed in metal battery cages supplied with separated feeders. Diets were offered *ad libitum* and fresh water was available all times from automatic nipple drinkers. The experimental period lasted for 8 weeks. Feed intake and body weight gain were weekly recorded and then feed conversion ratio was calculated. All rabbits were vaccinated against diseases and they were under veterinary control.

Digestion trials: At the end of the experimental period (14 weeks of age), digestibility trials were carried out using 12 NZW males (3 from each treatment) to determine the digestion coefficient of nutrients and nutritive value of

	Experimental diets (%)							
ltem	C (Control)	D1 (20% soybean replacement)	D2 (40% soybean replacement)	D3 (60% soybean replacement				
Diets structure								
Barley grains	15	12	9	7				
Soybean meal	15	12	9	6				
Spirulina	0	3	6	9				
Yellow corn	20	23	26	28				
Wheat bran	10	10	10	10				
Alfa alfa hay	35	35	35	35				
Methionine	0.25	0.25	0.25	0.25				
Limestone	0.75	0.75	0.75	0.75				
Molasses	3	3	3	3				
Salt	1	1	1	1				
Chemical composition								
DM	90.95	90.15	89.47	89.82				
OM	88.74	90.39	90.53	89.65				
Ash	11.26	9.61	9.47	10.35				
EE	3.510	3.70	3.53	3.72				
СР	17.89	19.56	20.04	20.32				
CF	10.54	9.78	9.86	10.04				
NFE	56.80	57.35	57.10	55.55				

Table 1: Formulation and chemical composition of experimental diets (%)

DM: Dry matter, OM: Organic matter, EE: Ether extract, CP: Crude protein, CF: Crude fiber and NFE: Nitrogen free extract

experimental diets as described by Abou-Raya *et al.*¹⁹. A plastic net was placed under the cages to retain feces during the collection period (4 days), feces were collected daily before the morning meal and weighed fresh and sprayed with 2% boric acid for trapping any ammonia released from feces and dried at 60°C for 24 h in an air drying oven. The feces were then ground and mixed, stored for subsequent chemical analysis. Data of quantities and chemical analysis of feed (input) and feces (output) were used to calculate the nutrient digestion coefficients and the nutritive value for each dietary treatment.

Chemical composition: Chemical analyses for determining moisture, Crude Protein (CP), Crude Fiber (CF), ethyl ether extract (EE) and ash for the tested diets, feces and meat were done according to the methods recommended by AOAC²⁰. Amino acids contents and fatty acids profile of rabbit meat were carried out according to Bailey²¹ and Christie²², respectively.

Blood parameters and immunoglobulin titer determination:

Blood samples were collected from rabbits at the end of digestion trail. The blood samples were taken in dry clean lasses tubes using heparin as anticoagulant and then centrifuged for 15 min at 4000 rpm to obtain plasma. Biochemical of blood plasma constituents were determined using commercial kits of total protein and creatinine^{23,24}, albumin²⁵ and blood plasma urea²⁶. Alanin amino transferase (ALT) and activity of aspartate transfearse (AST)

were determined by the methods of Young²⁷. Plasma total immunoglobulin titres was determined according to Van der Zijpp *et al.*²⁸.

Statistical analysis: The data were analyzed using General Linear Model (GLM) of SAS[®] software statistical analysis²⁹. Means were separated using Duncan's multiple range test³⁰ when the main effect was significant.

RESULTS

Digestion coefficients and nutritive values: Nutrients digestibility and nutritive values of the experimental diets are illustrated in Table 2. In general, Dry Matter (DM) and Organic Matter (OM) digestibility showed a positive significant effect by adding *Spirulina* in diets when compared with control diet. There was a clear effect of *Spirulina* on crude fiber digestibility, where D3 recorded the highest value (47.55%) with difference by 17% when compared with control one (30.43%). No significant differences observed among diets with EE and NFE.

Also, nutritive values expressed as Total Digestible Nutrients (TDN) and Digestible Crude Protein (DCP) exhibited a significant effect by adding *Spirulina* in rabbit diets. The addition of *Spirulina* in D2 and D3 significantly increased TDN values when compared with control (C), whereas, DCP values increased significantly in all treatments.

Blood parameters: Table 3 illustrates blood parameters and immune response of rabbits fed on diet containing

ltem	Experimental diets					
	C (Control)	D1 (20%)	D2 (40%)	D3 (60%)	±SEM	p-value
Digestibility (%)						
DM	60.29 ^b	64.35 ^{ab}	67.16ª	67.92ª	1.10	0.250
OM	62.93 ^b	66.13ªb	68.86ª	69.84ª	1.05	0.057
EE	73.88ª	74.26ª	75.62ª	76.14ª	0.51	0.388
СР	62.54 ^b	69.77ª	69.51ª	71.12ª	1.12	0.003
CF	30.43°	34.95 ^{bc}	43.35 ^{ab}	47.55ª	2.32	0.007
NFE	68.55ª	69.21ª	72.10 ^a	72.44ª	0.88	0.320
Nutritive values (%)						
TDN	59.16 ^b	62.93 ^{ab}	65.37ª	65.84ª	1.01	0.042
DCP	11.19 ^c	13.64 ^b	13.93 ^{ab}	14.45ª	0.38	0.0001

Table 2: Effect of inclusion *S. platensis* in growing rabbit diet on digestion coefficients and nutritive values

Means in the same row with different superscript are significantly different (p<0.05), TDN: Total digestible nutrients, DCP: Digestible crude protein, C: Control diet (without *S. platensis*), D1: Control diet with replacing 20% from soybean meal by *S. platensis*, D2: 40% from soybean meal by *S. platensis*, D3: 60% from soybean meal by *S. platensis*

Table 3: Effect of inclusion S. platensis in growing rabbit diet on blood parameters and immune response

ltem	Experimental diets							
	C (Control)	D1 (20%)	D2 (40%)	D3 (60%)	±SEM	p-value		
Blood parameters								
Total proteins (g dL ⁻¹)	6.83ª	5.90ª	6.47ª	6.67ª	0.26	0.674		
Albumin (g dL ⁻¹)	4.13ª	4.20ª	3.90ª	3.93ª	0.06	0.229		
Globulin (g dL ⁻¹)	2.70ª	2.70ª	2.57ª	2.73ª	0.29	0.617		
Urea (mg dL^{-1})	25.12 ^b	32.90ª	28.25 ^{ab}	20.00 ^c	2.84	0.0506		
Creatinine (mg dL ⁻¹)	0.93ª	1.20ª	0.90ª	1.20ª	0.09	0.629		
ALT (IU L ⁻¹)	12.33ª	10.41ª	10.92ª	11.85ª	3.27	0.576		
AST (IU L^{-1})	29.68ª	26.55ª	26.96ª	26.82ª	3.00	0.608		
Immune response								
lgG*	18.93ª	19.80ª	16.86 ^{ab}	16.30 ^b	1.80	0.018		
lgM**	0.93ª	1.06ª	0.93ª	0.99ª	0.50	0.803		

ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, *Immunoglobulin G, ** Immunoglobulin M, means in the same row with different superscript are significantly different (p<0.05), C: Control diet (without *Spirulina platensis*), D1: Control diet with replacing 20% from soybean meal by *S. platensis*, D2: 40% from soybean meal by *S. platensis*, D3: 60% from soybean meal by *S. platensis*

S. platensis. In general, inclusion of *S. platensis* in rabbit's diet significantly has no effect on blood parameters and immune response. Interestingly, 60% substitution in D3 significantly decreased urea concentration and IgG as well when compared with control. On the other hand, rabbits fed on D1 significantly exhibited an increase in urea.

Growth performance: Data of growth performance are represented in Table 4. No significant differences among the experimental animals in body weight parameters were noticed. The parameters of feed intake statistically affected by the addition of *Spirulina*. While, TDN and DCP values of all treatments recorded a significant increase, significant decrease was observed in DM value of D1 and D3 when compared with control. Feed conversion of the same parameter also affected by *Spirulina* addition in rabbit diet. All treated diets showed a significant increase in DCP. These results attributed to nutritive values of the experimental diets.

Carcass traits and rabbits meat composition: Results of carcass traits and chemical composition of rabbits' meat are illustrated in Table 5. Generally, no significant differences among diets in empty carcass (ECW) and Edible Parts Weight (EPW) were observed. Also, no differences (p<0.05) were observed in dressing and edible organs percentage. Regarding to the chemical composition data, D2 and D3 showed a significant increase in protein content and a significant decrease in total lipids when compared with control.

Fatty acids content in rabbits meat: Meat fatty acids content are illustrated in Fig. 1. Inclusion *Spirulina* in rabbit's diets significantly effected on the content of measured fatty acids. Myristic (C14:0), palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2) and γ -linolenic (C18:3) acids were the representing fatty acids. Significant decrease was noticed in stearic acid content by adding *Spirulina* at all tested substitutions. The D2 and D3 exhibited a significant

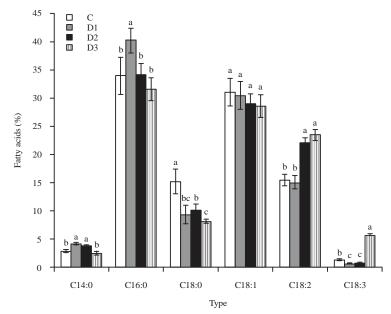


Fig. 1: Effect of inclusion *spirulina platensis* in growing rabbit diet on fatty acids content in meat

ltem	Experimental die	Experimental diets				
	C (Control)	D1 (20%)	D2 (40%)	D3 (60%)	±SEM	p-value
Body weight change (g)					
IBW	540.00ª	541.00ª	539.00ª	541.00ª	11.28	1.000
FBW	1805.90ª	1907.70ª	1809.98ª	1866.70ª	40.77	0.817
TG	1265.89ª	1366.66ª	1270.54ª	1325.58ª	41.77	0.817
ADG	21.00ª	23.00 ^a	21.00ª	22.00ª	0.69	0.790
Feed intake (g day ⁻¹)						
DM	81.09ª	78.43 ^b	80.82ª	77.42 ^b	0.52	0.005
TDN	47.97 ^d	49.36°	53.12ª	50.97 ^b	0.60	0.0001
DCP	9.07°	10.69 ^b	11.26ª	11.18ª	0.26	0.0001
Feed conversion (g feed	d g ^{−1} gain)					
DM	3.86ª	3.41°	3.85ª	3.52 ^b	0.06	0.0001
TDN	2.28 ^b	2.15°	2.53ª	2.32 ^b	0.04	0.0001
DCP	0.43 ^c	0.47 ^b	0.54ª	0.51 ^b	0.01	0.0001

Table 4: Effect of inclusion S.	<i>platensis</i> in growing	a rabbit diet on o	arowth	performance and feed intake

Means in the same row with different superscript are significantly different (p<0.05), IBW: Initial body weight, FBW: Final body weight, TG: Total gain, ADG: Average daily gain, DM: Dry matter, TDN: Total digestible nutrients, DCP: Digestible crude protein, C: Control diet (without *S. platensis*), D1: Control diet with replacing 20% from soybean meal by *S. platensis*, D2: 40% from soybean meal by *S. platensis*, D3: 60% from soybean meal by *S. platensis*

Table 5: Effect of inclusion spirulina platensis in growing rabbit diet on carcass traits and chemical composition of meat

	Experimental diet					
ltem	C (Control)	D1 (20%)	D2 (40%)	D3 (60%)	±SEM	p-value
Carcass traits						
LBW (g)	1805.90ª	1907.70ª	1809.98ª	1866.70ª	40.77	0.817
ECW (g)	914.76ª	959.71ª	890.53ª	882.21ª	20.77	0.616
EPW (g)	110.39ª	130.02ª	117.86ª	141.21ª	7.60	0.557
Dressing (%)	56.83ª	57.15ª	55.78ª	54.78ª	0.56	0.489
Edible (%)	6.15ª	6.82ª	6.43ª	7.61ª	0.38	0.627
Chemical composition						
Moisture	70.08ª	70.07ª	69.47ª	68.62 ^b	0.21	0.007
Ash	1.30ª	1.19ª	1.16ª	1.41ª	0.06	0.481
Protein	20.43 ^b	20.91 ^b	22.17ª	22.48ª	0.27	0.0001
Total lipids	8.19ª	7.86 ^{ab}	7.22 ^c	7.51 ^{bc}	0.13	0.014

Means in the same row with different superscript are significantly different (p<0.05), LBW: Live body weight, ECW: Empty carcass weight, EPW: Edible parts weight, C: Control diet (without *S. platensis*), D1: Control diet with replacing 20% from soybean meal by *S. platensis*, D2: 40% from soybean meal by *S. platensis*, D3: 60% from soybean meal by *S. platensis*

	Experimental diet					
Item	C (Control)	D1 (20%)	D2 (40%)	D3 (60%)	±SEM	p-value
Essential amino acids						
Valine	7.43 ^b	7.19 ^b	8.58 ^b	12.42ª	0.68	0.001
Isoleucine	21.41 ^b	22.63 ^b	20.78 ^b	32.04ª	1.40	0.0001
Lysine	14.99 ^b	13.80 ^b	13.30 ^b	20.30ª	0.87	0.0001
Methionine	1.02 ^c	2.71 ^{bc}	3.71 ^{ab}	5.39ª	0.54	0.004
Tyrosine	5.7°	5.48°	7.69 ^b	10.69ª	0.68	0.001
Threonine	6.12 ^b	4.93 ^b	5.41 ^b	8.61ª	0.49	0.008
Phenylalanine	14.29 ^c	13.57°	18.44 ^b	28.75ª	1.84	0.0001
Histidine	9.99 ^b	7.91°	8.39 ^{bc}	13.64ª	0.72	0.0001
Non-essential amino acids						
Arginine	20.52 ^b	23.19ª	19.35 ^b	19.87 ^b	0.51	0.007
Aspartic acid	16.22 ^b	11.97°	12.87 ^c	20.10ª	0.99	0.0001
Glycine	11.96 ^b	11.42 ^b	12.51 ^b	17.57ª	0.78	0.0001
Glutamic acid	46.21 ^b	38.22 ^d	43.40 ^c	70.01ª	3.69	0.0001
Proline	4.31ª	5.40 ^a	5.41ª	5.17ª	0.28	0.522
Serine	8.26 ^b	6.97 ^b	7.47 ^b	12.21ª	0.69	0.001

Table 6: Effect of inclusion Spirulina in growing rabbit diet on amino acids content in meat (mg g^{-1})

Means in the same row with different superscript are significantly different (p<0.05), C: Control diet (without *S. platensis*), D1: Control diet with replacing 20% from soybean meal by *S. platensis*, D2: 40% from soybean meal by *S. platensis*, D3: 60% from soybean meal by *S. platensis*

increase in linoleic acid recording 43 and 52% when compared with control. Moreover, γ -linolenic acid increased more than 4 folds when compared with control diet.

Amino acids content in rabbits meat: Amino acids in rabbit meat affected by inclusion of *S. platensis* in diets are shown in Table 6. A significant increase in methionine, tyrosine and phenylalanine contents was observed in D2 and D3 when compared with control. While, no significant difference noticed among diets in proline amino acid. Generally, concentrations of essential and non-essential amino acids in meat statistically affected by inclusion *S. platensis* in diets. The D3 recorded a significant increase in all essential amino acids. Similarly, D3 significantly achieved a noticeable increase in non-essential amino acids with exception of arginine and proline when compared with control.

DISCUSSION

Many studies have recently focused on using *Spirulina* as an alternative source of protein in rabbit feed instead of soybean^{1,11,12}. However, none of these studies examined the effect of different concentrations on the performance and meat quality of the growing rabbits. So, soybean was replaced by *Spirulina* in diets with percentage of 20, 40 and 60%. Generally, a noticeable increase in the nutritive values was observed notably the diet D3, 60% replacement. These results might be due to the rich content of *Spirulina* in protein, polyunsaturated fatty acids and minerals when compared with soybean content. Consequently, digestion

coefficients were increased especially CP and CF. The obtained results were in agreement with the investigation of Peiretti and Meineri¹¹, who found an enhancement in the digestibility nutrients by dietary inclusion of 1% *Spirulina*. In contrast, Gerencser *et al.*³¹ noticed that, no significant effect of *Spirulina* supplementation (5%) on dry matter and organic matter total tract digestibility, whereas, a significant increase in the CP total tract digestibility was noticed when compared with control fed rabbits. Alvarenga *et al.*³² observed that *Spirulina* species had a higher nutritional value compared to soybean meal in terms of crude protein content, minerals, metabolisable energy and amino acids profile.

The lower concentrations of *Spirulina* inclusion had no effect on blood parameters and immune response of rabbits. However, the diet 60% substitution significantly decreased urea concentration and IgG referring to good physiological properties. Generally, the blood results indicated that all parameters were within the normal range of rabbits according to Ozkan *et al.*³³ and Merck³⁴.

Regarding to the growth performance parameters, body weight did not affect by inclusion, whereas, TDN and DCP values significantly increased. These observations attributed to nutritive values of the experimental diets. These results were matched with Heidarpour *et al.*³⁵, Moreira *et al.*³⁶ and Seyidoglu and Galip³⁷ where the effect of *S. platensis* on growth performance of calves and rabbit was determined. Seyidoglu and Galip³⁷ reported that no significant difference between rabbits fed diet with 5% *S. platensis* and control one was observed. Also, Dernekbasi *et al.*³⁸ used 10, 20, 30 and 40% *S. platensis* into

diet of fish and no differences were recorded in growth parameters among groups. Also, other studies indicated that no differences in rabbit live body weight changes were noticed when fed *Spirulina* supplements at levels of 0.5%³⁹, 1%¹¹, 3%⁴⁰, 5%³¹ or 5, 10 and 15%¹. However, Grinstead *et al.*⁴¹ found a significant increase in growth rate by adding *S. platensis* in big diets. Also, Promya and Chitmanat⁴² noticed a significant increase in the body weight gain by dietary *S. platensis* supplementation in catfish diets.

No differences were observed between the parameters of carcass traits of treated rabbits and control ones, whereas, significant increase in protein content and a significant decrease in total lipids. The observation was not in agreement with Seyidoglu and Galip³⁷ who found that chemical composition significantly unaffected by adding *Spirulina* to rabbit diets.

The addition of *Spirulina* to the diet increased the nutritional values of diet also throughout the increasing of linoleic acid (50%) and γ -linolenic acid (200%) when compared with control diet. Many researchers emphasized these results. Estrada *et al.*⁴³ reported that adding *Spirulina* to the diet components had therapeutic properties acting as antioxidant agent. They attributed this activity to presence of polyunsaturated fatty acids and phycocyanin in *Spirulina*. Also, Sarada *et al.*⁴⁴ found that *Spirulina* contained content of linolenic acid (C18:3). Peiretti and Meineri¹ and Meineri *et al.*¹² revealed that *Spirulina* in rabbits diets act as a causal factor for increasing γ -linolenic acid and n-6/n-3 PUFA ratios within rabbit muscle lipid contents.

Changing part of protein source in diets by substitution of soybean by *Spirulina* affected on the amino acid types and contents notably in D2 and D3. Methionine, tyrosine and phenylalanine were the most affected once, whereas, proline didn't change. In general, D3 significantly increased both essential and non-essential amino acids in meat. That increase could be attributed to high content of amino acids in *Spirulina* when compared with soybean content^{45,9}.

CONCLUSION

Substitution of soybean by *Spirulina platensis* had not noticeably effect on growth performance of growing rabbits. However, there was valuable effect on meat quality by increasing of protein and polyunsaturated fatty acids content. At the same time, there was no effect in kidney, liver functions and immune response by increasing *Spirulina* in diets and all parameters were within normal range of rabbits. The substitution 60% was chosen as the best diet for rabbit.

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