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Article:

Appraisal of addition of *Bacillus subtilis* and *Saccharomyces cerevisiae* to Japanese quail diet on growth performance, biochemical parameters, and digestive enzymes.

Marwa A. Fwaz[1]*, Reham Nabil[2] , Ahmed S. Asman[3], Gamal M. Mehani[4] and Mohamed A. Mousa[1]

[1]Department of Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Sohag University.[2] Department of Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Sadat city University.[3] Department of Biochemistry, Faculty of Veterinary Medicine, Sohag University.[4] Department of Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Minya University.

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Abstract

The present study aimed to investigate effects of adding some probiotics as *Bacillus subtilis (B. subtilis)* and *Saccharomyces cerevisiae (S. cerevisiae)* on the growth performance, blood biochemical parameters, and specific activity of some digestive enzymes as (trypsin, chymotrypsin and α amylase). A total of 150 Japanese quail chicks were randomly distributed into three groups. The first group (control group) received the basal diet that contains recommended requirements; the second group received the basal diet with (1×108 CFU /kg DM *Bacillus subtilis*) and the third group received the basal diet with (3×108 CFU /kg DM *Saccharomyces cerevisiae*). There was a significant improvement ($p<0.05$) in body weight gain, feed conversion ratio (FCR) and specific growth rate (SGR) in the group supplemented with *Bacillus subtilis* and *Saccharomyces cerevisiae* when compared with the control group, while feed consumption not affected among groups. Serum cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL) were significantly improved in the second group, while triglycerides slightly increased in this group. The addition of *Bacillus subtilis and Saccharomyces cerevisiae* significantly decreased the serum creatinine and urea levels ($p<0.05$) compared with the control group. All groups had no significant difference in serum total protein, albumin, and globulin. However, blood and aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were significantly ($p<0.05$) decreased in the second group. *Bacillus subtilis* and *Saccharomyces cerevisiae* improved digestive enzymes activity especially α amylase enzyme. It is recommended to add these additives during quails fattening, as they could improve birds' health and production.

Keywords: *Bacillus subtilis*, Biochemical parameters, Growth performance, Japanese quail, *Saccharomyces cerevisiae.*

Introduction

Antibiotics have long been used in the poultry industry as growth promoters, in 2003 their usage was banned **[1]** because the use of these additives in poultry diets can lead to adverse effects such as antimicrobial resistance and drug residue issues **[2]**. This tragedy prompted the search for promising alternatives to antibiotics. Although there are many alternatives to them, such as probiotics, prebiotics, and medicinal plants **[3]**.

Probiotics, called direct-feeding live microbes, can alter the microbial profile of the gut ecosystem and improve the health and growth performance of host animals **[4]**. *Bacillus subtilis* spores, a Gram-positive spore-forming bacterium, are one of the most used probiotics in poultry production due to their thermal stability during feed processing and their resistance to the physiological conditions of the gut ecosystem, which produce anti-clostridial substances, so they mainly improve immunity and increase production of

IJCVR **Corresponding author:* *Marwa A. Fwaz, Email: marwaahmedfwaz@gmail.com Address: Department of Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Sohag University, Sohag, Egypt*

immunoglobulins **[5]**. Appropriate use of feed additives can improve feed conversion, production, and public health for Japanese quail. Probiotics such as yeast (Saccharomyces cerevisiae) are one of the most important feed additives used to improve animal health and performance **[4]**. *Saccharomyces cerevisiae* is evaluated as potential feed additives aimed at improving feed conversion, digestibility, reducing pathogen numbers, and improving animal performance **[6]**. It has beneficial effects on host health through its direct nutritional effects, source of vitamins and minerals **[7]**. Budding yeast produces alpha-amylase and protease that break down starch and protein molecules, respectively, to aid digestion and efficient utilization **[7]**. Japanese quail is the smallest of the poultry species in terms of meat output, making it simple to handle and allowing for rearing large number of them in a small area. Because of these factors, the Japanese quail has also become more significant on a global scale due to its usage in experiments involving animal models for biological and genetic investigations **[8]**. Generally, the use of such probiotics in the poultry industry is pronouncedly increased, while its usage on quail is limited. Therefore, this study aimed to appraise effects of such supplements on growing quail chicks, biochemical parameters, and digestive enzymes activity.

Materials and Methods

The study was conducted at the Nutrition and Clinical Nutrition Research Center at the Faculty of Veterinary Medicine, Sohag University, Sohag, Egypt. The ethical approval number: VUSC-026-1-23 of the Faculty of Veterinary Medicine, Sadat city University.

1. Japanese quail

A total of one hundred and fifty (150) unsexed 5- day old Japanese quail chicks (*Coturnix coturnix japonica*) were obtained from a commercial company for quail production, Sohag Governorate.

2. Feeding

The experimental diets were formulated from commercially available ingredients. The ingredients in the diets were corn, soybean meal, and corn gluten meal. The starting and growing diets were formulated to contain 24% crude protein and 2900 Kcal/Kg (ME) **[9]** (NRC1994) as shown in **Table 1.** The quails were fed a mash diet on an ad libitum basis, and the health status of quails was monitored daily.

Table (1): Chemical composition of the ingredients used in the experimental diets (as fed basis).

Stages	Starter – grower
Ingredients, %	
Corn	56.00
Soybean meal 44% CP	34.00
Corn gluten meal	6.00
Limestone	0.52
Dicalcium phosphate	2.15
NaCl	0.30
DL-Methionine	0.18
L-Lysine HCL	0.60
Vitamin-Mineral Premix	0.25
Chemical analysis	
DM%	82.27
CP%	24.27
CF%	2.64
Ash%	2.99
EE%	2.62
ME, Kcal/g	2960
Lysine	1.33
Methionine	0.62

Vitamins and minerals mixture provide per kilogram of diet: Vitamin A (as all-trans-retinyl acetate); 12000 IU; Vitamin E (all rac-α-tocopheryl acetate); 10 IU; k3 3mg; Vit.D3, 2200 ICU; riboflavin, 10 mg; Ca pantothenate,10 mg; niacin, 20 mg; Choline chloride, 500 mg; Vitamin B12, 10μg; Vitamin B6, 1.5 mg; Thiamine (as thiamine mononitrate); 2.2 mg; Folic acid, 1 mg; D-biotin, 50μg. Trace mineral (milligrams per kilogram of diet) Mn, 55; Zn, 50; Fe, 30;Cu, 10; Se, 0.1 and Ethoxyquin 3mg.

3. Experimental groups

After an adaptation period that lasted for 8 days, one hundred and fifty Japanese quail (*Coturnix coturnix japonica*), 14-days old, were divided into three groups. The first group (control group) received the basal diet that contains recommended requirements without probiotics; the second group received the basal diet plus (1×10^8CFU/kg DM *Bacillus subtilis*), which was recommended by **Mojgani [10]** for improvement of growth performance and biochemical parameters in Japanese quail. and the third group received the basal diet with (3×10^8CFU/kg DM *Saccharomyces cerevisiae)* which was recommended by **Celik [11]** to improve the performance and biochemical parameters of broiler chickens.

4. Experimental design and measurement

Quails were housed in an environmentally controlled room. Housing temperature was initially maintained at 36 ˚C and then gradually reduced to 23-24 ˚C for the remaining of the experimental period. A continuous lighting program was

applied during the experiment (The light regime used was about 16 hours/day, artificial light). Feed and water were provided ad libitum to birds. The birds were kept under the same environmental and management conditions. Quails were kept for an adaptation period from 5 days to 13 days of age. In this period, quail were fed a basic diet. At 14 days of age quails' chicks were weighted to determine the initial weight. Serum samples and duodenum tissue were taken from three birds for biochemical analysis and digestive enzymes activities evaluation at 14, 24 and 36 days of age.

5.Proximate analysis of experimental rations

The experimental feed was analyzed using the standard analysis method of the Association of Official Analytical Chemist **[12]**. The dry matter of the samples was determined using a hot air oven at 105°C, crude protein analyzed by using Micro-Kjeldahl method. The ash content was determined using a muffle furnace (Nabertherm) at 600°C for two hours. Crude fiber was determined by fiber analyzer (Ankom 2000).

6. Biochemical parameters determination:

6.1 Samples collection

During slaughtering, three blood samples per group were collected in sterile tubes without anticoagulant, left to clot for 10 minutes and centrifuged at 4000 rpm for 15 minutes at 20ºC until clear serum was obtained. The separated serum was stored at -80°C for later biochemical parameters analysis.

6.2 Methods of biochemical parameters determination

The serum samples were used for analysis of total protein (TP) **[13]**, albumin **[14]**, urea **[15]**, creatinine **[16]**, aspartate aminotransferase (AST), alanine aminotransferase (ALT) **[17]**, triglycerides **[18]**, cholesterol **[19]**, high-density lipoprotein (HDL) **[20]**, low-density lipoprotein (LDL) **[21]**. Biochemical analyses were measured by using commercial kits purchased from bio-diagnostic company (Address: 29 Tahrir St., Doki, Giza, Egypt) using a T80+ UV/VIS spectrometer (PG instruments Ltd).

7. Performance parameter:

7.1 Growth performance

Quails were weighed at the beginning of the experimental period (day 14) to determine the initial weight. Every 10 days, body weight (BW), body weight gain (BWG), specific growth rate (SGR), feed consumption and feed conversion ratio (FCR) were calculated **[22,23 and 24]**.

7.1.1Body weight gain

Body weight gain was calculated using the formula:

$$\text{BWG} = \frac{\text{final weight} - \text{initial weight}}{\text{number of days from initial to final weight}}$$

7.1.2 Feed Conversion Ratio

The feed conversion ratio (FCR) was calculated as gram of feed per gram of live weight gain.

$$\text{FCR} = \frac{\text{feed intake}}{weight\ gain}$$

7.1.3 Specific growth rate

$$\text{SGR} = \frac{\text{Final weight} - \text{initial weight}}{\text{time of experiment}} \times 100$$

7.2 Carcass trait parameters

Every 10 days, 9 quails (3 per group) were randomly taken and weighed, and then slaughtered to obtain the carcass and organs such as intestine and liver. Carcass and organs percentage were calculated based on live body weight, which was calculated as described previously **[25]**.

$$\text{Carcass yield} = \frac{\text{slaughter weight}}{\text{living BW}}$$

8. Digestive enzymes activity assay

Throughout the experiment, three quails were randomly collected from each experimental group and slaughtered by severing the jugular vein, and then duodenum tissues were immediately collected after the birds' evisceration. Based on the sample weight, samples were diluted 10× with monobasic and dibasic phosphate buffer, subsequently homogenized using a tissue homogenizer and centrifuged at 10000 rpm for 30 min at 4 °C. The supernatants were separated and stored at -80°C until analysis. Total protein, trypsin, chymotrypsin, and α-amylase activities were determined. Protein by Folin Reaction **[26]** has been used to estimate the amount of protein in biological samples. The amount of protein in the sample can be estimated by reading the absorbance (at 750 nm) of the product of the Folin reaction against a standard curve of a selected standard protein solution (Bovine Serum Albumin-BSA- solution). After homogenizing and centrifuging, the crude enzyme extract in the supernatant was kept at -80ºC. Trypsin activity **[27]**, chymotrypsin activity **[28]** and α-amylase activity **[29]** were determined using a T80+ UV/VIS spectrometer (PG instruments Ltd).

Statistical analysis

The experimental results were expressed as mean ± standard error of triplicate measurements. The statistical analysis was performed by one-way ANOVA, followed by Ducan's multiple range test using the IBM SPSS statistical package **[30]** (version 22, SPSS Inc., Chicago, IL, USA) to determine the effect of the treatments. The differences were considered statistically significant at $p < 0.05$, using IBM SPSS statistical package.

Results

In this study, we observed the effect of using probiotics such as *Bacillus subtilis* and *Saccharomyces cerevisiae* on growth performance, biochemical parameters, and some digestive enzymes activities.

As we observed, there was a significant increase (P<0.05) in the body weight of the *B. subtilis* (238±2.64 g) and *S. cerevisiae* (236.66±1.45 g) groups when compared with the control group (208.66±4.66 g)*;* however, there was no significance between two supplemented groups. Also, the addition of *B. subtilis* and *S. cerevisiae* to Japanese quails' diet had a positive effect on the body weight gain, especially in *Bacillus subtilis* supplemented group. Our results showed that there was a significant difference (P<0.05) in feed conversion ratio and specific growth rate in the group supplemented with *B. subtilis* when compared with other groups, as shown in **Table 2.**

Carcass characteristics

As it was shown in **Table 3**. there was a significant increase (P<0.05) in relative weights percent of carcass, intestine, and liver in the second group (70.96±1.9, 4.37±0.3 and 2.59±0.22 respectively) which supplemented with *B. subtilis*. However, it was (65.11±1.06, 3.7±0.05 and 2.1±0.01 in carcass, intestine, and liver weight percent) in the control group, while there was a significant decrease in carcass and organs weight in the *Saccharomyces cerevisiae* supplemented group where it was 65.48±0.96, 3.25±0.06 and 1.7±0.09 in carcass, intestine, and liver weight percent respectively.

Biochemical parameters

Table 4. shows the effect of *B. subtilis* and *S. cerevisiae* on lipid profile as there was a significant increase (P<0.05) in triglyceride level in the group supplemented with *B. subtilis* by 271.85±8.26 mg/dl. Serum cholesterol (194.21±11.25 mg/dl) and LDL (112.62±9.7 mg/dl) levels were significantly decreased in *B. subtilis* supplemented group when compared with other groups.

Body performance

The protein profile as presented in **Table 5.** showed that there was no difference in total protein, albumin, and globulin among all groups. Serum total protein was (6.84±0.91, 5.3±0.64 and 6.8±0.68 g/dl) and albumin was (4.78±1, 3.88±0.48 and 5±0.2 g/dl) in the control group, *B. subtilis,* and groups, respectively. The kidney function was improved when the diet was supplemented with probiotics, as explained in **Table 6.** Where urea values were 21.39±1.32 and 25.86±1.72 mg/dl in *B. subtilis* and *S. cerevisiae* groups, respectively during the experiment when compared to control group (36.16±2.04 mg/dl), however creatinine level were 0.73±0 and 0.68±0.02 mg/dl in *B. subtilis* and *S. cerevisiae* groups, respectively, while it was 0.9±0.03 mg/dl in control group. *Bacillus subtilis* significantly reduced AST (18.94±1.62 U/L) at day 36 compared to the control (25.44±1.47 U/L) and *S. cerevisiae* (27.39±1.80 U/L) groups. However, ALT (34.95±1.39 U/L) was not significantly decreased compared to the control (36.99±2.90 U/L) and S. cerevisiae (46.48±3.73 U/L) groups **Table 7.**

Digestive enzymes activities

There was a significant improvement (P<0.05) in trypsin enzyme activity in *B. subtilis* and *S. cerevisiae* supplemented groups which were 20.08±0.48 and 22.14±1.77, respectively and chymotrypsin activities by 20.27±0.41 and 20.75±0.82 in *B. subtilis* and *S. cerevisiae*, respectively. However, α amylase enzymes activity was slightly increased in *B. subtilis* group (1116.27±32.98) compared to *S. cerevisiae* group (1038.33±36.71) **Table 8.**

Table (2): Effect of *Bacillus subtilis* and *Saccharomyces cerevisiae* supplemented to Japanese quails' diet on the growth performan

Criteria	Control	*Bacillus subtilis*	*Saccharomyces cerevisiae*	p value
Initial body weight, (g/bird) 14d	71.80±2.36			
Body weight, (g/b/period)				
At 24 days	144.86±6.07b	164.66±2.34a	163.33±2.6a	0.023
At 36 days	208.66±4.66b	238±2.64a	236.66±1.45a	0.001
Body weight gain, (g/b/period)				
from 14 to 24 days	76.8±6.08b	96.6±1.47a	95.27±3.4a	0.025
from 25 to 36 days	63.78±5.68a	73.33±4.74a	73.33±2a	0.560
Feed conversion ratio				
from 14 to 24 days	3.05±0.277b	4.40±0.15a	4.12±0.063a	0.370
from 25 to 36 days	2.25±0.377a	2.64±0.072a	2.53±0.16a	0.520
Specific growth rate				
SGR	3.45±0.056b	4.41±0.12a	4.29±0.02a	0.080
Feed consumption				
from 14 to 24 days	21.90±0.08b	23.41±0.11a	21.61±0.12b	0.001
from 25 to 36 days	28.30±0.11b	28.90±0.11a	27.70±0.11c	0.010

Values are expressed as mean ± standard errors. a, b means in the same row within each item bearing different superscripts are significantly different (P <0.05).

Table (3): Effect of *Bacillus subtilis* and *Saccharomyces cerevisiae* supplemented to Japanese quails' diet on the carcass traits:

Criteria	Control	*Bacillus subtilis*	*Saccharomyces cerevisiae*	p value
Carcass, (g/100g LBW)				
At 24 days	66.88±4.42b	76.30±0.5a	67.04±2.49b	0.01
At 36 days	65.11±1.06b	70.96±1.9a	65.48±0.96b	0.034
Intestine, (g/100g LBW)				
At 24 days	5.61±0.28b	6.38±0.02a	5.97±0.41b	0.015
At 36 days	3.70±0.05b	4.37±0.30a	3.25±0.06c	0.002
Liver, (g/100g LBW)				
At 24 days	2.48±0.27b	3.18±0.32a	2.59±0.10b	0.001
At 36 days	2.10±0.01b	2.59±0.22a	1.70±0.09c	0.008

Values are expressed as mean ± standard errors. a, b means in the same row within each item bearing different superscripts are significantly different (P <0.05).

Table (4): Effect of *Bacillus subtilis* and *Saccharomyces cerevisiae* supplemented to Japanese quails' diet on lipid profile:

Criteria	Control	*Bacillus subtilis*	*Saccharomyces cerevisiae*	p value
		Triglyceride (mg/dl)		
At 14 days	132.00±3			
At 24 days	118.67±4.05b	143±1.52a	114.67±1.45b	0.001
At 36 days	245.75±16.44c	271.85±8.26a	257.89±13.69b	<0.01
		Cholesterol, (mg/dl)		
At 14 days	175.00±7.50			
At 24 days	150.00±3.38a	146.47±3.52a	155.33±3.48a	0.280
At 36 days	307.00±5.82a	194.216±11.25c	236.82±7.52b	<0.01
		HDL, (mg/dl)		
At 14 days	51.60±0.6			
At 24 days	37.01±2.08b	37.06±3.27b	41.33±4.96a	0.001
At 36 days	39.20±2.41a	40.57±2.18a	31.50±1.15b	0. 036
		LDL, (mg/dl)		
At 14 days	97±7.5			
At 24 days	98.3±1.45ab	105±7.23a	80.33±7.53b	0.067
At 36 days	205.85±5.51a	112.62±9.70c	137.07±6.66b	<0.01

Values are expressed as mean ± standard errors. a, b means in the same row within each item bearing different superscripts are significantly different (P <0.05).

Table (5): Effect of *Bacillus subtilis* and *Saccharomyces cerevisiae* supplemented to Japanese quails' diet on protein profile:

Criteria	Control	*Bacillus subtilis*	*Saccharomyces cerevisiae*	p value
		Total protein, (g/dl)		
At 14 days	4.30±0.1			
At 24 days	2.76±0.27ab	3.23±0.06a	2.23±0.033b	0.014
At 36 days	6.84±0.91a	5.30±0.64a	6.80±0.68a	0.328
		Albumin, (g/dl)		
At 14 days		1.60±0.1		
At 24 days	1.30±0a	1.10±0.25a	1.13±0.06a	0.630
At 36 days	4.78±1a	3.88±0.48a	5.00±0.2a	0.480
		Globulin, (g/dl)		
At 14 days	2.8±0.1			
At 24 days	1.46±0.27b	2.13±0.28a	1.1±0.1b	0.053
At 36 days	2.06±0.79a	1.42±0.61a	1.8±0.66a	0.812

Values are expressed as mean ± standard errors. a, b means in the same row within each item bearing different superscripts are significantly different (P <0.05).

Table (6): Effect of *Bacillus subtilis* and *Saccharomyces cerevisiae* supplemented to Japanese quails' diet on kidney function:

Criteria	Control	*Bacillus subtilis*	*Saccharomyces cerevisiae*	p value
		Urea, (mg/dl)		
At 14 days	12.70±0.5			
At 24 days	19.60±0.3a	9.90±0.057b	7.85±0.7c	<0.01
At 36 days	36.16±2.04a	21.39±1.32b	25.86±1.72b	0.002
		Creatinine, (mg/dl)		
At 14 days	0.4±0			
At 24 days	0.50±0a	0.40±0ab	0.30±0b	0.079
At 36 days	0.90±0.033a	0.73±0ab	0.68±0.029b	0.062

Values are expressed as mean ± standard errors. a, b means in the same row within each item bearing different superscripts are significantly different (P <0.05).

Table (7): Effect of *Bacillus subtilis* and *Saccharomyces cerevisiae* supplemented to Japanese quails' diet on Liver enzymes:

Criteria	Control	*Bacillus subtilis*	*Saccharomyces cerevisiae*	p value
		ALT, (U/L)		
At 14 days	21.60±5.2			
At 24 days	76.00±1.52a	26.00±1b	28.33±1.45b	0.001
At 36 days	36.99±2.9ab	34.95±1.39b	46.48±3.73a	0.060
		AST, (U/L)		
At 14 days	36.96±2.15			
At 24 days	28.66±0.57a	26.90±1.32a	21.80±0.95b	0.070
At 36 days	25.44±1.47a	18.94±1.62b	27.39±1.8a	0.025

Values are expressed as mean ± standard errors. a, b means in the same row within each item bearing different superscripts are significantly different (P <0.05).

Table (8): Effect of *Bacillus subtilis* and *Saccharomyces cerevisiae* supplemented to Japanese quails' diet on digestive enzymes specific activity:

Criteria	Control	Bacillus subtilis	Saccharomyces cerevisiae	p value
		Trypsin		
At 14 days	13.09±0.024			
At 24 days	17.63±0.165b	20.08±0.489ab	22.14±1.77a	0.064
At 36 days	15.43±0.36a	17.04±0.90a	17.44±0.658a	0. 167
		Chymotrypsin		
At 14 days	11.97±0.7			
At 24 days	16.24±0.92b	20.27±0.41a	20.75±0.82a	0.010
At 36 days	16.11±1.04a	17.21±0.25a	17.58±0.19a	0.297
		α-amylase		
At 14 days	624.79±28.07			
At 24 days	667.63±14.89b	964.39±14.68a	933.91±11.54a	<0.01
At 36 days	763.72±24.66b	1116.27±32.98a	1038.33±36.71a	0.001

Values are expressed as mean ± standard errors. a, b means in the same row within each item bearing different superscripts are significantly different (P <0.05).

Discussion:

We observed that adding *B. subtilis* and *S. cerevisiae* to Japanese quails' diet could increase body weight. As we found, probiotic dietary supplementation in broilers has a positive effect on growth performance **[31]**. Also, we noticed that diet supplemented with *Bacillus subtilis* has a positive effect on FCR when compared with control group. *Bacillus subtilis* spores are effective and economical exclusion agents, it enhances the digestion and absorption of consumed feed, and consequently, improving body weight and feed conversion ratio. The improvement in growth performance of birds fed diets supplemented with *B. subtilis* may be associated with their ability in fine-tuning gut ecosystem **[32]**. This may happen through competitive adhesion and synthesis of the antimicrobial compound, immunomodulation, improving intestinal integrity and function, and secretion of digestive enzymes **[33].** *Saccharomyces cerevisiae* had increased body weight and body weight gain and improved feed conversion rate and economic efficiency in quails fed diets supplemented with different levels of *Saccharomyces cerevisiae* compared to the control group **[34]** which consistent with our results. The better growth performance of broilers due to *Saccharomyces cerevisiae* supplementation could be attributed to numerous useful impacts of yeast such as its rich content of protein, vitamin B-complex, trace minerals and numerous other useful impacts **[35]**. Quail chicks fed basal diet supplemented with *Bacillus subtilis* and *Saccharomyces cerevisiae* had no effect on feed intake. Other study agreed with our results that the dietary supplementation with *Bacillus subtilis* had no effect on daily feed intake **[36].** Contrary, **Ahmed *et al.* [37]** observed that 3% dietary *Saccharomyces cerevisiae* significantly ($P<0.05$) increased feed intake when compared to the control, but this might be due to different CFU/g. In this study we observed that there was a significant increase in the relative weights of carcass, intestine, and liver in the second group which supplemented with *Bacillus subtilis* compared to the control group while there was a significant decrease in carcass and organs weight in *Saccharomyces cerevisiae* supplemented group. In agreement with our result, probiotic as dietary supplementation increased the relative weight of organs. Also, there were no significant differences in the relative weights of carcass and liver *in Saccharomyces cerevisiae* supplemented group **[38,39].** Addition of probiotics especially *B. subtilis* and *S. cerevisiae* have many beneficial effects on growth that could be contributed to enzyme production, competitive action with pathological bacteria, nutrient sources, fine-tunning gut ecosystem **[33]** but, it depends mainly on (concentration/g).

The inclusion of *Bacillus subtilis* and *Saccharomyces cerevisiae* in quails' diets significantly increased triglyceride ($P<0.05$) during all periods of the experiment compared with control group and a significant decrease in cholesterol in *B. subtilis* group when compared with control and *S. cerevisiae* group. Also, we noticed that there was a significant increase ($P<0.05$) in HDL values in the second group which supplemented with *B. subtilis* compared with other groups and there was a significant decrease in LDL at day 36 specially in *B. subtilis* group. These findings detect the mechanism of the cholesterol-lowering effects of probiotics is the enzymatic deconjugation of bile acids by the hydrolysis of bile salts which allows them to bind to cholesterol in the small intestine **[40].** On the other hand, the serum analysis indicated that *B. subtilis* consumption could significantly decrease the triglycerides level in *B. subtilis* group compared to the control group **[41].** Also, yeast supplementation to chickens' diets caused decreased plasma cholesterol, this reduction may be due to the ability of bacteria to assimilate or degrade the cholesterol to bile acids followed by deconjugation to prevent re-synthesis **[42].** Additionally, in consistent with our results, dietary probiotic supplementation did not influence the concentration of total protein and albumin when compared with the control **[43,44].** This result may clarify that dietary yeast supplementation had no adverse effect on the immune system or the osmoregulatory system of animal body as the most important role of albumin is control osmotic pressures in the blood. In contrast to our results, a significant increase ($P<0.05$) in serum proteins was detected due to probiotic treatments **[45].** The increase in serum TP and ALB could be explained by the inhibition exclusion mechanism, where *B. subtilis* improves dietary protein utilization through its ability to inhibit pathogens growth, which reduces protein breakdown into nitrogen and diminishes dietary protein efficiency and increases the surface area for nutrient absorption **[36]** The kidney function and its health state were evaluated through measuring serum urea and creatinine levels. From our finding, the result shown that serum urea and creatinine level were significantly decreased in the *B. subtilis* and *S. cerevisiae* groups. *Our results proved that Saccharomyces cerevisiae* enhanced the kidney function in the supplemented group. Consistent with the current study, uric acid was decreased significantly by feeding probiotics **[46].** In contrast, there was no effect on serum uric acid levels with the addition of probiotics **[47].** However, in another study, the dietary probiotic supplementation had higher serum uric acid than the control **[48].** From our results and the previously explanation of addition of *B. subtilis* and *S. cerevisiae* , we could contribute the increased of creatinine and uric acid in blood with *S. cerevisiae* to the action of digestion of nutrients and

increase amino acids pass to the liver which in return increase deamination, this action is an energy consuming mechanism, so our results of decreased with of such group is due to consuming energy to deaminate protein in liver. While *B. subtilis* mainly enhances immunity and fine-tunning of gut ecosystem and improve both energy and protein, so its result was better than control and *S. cerevisiae* groups. The measurement of serum ALT and AST levels revealed that the administration of probiotics especially *Bacillus subtilis* could reduce AST significantly ($P<0.05$) specially at day 36 when compared to control and *S. cerevisiae* group but ALT values was insignificantly decreased compared to control group. Also, we found that ALT and AST enzymes showed an insignificant increase of activity in *S. cerevisiae* fed quails at day 36. The administration of probiotics could reduce these hepatic enzymes significantly in Ross broiler chickens fed with basal diet with (*Bacillus toyonensis*) **[44],** also the same result when fed male Wistar rats with *B. subtilis* **[41].** Also, we noticed that ALT, and AST were significantly decreased with dietary inclusion of probiotic. Contrary, ALT and AST were not affected by the administration of *Bacillus subtilis* **[46].** In agree with our results the concentrations of AST and ALT were increased ($P<0.05$) when yeast culture was added to the diet of Damascus goats at rates of 2.5 and 5 g/h/d **[49].**

Gastrointestinal enzyme activities such as trypsin, chymotrypsin and α amylase have an important role in nutritional digestion, which improves growth performance. Probiotics could participate in digestive processes by producing enzymes, such as amylases, lipases, and proteases **[50].** Presently, *Bacillus subtilis* and *Saccharomyces cerevisiae* supplemented groups experienced improved trypsin, and chymotrypsin activities especially at day 24 however α amylase was improved during the experiment especially at *B. subtilis* group. *Bacillus* spp. contributes to the excretion of exogenous enzymes together with producing the host from the endogenous enzymes **[51].** Consistent with our results, dietary supplementation of *B. subtilis* spores led to increased protease, lipase, and amylase activities when compared with the control **[52].** The probiotic *Bacillus* strains increased digestive enzymes in Indian shrimp **[53]** , also, there was a significant increase in some digestive enzymes in white leg shrimp when fed on *Bacillus subtilis* and *B. licheniformis* **[54]**.The supplemented *B. subtilis* group improved α-amylase activity significantly **[46].** Higher activity of α-amylase enhanced the digestion of starch, and this might be a possible cause for growth improvement *S. cerevisiae* might have stimulated pancreatic α-amylase secretion compared with control group. In fact, it has been reported that a component of Cerevisiae, β-glucan, stimulates cholecystokinin from enteroendocrine cells and cholecystokinin's effective on the stimulation of pancreatic secretion **[55].** On the contrary, there are non-significant alternations in amylolytic, or proteolytic activities as influenced by probiotic administration **[56].**

Conclusion

We could conclude that, addition of such probiotics has beneficial effects on growing quail chicks, but we could recommend the usage of *Bacillus subtilis* when we are looking for growth and general health, and *Saccharomyces cerevisiae* when we have high energy and less protein diets. Finally, both have better growth performance when added to quail chicks' diet and positive effects on digestion and metabolism.

Authors' contribution

The work was equally distributed between authors. All authors have read and approved the final version of the manuscript.

Conflict of interest

There is no conflict of interest.

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