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Effects of encapsulated *Lactobacillus acidophilus* and *Spirulina platensis* on growth performance, blood metabolites, jejunal morphology, and ileal microbial populations of heat-stressed broilers

Zahra Ranjbarinasab[1] Omidali Esmaeilipour[1]* Mozhgan Mazhari[1] Fatemeh Shahdadi[2] and Arsalan Barazandeh[1]

[1]*Department of Animal Sciences, Faculty of Agriculture, University of Jiroft, 7867155311 Kerman, Iran.*
[2]*Department of Food Science and Technology, Faculty of Agriculture, University of Jiroft, 7867155311 Kerman, Iran.*

***Correspondence** should be addressed to Omidali Esmaeilipour: Omid.esmaeili1@gmail.com, omid.esmaeilipour@ujiroft.ac.ir

Abstract

Aim of study: The goal of this study was to investigate the effects of encapsulation of *Lactobacillus acidophilus* (LA) *and Spirulina platensis* (SP) on the growth performance, blood metabolites, intestinal morphology, and gut microflora of broilers under heat stress.

Area of study: The work was performed at the University of Jiroft, Iran.

Material and methods: A total of 240, one-day-old Ross 308 male broilers were allocated to six treatments, with four replicates (10 birds each) per treatment. The six dietary groups were as follows: (1) corn-soybean as control diet (Control), (2) control diet including 0.02% LA (LA), (3) control diet including 1% SP (SP), (4) control diet including 0.02% LA + 1% SP (LA+SP), (5) control diet including 0.02% LA encapsulated (encapsulated LA), and (6) control diet including 0.02% encapsulated LA and 1% encapsulated SP (encapsulated LA+SP). To induce chronic heat stress, birds were exposed to an ambient temperature of 34 ± 2 °C for 8 h daily (from 10:00 am until 18:00 pm) from 25 to 42 d of age. At the end of the trial, two chicks/pen were randomly selected, and the performance, blood metabolites, jejunal morphology, and ileal microbial populations were evaluated.

Main results: All experimental groups had higher feed intake (FI), body weight gain (BWG) and lower feed conversion ratio (FCR), compared to the control. The highest FI and BWG were seen in birds fed by LA+SP and encapsulated LA+SP ($P<0.05$). The lowest cholesterol and the highest activity of glutathione peroxidase enzyme in blood serum were seen in the LA+SP treatment ($P<0.05$). Dietary treatments with LA, SP or both significantly increased villus height (VH), crypt depth (CD) and villus height/crypt depth ratio and decreased villus width compared to the control ($P<0.05$). The effect of dietary treatments on ileal microbial community was significant since *Lactobacillus* spp. counts increased and *Escherichia coli* counts decreased in all dietary treatments compared to the control group ($P<0.05$).

Research highlights: In general, adding LA, SP, and a combination of both, encapsulated or not in broiler's feeds alleviated the negative effects of heat stress on growth performance, and ameliorate blood metabolites, jejunal morphology and ileal microbial populations of broilers.

Keywords: body weight gain; broilers; glutathione peroxidase; gut microbiota; heat stress; microalgae; villus height.

Efectos de *Lactobacillus acidophilus* y *Spirulina platensis* encapsulados en el rendimiento de crecimiento, metabolitos sanguíneos, morfología yeyunal y poblaciones microbianas ileales de los pollos de engorde sometidos a estrés por calor

Resumen

Objetivo del estudio: El objetivo de este estudio fue investigar los efectos de la encapsulación de *Lactobacillus acidophilus* (LA) y *Spirulina platensis* (SP) sobre el rendimiento del crecimiento, los metabolitos sanguíneos, la morfología yeyunal y la microflora intestinal de los pollos de engorde bajo estrés por calor.

Área de estudio: El trabajo se realizó en la Universidad de Jiroft, Irán.

Material y métodos: Un total de 240 pollos de engorde machos Ross 308 de un día de edad fueron asignados a seis tratamientos, con cuatro réplicas (10 aves cada una) por tratamiento. Los seis grupos dietéticos fueron los siguientes: (1) maíz-soja como dieta de control (Control), (2) dieta de control que incluye 0.02% de LA (LA), (3) dieta de control que incluye 1% de SP (SP), (4) dieta de control que incluye 0.02% de LA + 1% de SP (LA+SP), (5) dieta de control que incluye 0.02% de LA encapsulado (LA encapsulado), y (6) dieta de control que incluye 0.02% de LA encapsulado y 1% de SP encapsulado (LA+SP encapsulado). Para inducir estrés térmico crónico, las aves fueron expuestas a una temperatura ambiente de 34 ± 2 °C durante 8 horas diarias (desde las 10:00 a.m. hasta las 6:00 p.m.) desde los 25 hasta los 42 días de edad. Al final del experimento, se seleccionaron aleatoriamente dos pollitos por jaula, y se evaluaron el rendimiento, los metabolitos en sangre, la morfología yeyunal y las poblaciones microbianas ileales.

Resultados principales: Todos los grupos experimentales tuvieron una mayor ingesta de alimento (IA), aumento de peso corporal (APC) y una menor relación de conversión alimenticia (RCA), en comparación con el control. La mayor IA y APC se observaron en aves alimentadas con LA+SP y LA+SP encapsulado (P<0.05). El colesterol más bajo y la mayor actividad de la enzima glutatión peroxidasa en suero sanguíneo se observaron en el tratamiento LA+SP (P<0.05). Los tratamientos dietéticos con LA, SP o ambos incrementaron significativamente la altura de las vellosidades, la profundidad de las criptas y la relación altura de vellosidad/profundidad de cripta, y disminuyeron el ancho de las vellosidades en comparación con el control (P<0.05). El efecto de los tratamientos dietéticos sobre la comunidad microbiana ileal fue significativo, ya que los recuentos de *Lactobacillus* spp. aumentaron y los recuentos de *Escherichia coli* disminuyeron en todos los tratamientos dietéticos en comparación con el grupo control (P<0.05).

Aspectos destacados de la investigación: En general, añadir LA, SP, y una combinación de ambos, encapsulados o no en los alimentos para pollos de engorde, mitigó los efectos negativos del estrés térmico en el rendimiento del crecimiento, y mejoró los metabolitos sanguíneos, la morfología yeyunal y las poblaciones microbianas ileales de los pollos de engorde.

Palabras clave: altura de vellosidades; aumento de peso corporal; estrés térmico; glutatión peroxidasa; microalgas; microbiota intestinal; pollos de engorde.

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Introduction

Rising temperatures worldwide has made the management of heat stress a major concern for the modern poultry industry (Shakeri et al., 2019). Broilers are susceptible to high environmental temperatures because of their fast growth rate, rapid metabolism, and absence of sweat glands, which results in a significant decline in growth performance and higher mortality in heat stress conditions (Shakeri et al., 2019). The deleterious effects of heat stress on growth performance of broilers include reduced feed intake, decreased nutrient digestion, increased maintenance needs, changes in hormonal systems, and decreased immunity (Cheng et al., 2018; Bai et al., 2019). Heat stress has also a deleterious effect on intestinal morphology, resulting in reduced nutrient utilization (Vimon et al., 2023). The researchers stated that the poultry digestive system is sensitive to stressors, which can lead to a wide range of changes, including changes in the intestinal microbial ecology, and damage to intestinal function (Attia et al., 2023). Also, it has been reported that heat stress decreased the serum antioxidant activity (Bai et al., 2019; Elbaz et al., 2022) and increased serum lipids (Elbaz et al., 2022) in broiler chickens. Many feed additives have been proposed to improve the performance of birds suffering from heat stress (Mirzaie et al., 2018; Li et al., 2020).

Probiotics are live bacteria that have positive effects on the host (Wang et al., 2020). *Lactobacillus acidophilus* (LA) is a Gram-positive, rod-shaped bacterium that is naturally present in the gastrointestinal tract of humans and animals. *Lactobacillus acidophilus* can ferment sugars to produce lactic acid, which helps create an acidic environment that inhibits harmful bacteria's growth and promotes beneficial bacteria's growth (Molina et al., 2024). Probiotics have been shown to optimize gut morphology, microflora population, nutrient absorption, body antioxidative capacity, and immune response, which ultimately improves the gut health and growth performance of broiler chickens (Bai et al., 2017; Wang et al., 2020; Bilal et al., 2021). In addition, it has been shown that the mixture of two strains of LA and *Lactobacillus pentosus* improved the growth performance and bacterial population of the cecal contents of broilers under heat stress (Faseleh Jahromi et al., 2016). One of the main points for selecting a preferable probiotic, is the ability to pass through the initial parts of the digestive system and reach the intestine in a healthy and sufficient amount, where they exert beneficial effects on the host (Rodklongtan et al., 2014). Several factors can reduce the viability of probiotics, such as exposure to oxygen, low pH, digestive enzymes, heat treatment, and water activity (Martín et al., 2015). It has been stated that the encapsulation of probiotics into polymeric microcapsules effectively preserved them from gastric juices (Gyawali et al., 2022; Vimon et al., 2023). Among the most commonly used polymers as encapsulating materials, sodium alginate is capable of making a highly versatile matrix that is biocompatible and non-toxic for the preservation of active components, cells, and probiotic microorganisms that are susceptible to pH, oxygen, high temperature, and other instances (Kumar et al., 2023).

On the other hand, *Spirulina platensis* (SP) is a blue-green microalga from the Cyanophyceae family with a quantity of minerals, protein, and vitamins superior to many feed ingredients such as soybeans (Jung et al., 2019). In addition, SP contains numerous bioactive components in high amounts, such as pigments (phycocyanins, chlorophyll, β-carotene) steroid, saponin, favonoid, triterpenoid, and phenolic acids (Mariey et al., 2012; Attia et al., 2023). It has been reported that dietary inclusion of SP to birds reared under heat stress conditions may reduce the adverse effects of stress on growth performance, nutrients digestibility, immunity, biochemical parameters and the antioxidant capacity of blood (Mirzaie et al., 2018; Abdel-Moneim et al., 2021; Attia et al., 2023). Furthermore, studies showed that the supplementation of SP and *Bacillus subtilis* PB6 resulted in improved growth performance, antioxidant capacity, and intestinal health in broiler chickens (Joya et al., 2020).

The present study aimed to determine the effects of including LA, encapsulated or not, or/and SP in the diet of heat-stressed broilers on growth performance, blood metabolites, jejunal morphology, and ileal microbiota.

Material and Methods

All experimental procedures used in this experiment were reviewed and approved by the Animal Care and Use Committee of the University of Jiroft, Iran (Ref. 2812-30009).

Birds, diets, and experimental design

A total of 240 one-day-old Ross 308 male broilers with an average initial body weight of 49.25 ± 1.13 g were obtained from a commercial hatchery and randomly distributed into 24-floor pens (1 m×1.5 m) in an environmentally controlled house with free access to feed and water until 42 days of age. The experiment was conducted as a completely randomized design with six treatments and four replicates of 10 birds each. Birds were raised commercially for the first 10 days before the beginning of the experiment.

From the age of 11 to 42 d, the birds were fed with the following six dietary treatments: (1) corn-soybean as control diet (Control), (2) control diet including 0.02% *Lactobacillus acidophilus* (LA), (3) control diet including 1% *Spirulina platensis* (SP), (4) control diet including 0.02% *Lactobacillus acidophilus* + 1% *Spirulina platensis* (LA+SP), (5) control diet including 0.02% encapsulated *Lactobacillus acidophilus* (encapsulated LA), and (6) control diet including 0.02% encapsulated *Lactobacillus acidophilus* and 1% encapsulated *Spirulina platensis* (encapsulated LA+ SP). The probiotic used in this experiment was the *Lactobacillus acidophilus*® (La-5) strain (3×10^9 CFU of bacteria), which was added to the experimental diet at a rate of 0.02% (Parsilact Company, Shiraz, Iran). Each gram of probiotic contained 3×10^9 CFU of bacteria. *Spirulina platensis*® microalgae were prepared in powder from Qeshm Sina Micro Algae Company (Qeshm, Iran).

The control diets were formulated to meet Ross 308 nutrition requirements during the starter (1-10 d), grower (11-25 d), and finisher (26-42 d) phases (Table 1) (Aviagen, 2014). From 1 to 7 d of age, birds were subjected to a 24-hour lighting period, followed by a 23-hour light (1-hour darkness cycle) until the end of the experiment. During the first 3 d, the temperature was initially set to 32°C and lowered by 2.5 °C per week till 25 d. Then in order to induce heat stress condition, from 26 d of age, the barn temperature was set at 34±2°C (with 50-60% relative humidity) for 8 h daily (from 10:00 am until 18:00 pm, day) and reduced to 22 ±2°C during the rest of the day (night), according to Chang et al. (2020).

Table 1. Ingredients and compositions of the control diet of heat stressed broilers.

	Starter (1-10 d)	Grower (11-25 d)	Finisher (26-42 d)
Ingredients (%)			
Corn	50.92	54.26	58.87
Soybean meal	41.71	37.84	33.44
Soybean oil	3.42	4.33	4.46
Limestone	1.30	1.21	1.18
Dicalcium phosphate	1.45	1.25	1.23
Sodium chloride	0.31	0.31	0.29
DL-Methionine	0.16	0.13	0.12
L-Lysine	0.24	0.17	0.22
Vitamin premix [1]	0.25	0.25	0.25
Mineral premix [2]	0.25	025	025
Calculated analysis (DM basis)			
Metabolizable energy (kcal/kg)	3000	3100	3200
Crude protein (%)	23.00	21.50	20.00
Calcium (%)	0.96	0.87	0.81
Available phosphorus (%)	0.48	0.43	0.41
Lysine (%)	1.44	1.29	1.19
Methionine (%)	0.56	0.51	0.48

[1] Vitamin premix supplied the following per kilogram of diet: retinol, 12,000 IU; cholecalciferol, 1500 IU; tocoferol, 60 IU; filokinon, 2 mg; thiamine, 2.4 mg; riboflavin, 4.8 mg; niacin, 30 mg; pantothenic acid, 16 mg; pyridoxine, 3 mg; folic acid, 1 mg; vitamin B12, 0.03 mg; biotin, 0.15 mg; and cholin chloride, 50 mg. [2] Mineral premix supplied the following per kilogram of diet: Mn, 80 mg; Fe, 120 mg; Zn, 60 mg; Cu, 100 mg; I, 0.95 mg; and Se, 0.25 mg.

Chemical analyses of *Spirulina platensis* microalgae

Spirulina platensis powder was analyzed to determine chemical compounds which are presented in Table 2. Total phenolic compounds were determined according to the Folin–Ciocalteu method using gallic acid as standard. Briefly, 20 µl of extract solution were mixed with 300 µL of Na2CO3 solution (20%), then 1.16 mL of distilled water and 100 µL of Folin–Ciocalteu reagent added to mixture after 1 min and 8 min, respectively. Subsequently, the mixture was incubated in a shaking incubator at 40 °C for 30 min and its absorbance was measured at 760 nm. Gallic acid was used as a standard for calibration curve (Arabshahi-Delouee & Urooj, 2007). Antioxidant activity was determined by DPPH free radical scavenging method according to Anandjiwala et al. (2008). Briefly, 1 mL of a 1 mM methanolic solution of DPPH (2, 2-Diphenyl-1-picrylhydrazyl) was mixed with 3 mL of extract solution (500 ppm concentration). The mixture was then homogenized vigorously and left for 30 min in the dark place (at room temperature). Its absorbance was measured at 517 nm and activity was expressed as percentage of DPPH scavenging relative to control. The DPPH-scavenging activity of SP extract was 69.4 % at a concentration of 500 ppm. Moisture, crude protein, lipid, and total ash contents in SP were analyzed according to the AOAC International procedures (AOAC, 1995).

Table 2. Chemical analysis of the *Spirulina platensis* used in feeds of heat stressed broilers

Parameters	Total phenolic contents[1]	Antioxidant activity	Protein	Moisture	Lipid	Ash
Spirulina platensis	75. 2 mg gallic acid/g dry weight	69.4%	59%	2.5%	3.33%	4.5%

[1] Total phenolic compounds were determined according to the Folin–Ciocalteu method using gallic acid as standard (Arabshahi-Delouee & Urooj, 2007). Antioxidant activity was determined by DPPH free radical scavenging method according to Anandjiwala et al. (2008). Moisture, crude protein, lipid, and total ash contents in SP were analyzed according to the AOAC International procedures (AOAC, 1995).

Microencapsulation of *Lactobacillus acidophilus* and *Lactobacillus acidophilus+Spirulina platensis*

Pure probiotic cultures of LA (3×10^9 CFU of bacteria) and a combination of LA (3×10^9 CFU of bacteria) and SP microalgae powder were added to Na–alginate solution (2% of Na–alginate in distilled water). Then, these mixtures were injected into a CaCl2 solution (0.1 M). The distance between the syringe and CaCl2 solution was 25 cm. Diameter of the resultant beads was 500–1000 µm (Pan-Utai & Iamtham, 2018). Then the calcium chloride solution was drained and isolated and the resulting beads were collected.

Sample collection and measurements

Growth performance

Feed intake and body weight gain of each replicate pen were recorded at 26 and 42 d of age. Mortality was recorded throughout the trial, and the feed conversion ratio for each pen was calculated by dividing feed intake by body weight gain from 26 to 42 d.

Blood metabolites

At the end of the experiment, two birds per replicate were randomly chosen, weighed, and slaughtered by cutting the jugular vein. Subsequently, blood samples were collected from the brachial vein. Serum biochemical parameters were measured using an auto-analyzer (Cobas Mira Plus, Roche Diagnostics Systems, Inc., Montclair, NJ, USA). Also, glutathione peroxidase enzyme (GSH-P_X) was measured using the protocol provided by the kit manufacturer (Zellbio, Germany) and was determined by ELISA reader (Biotek, ELx800, USA).

Jejunal morphology

The same two birds per replicate slaughtered for blood sampling were dissected to obtain jejunum. A three cm segment of tissue, from the midpoint of the jejunum of each bird, was removed and placed in a 10% formalin solution. Three stages of dewatering, clarification, and paraffinization were performed to prepare tissue samples. Incisions 5-6 µm thick were made with a rotary microtome (SLEE, CUT 5062, Germany) and were floated in 40 °C water so that they could be easily placed on the slide after the possible wrinkles had been smoothed out. After paraffinization with xylan and irrigation with decreasing degrees of ethanol, staining of the stabilized tissues on the slide was performed with hematoxylin (Merck®, Germany) and eosin (Merck®, Germany), and the desired parameters were measured using the relevant device (Nikon microscope, Eclipse 50i, Japan). Thus, the villus height (from the tip of the villus to the crypt junction), the villus width, and the crypt depth were measured according to the method of Iji et al. (2001).

Ileal microbial populations

After slaughtering, the small intestine was instantly removed, and fresh digesta from the ileum was aseptically collected, put on ice, and immediately transported to the laboratory for enumeration of the microbial population. One gram of ileal contents was homogenized in 9 mL of sterile water. The *Lactobacillus* spp. and *Escherichia coli* were determined by serial dilution (10-3 to 10-4) of ileal samples before inoculation onto Petri dishes. Using these diluted subsamples, the *Lactobacillus* bacteria population was enumerated on MRS agar (Merck®, Germany), and *E. coli* were counted on MacConkey agar (Merck®, Germany) after incubation at 37 °C in an anaerobic chamber for 48 h and in an aerobic chamber for 24 h, respectively. The number of the bacterial groups was transferred to the Log number before statistical analysis, according to Salim et al. (2013).

Statistical analysis

Data were analyzed by ANOVA using the GLM procedure of SAS (SAS, 2005). Analysis of variance was performed using a completely randomized design of treatments. The Tukey multiple range test was used to determine differences among the treatment means. Statistical significance was considered at $P<0.05$.

Results

Effects of dietary treatments on growth performance (FI, BWG, and FCR) of broilers exposed to heat stress from 26 to 42 d of age are presented in Table 3. The results showed that all the experimental groups significantly increased FI and BWG and decreased FCR, compared to the Control group, so that the highest FI was observed in the birds fed with LA+SP and encapsulated LA+SP, and the highest BWG and the lowest FCR were seen in the birds fed with LA+SP, encapsulated LA+SP and SP (P<0.05) compared with the rest of the groups.

Table 3. The effects of *Lactobacillus acidophilus* (LA) and *Spirulina platensis* (SP) addition with or without encapsulation in feeds on growth performance of broilers exposed to heat stress from 26 to 42 d of age.

Treatments	FI (g/bird)	BWG (g/bird)	FCR (feed/gain)
Control	2257.56^{c}	1189.28^{c}	1.90^{a}
LA	2317.39^{b}	1244.41^{b}	1.86^{b}
SP	2325.25^{b}	1329.00^{a}	1.75^{c}
LA+SP	2398.95^{a}	1355.82^{a}	1.77^{c}
encapsulated LA	2326.98^{b}	1274.16^{b}	1.83^{b}
encapsulated LA+SP	2347.42^{ab}	1328.54^{a}	1.77^{c}
SEM	22.5	19.9	0.016
p-value	0.03	<0.001	<0.001

$^{a-c}$ Means within the same column with uncommon superscript differ significantly (P<0.05).
FI: Feed intake; BWG: body weight gain; FCR; Feed conversion ratio; SEM: Standard error of mean

The biochemical blood metabolites analysis of broilers (Table 4) revealed that the dietary treatments did not affect serum glucose and triglyceride levels (P>0.05). Serum cholesterol was lower in the groups of birds fed with LA, SP or the combination of both and the lowest serum cholesterol concentration was observed in the birds fed with the diet supplemented with LA+SP and SP (P<0.05). Also, the highest of GSH-PX activity was observed in LA+SP, SP and encapsulated LA+SP groups (P<0.05).

Table 4. The effects of *Lactobacillus acidophilus* (LA) and *Spirulina platensis* (SP) addition with or without encapsulation in feeds on serum glucose (mg/dl), triglyceride (mg/dl), cholesterol (mg/dl), and glutathione peroxidase enzyme (GSH-P_X; U/ml) of broilers exposed to heat stress at 42 d of age.

Treatments	Glucose	Triglyceride	Cholesterol	GSH-P_X
Control	244	72	132^{a}	130^{c}
LA	240	67	122^{b}	146^{bc}
SP	233	62	115^{bc}	152^{ab}
LA+SP	237	61	114^{c}	160^{a}
Encapsulated LA	239	69	120^{b}	148^{bc}
Encapsulated LA+SP	234	60	116^{bc}	153^{ab}
SEM	4.570	5.934	3.263	3.906
p-value	0.572	0.658	0.017	0.003

$^{a-c}$ Means within the same column with uncommon superscript differ significantly (P<0.05).
SEM: Standard error of mean.

The effects of the diet on the morphology of the intestinal jejunal villus of broilers reared under heat stress are shown in Table 5. The results showed that, all supplemented treatments significantly increased VH, CD and VH/CD ratio and decreased villus width; so that the birds fed LA+SP, encapsulated LA+SP and SP groups had the highest VH and VH/CD ratio (P<0.05).

Table 5. The effects of *Lactobacillus acidophilus* (LA) and *Spirulina platensis* (SP) addition with or without encapsulation in feeds on jejunal morphology of broilers reared under heat stress at 42 days of age.

Treatments	Villus height (µm)	Villus width (µm)	Crypt depth (µm)	Villus height/crypt depth ratio
Control	1318^{d}	172^{a}	157^{d}	8.4^{c}
LA	1550^{c}	160^{b}	161^{c}	9.6^{b}
SP	1863^{ab}	157^{b}	167^{b}	11.1^{ab}
LA+SP	1954^{a}	155^{b}	174^{a}	11.2^{a}
Encapsulated LA	1710^{b}	154^{b}	166^{b}	10.3^{ab}
Encapsulated LA+SP	2014^{a}	152^{b}	178^{a}	11.3^{a}
SEM	55.872	2.240	1.753	0.329
p-value	<0.001	<0.001	<0.001	<0.001

[a-c] Means within the same column with uncommon superscript differ significantly (P<0.05).
SEM: Standard error of mean

The effects of dietary supplementation with LA (encapsulated or not) and SP on ileal microbial populations are presented in Table 6. The supplemented groups significantly affected the population of *Lactobacillus* spp. and *E. coli* (P<0.05). Dietary inclusion of LA, SP, LA+SP, encapsulated LA, and encapsulated LA+SP increased ileal *Lactobacillus* spp. and decreased *E. coli* counts compared to the control group (P<0.05).

Table 6.The effects of *Lactobacillus acidophilus* (LA) and *Spirulina platensis* (SP) addition with or without encapsulation in feeds on ileal microbial populations (Log10 CFU/gr digesta) of broilers reared under heat stress at 42 days of age.

Treatments	*Lactobacillus* genus	*Escherichia coli*
Control	4.63^{b}	6.40^{a}
LA	5.76^{a}	5.69^{b}
SP	5.68^{a}	5.84^{b}
LA+SP	5.75^{a}	5.83^{b}
Encapsulated LA	5.84^{a}	5.63^{b}
Encapsulated LA+SP	5.65^{a}	5.59^{b}
SEM	0.217	0.157
p-value	0.017	0.033

[a-b] Means within the same column with uncommon superscript differ significantly (P<0.05).
SEM: Standard error of mea

Discussion

High ambient temperature reduces broilers' growth performance by causing oxidative stress and damage to the intestinal brush border membrane (Santos et al., 2015). Similar to the results of this study, Liu et al. (2023) and El-Shobokshy et al. (2024) showed that heat stress could significantly decrease the average daily gain and, average daily feed intake and increase the FCR of broiler chickens. The poor growth performance observed in broilers raised under high ambient temperatures may be attributed to higher energy loss via increased respiration, reduced feed intake, and retardation of nutrient digestion (Mello et al., 2015). Other researchers have stated that lower growth rate in broilers under heat stress could be due to a poor appetite and lower FI, which is a protection mechanism to reduce the heat increment of broiler chickens (Song et al., 2013). He et al. (2019) attributed the decrease in FI under heat stress conditions to a decrease in the orexigenic neuropeptide Y expression in the hypothalamus. On the other hand, the present findings showed that LA, SP, LA+SP, encapsulated LA, and encapsulated LA+SP improved the growth performance of broilers. Vimon et al. (2023) also found that feeding *Lactobacillus plantarum* MB001 and encapsulated *Lactobacillus plantarum* MB001 (1×10^{8} CFU/kg of diet) increased the final body weight and average daily gain and decreased the FCR of broilers under heat stress. Li et al. (2020) also reported that the addition of a probiotic mixture (1.5×10^{8} CFU/kg of diet) to the diet of heat-stressed broilers significantly improved FI and average daily gain, and reduced FCR. It has been suggested that probiotics increased performance of broiler chickens by increasing feed intake, as observed in the present study, and nutrient digestibility (Awad et al., 2009) and also by increasing the activity of digestive enzymes (Sugiharto, 2016).

Additionally, the data of the current study are concomitant with the results of Shanmugapriya et al. (2015) and Fathi et al. (2018), which showed that adding SP to the diet of broilers can improve their growth performance. In the same line, Elbaz et al. (2022) found that feeding 2 g/kg of SP powder significantly increased body weight and BWG and decreased the FCR of heat-stressed broilers; this significant effect may be due to an improvement of the efficiency of feed utilization. Also, Abdel-Moneim et al. (2021) found that feeding 10 g/kg of SP improved the growth performance of broiler chickens raised under heat stress. SP has 70% antioxidant activity (Ranjbarinasab et al., 2024), which can decrease the oxidation reactions and reduce the adverse effect of heat stress on broiler performance. The high content of carotenoids, pigments, and polyphenols in SP, exhibit strong antioxidative potential and contribute to improving the health and growth of birds raised under high ambient temperatures and contribute to improving the health and growth of birds raised under high ambient temperatures (Tavernari et al., 2018; Abdel-Moneim et al., 2020). Additionally, it has been proposed that dietary SP supplementation improved the microbial equilibrium in broiler chickens' intestine, resulting in improved nutrient digestion and absorption (Alwaleed et al., 2021). In accordance with the results of other researchers, part of the interaction effect between SP and LA treatments appeared in this study can be explained by the synergistic effect between these two additives (Joya et al., 2020; Khalilnia et al., 2023).

Regarding blood parameters, in the present study, heat stress increased serum cholesterol concentration, which is in agreement with earlier studies (Attia et al., 2023). According to Rashidi et al. (2010), high ambient temperatures decrease feed intake in broiler chickens, so they compensate their requirement for energy through lipolysis of body lipids, resulting in rising blood cholesterol levels. In the present study, LA, SP, LA+SP, encapsulated LA, and encapsulated LA+SP significantly reduced serum cholesterol concentration compared to the control group. Similarly, several studies reported that feeding SP decreased the serum cholesterol concentration of broilers (Fathi et al., 2018; Mirzaie et al., 2018) and local laying hens (Mariey et al., 2012). The hypolipidemic effect of SP has been ascribed to C-phycocyanin, a blue color pigment capable to prevent the pancreatic lipase activity in a dose-dependent way (Deng & Chow, 2010). Adding probiotics to the diet of heat-stressed birds also decreased the serum cholesterol concentration compared to the Control group, which was also stated by Ahmed et al. (2019). This reduction may be due to the fact that some microorganisms in probiotics use cholesterol in the gastrointestinal tract for their metabolism, which reduces the amount of cholesterol absorbed (Ahmed et al., 2019). It has been proven that beneficial bacteria increase bile salt bile salt excretion by producing bile salt hydrolase, which in turn reduces bile salts and lipid absorption efficiency (Jazi et al., 2018). Although the mechanism of cholesterol reduction with the addition of probiotics and prebiotics is not fully known, in the present experiment our findings suggest that the reduction of cholesterol levels in the diets with SP and LA might be due to the absorption of cholesterol by beneficial microorganisms (*Lactobacillus and Bifidobacteria*).

The high temperature reduced the serum GSH-P_X activity of broiler chickens in the present study, which is in agreement with Bai et al. (2019). This decrease could be partly attributed to the fact that chronic heat stress leads to an increase in body temperature, metabolic rate, and metabolic enzyme activity, which in turn leads to decreased levels of antioxidant enzymes and reduced ability to respond to oxidative stress in broilers (Zhang et al., 2017). In the current study, dietary inclusion of LA+SP, SP, and encapsulated LA+SP increased serum GSH-P_X activity compared to the Control group. In line with the current experiment, Mirzaie et al. (2018) reported that using SP (0.5, 1, and 2%) in the diet of broiler chickens reared under high ambient temperature increased serum GSH-P_X activity. One of SP's main constituents is C-phycocyanin, which is a strong antioxidant (Deng & Chow, 2010). Also, SP contains antioxidants such as carotene, tocopherol, selenium, polypeptide pigments, and phenolic acids that may affect the antioxidant status (El-Desoky et al., 2013). On the other hand, Bai et al. (2017), also showed that the dietary supply of probiotics (Bacillus subtilis, 3×10^{10} CFU/kg) to broilers increased serum GSH-P_X activity. Probiotics are considered natural sources for eliminating the excess of free radicals and improving the antioxidant capacity of animals (Amaretti et al., 2013). Therefore, part of the interaction effect between SP as a prebiotic in combination with a probiotic in increasing serum GSH-P_X activity that appeared in this study can be explained by the stimulating role of these two additives in the production of antioxidants and destruction of free radicals.

Heat stress can harm intestinal epithelial cells by forming intestinal lesions, vacuolization of epithelial cells, increasing villus tips sloughing rate, and damaging the luminal membrane with microvilli loss (Lambert et al., 2002). In addition, high ambient temperatures are likely to reduce the amount of FI by the birds, thus greatly decreasing the amounts of energy delivered to the gastrointestinal tract cells during heat stress periods. As a result, low FI will reduce the absorptive area of the intestinal mucosa and decrease the secretion of digestive enzymes (Porto et al., 2015). In the current study, heat stress reduced the VH, CD and VH/CD ratio, which is consistent with the findings of Huang et al. (2020) and El-Shobokshy et al. (2024). Additionally, in the present experiment, LA supplementation improved the jejunal morphology of heat-stressed broilers. Similar results were reported by Ashraf et al. (2013) who showed that *Lactobacillus*-based probiotics significantly increased the VH, CD, and VH/CD ratio, while decreasing villus width, of different parts of the small intestine (duodenum, jejunum, and ileum) in broilers under heat stress. Song et al. (2014) found that supplementing a probiotic mixture (*Bacillus licheniformis*, *Bacillus subtilis*, and *Lac-*

tobacillus plantarum) in heat-stressed broiler diet increased VH, which is consistent with the current finding. Probiotic bacteria compete with pathogens for binding sites in the gut and occupy common receptors in the intestinal mucosa (Abdelrahman et al., 2014), which prevents the attachment of pathogens to the intestinal mucosa, thereby reducing villi damage and increasing their health. Our study revealed that the addition of encapsulated LA has a positive influence on the jejunal morphology of broilers reared under heat stress, which is in harmony with the data obtained by Vimon et al. (2023), who clarified that dietary *Lactobacillus plantarum* MB001 (encapsulated or not, 1×10^8 CFU/kg of diet) supplementation increased VH and VH/CD ratio and decreased CD of broilers under heat stress. On the other hand, Shanmugapriya et al. (2015) reported that the use of SP supplementation increased the VH in broilers. Attia et al. (2023) also found that adding one g/kg of SP supplement to the diet of broiler chickens under heat stress increased VH and VH/CD ratio and decreased CD. Therefore, higher body weight gains in broilers consumed SP and LA may be due to their positive effect on gut morphology in the present study.

The stability of a balanced gut microflora plays an important role in improving gut health and functions (Song et al., 2014). Stressors can cause a variety of changes in the gastrointestinal tract, including changes in the normal protective microflora (Bailey *et al*., 2004) and decreased integrity of the intestinal epithelium (Soderholm et al., 2002). It has been reported that heat stress led to changes in the intestinal microbial population and some disorders in intestinal permeability and morphology (Zhang et al., 2017; Vimon et al., 2023). In support of the positive results obtained in the present study regarding probiotic and prebiotic supplementation in diets and microbiota balance, Alwaleed et al. (2021) reported that adding 5 and 10 g/kg SP to the broiler diet increased the ileocecal population of *Lactobacillus* and decreased the population of *E. coli*. Also, it has been reported that SP (1 g/kg diet) supplementation increased the cecal and fecal *Lactobacillus* and reduced the coliform contents of broilers under heat stress (Attia et al., 2023). The antimicrobial activity of SP could be attributed to the presence of gamma-linolenic acid, active fatty acids, and synergistic effects between lauric acid and palmitoleic acid (Kumar et al., 2011). It has been stated that probiotics repress pathogen growth by releasing antimicrobial factors such as bacteriocins, hydrogen peroxide, nitric oxide, and short-chain fatty acids, which lower the pH of the lumen like acetic and lactic acids (Gogineni et al., 2013). Dong et al. (2019) demonstrated that microencapsulated *E. faecalis* supplementation increased ileal *Lactobacillus* counts and decreased ileal *E. coli* counts in *E. coli* K88-challenged broiler chickens. Also Vimon et al. (2023) found that feeding *Lactobacillus plantarum* MB001 and encapsulated *Lactobacillus plantarum* MB001 (1×10^8 CFU/kg of diet) increased Cecal *Lactobacillus* and *Enterococcus* of broilers under heat stress on days 21 and 42 of age. In this regard, our findings also suggest that the encapsulation process could enhance host health status by improving the jejunal morphology, and ileal microflora balance of heat-stressed broilers.

Conclusions

The results obtained in the present study indicated that dietary supplementation with LA, encapsulated or not, or/and SP significantly improved the growth performance, intestinal morphology, and intestinal bacterial community balance, as evidenced by increasing the FI, BWG, villus height, crypt depth, and VH/CD ratio, decreasing FCR, and suppressing the growth of harmful bacteria in heat-stressed broiler chickens. The current findings also indicated that the diets with SP and LA+SP were more effective in overcoming the negative effect of heat stress suggesting that its use can be recommended for improving chicken production performance, health, and welfare during hot seasons, especially in tropical and sub-tropical. Additionally, the encapsulation of LA or LA+SP has limited advantages compared with non-encapsulated additives.

Ethical approval: All experimental procedures used in this experiment were reviewed and approved by the Animal Care and Use Committee of the University of Jiroft, Iran (Ref. 2812-30009).

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