Spanish Journal of Agricultural Research
22 (1), e0601, 9 pages (2024)
eISSN: 2171-9292
https://doi.org/10.5424/sjar/2024221-20223
INIA-CSIC

RESEARCH ARTICLE

OPEN ACCESS

Effects of the encapsulation of *Lactobacillus acidophilus* and *Spirulina platensis* on carcass yield and meat quality of broilers under heat stress conditions

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Abstract

Aim of study: To evaluate the effects of adding *Lactobacillus acidophilus* (LA), *Spirulina platensis* (SP) and the encapsulation of LA on the relative weights of carcass parts and meat quality of broilers subjected to heat stress.

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

Material and methods: Two hundred forty 1-day-old male broilers (Ross 308) were used in a completely randomized design, with six treatments and four replicates (cages, 10 birds per cage). Dietary treatments included: (i) corn-soybean as control diet (CON), (ii) 0.02% LA, (iii) 1% SP, (iv) 0.02% LA + 1% SP, (v) 0.02% of encapsulated LA, and (vi) 0.02% encapsulated LA + 1% SP.

Main results: The relative weight of the carcass increased in all experimental groups except in the 0.02% LA encapsulated group ($p<0.05$). Birds fed diets containing LA+SP (0.02% LA+ 1%SP and 0.02% LA encapsulated+1% SP) had a significantly higher relative weight of the breast ($p<0.05$). Dietary supplementation with SP, LA+SP, and encapsulated LA+SP significantly increased water holding capacity and decreased cook loss, respectively ($p<0.05$), whereas dietary LA+SP and encapsulated LA+SP decreased drip loss ($p<0.05$), compared to the CON group. Moisture and pH were not significantly affected by the dietary treatments ($p>0.05$). The malondialdehyde content of thigh and breast meat at 30 and 37 days after the slaughter was reduced ($p<0.05$) in the SP, LA+SP, and LA encapsulated +SP groups.

Research highlights: Based on results, including LA, SP and encapsulated LA in broiler's feeds were effective in improving carcass yield, quality and oxidative stability of broiler meat under heat stress condition.

Additional key words: breast weight; cooking loss; meat oxidative stability; microalgae; probiotic.

Abbreviations used: DPPH (diphenyl-1-picrylhydrazyl); LA (*Lactobacillus acidophilus*); MDA (malondialdehyde); ROS (reactive oxygen species); SP (*Spirulina platensis*); TBARS (thiobarbituric acid reactive substances); WHC (water-holding capacity).

Citation: Ranjbarinasab, Z; Mazhari, M; Esmaeilipour, O; Shahdadi, F; Barazandeh, A (2024). Effects of the encapsulation of *Lactobacillus acidophilus* and *Spirulina platensis* on carcass yield and meat quality of broilers under heat stress conditions. Spanish Journal of Agricultural Research, Volume 22, Issue 1, e0601. https://doi.org/10.5424/sjar/2024221-20223

Received: 05 Feb 2023. **Accepted**: 27 Nov 2023. **Published**: 23 Jan 2024.

Introduction

In recent decades, genetic selection has greatly improved broiler growth efficiency to meet the increasing demands of poultry meat, but this has made modern broilers more vulnerable to environmental challenges, especially high temperatures (Chang et al., 2020). Broilers are particularly susceptible to heat stress due to abundant feathers, lack of sweat glands, and high metabolic activity (Cramer et al., 2018). Heat stress reduced the carcass and breast yield of broilers by decreasing protein deposition mainly due to restraining protein synthesis in the breast muscle of broilers (Zuo et al., 2015). Besides, in poultry, overheat-induced oxidative stress overproduces reactive oxygen species (ROS), which cause oxidative damages in skeletal muscles and also in the lipids and proteins of muscles and decrease meat quality and flavor (Cheng et al., 2018), which depreciates its value from a commercial point of view.

Poultry farmers have commonly utilized synthetic antioxidants to reduce the negative impact of stress. However, natural antioxidants from plant origin do also show strong potential antioxidant effects without negative side effects in the animals and can also be nutritionally beneficial to the consumer (Gulcin, 2012). In addition to plants, *Spirulina* and *Chlorella* microalgae have been utilized as natural antioxidants for poultry (Sugiharto, 2020). *Spirulina platensis* (SP) is a blue-green microalgae or cyanobacteria from the Cyanophyceae family (Demisu & Benti, 2018), which due to its antimicrobial properties, positive effects on intestinal morphology and immune system, can be regarded as a prebiotic (Shanmugapriya et al., 2015). Kaoud (2012) It has been reported that dietary inclusion of *Spirulina* increases carcass yield of broilers. Additionally, Park et al. (2018) suggested that dietary *Spirulina* supplementation improved the microbial balance in the intestine resulting in better digestion and nutrient absorption in broilers which can lead to higher weight gain and carcass weight. Also, El-Bahr et al. (2020) reported that feeding *Spirulina* decreased the malondialdehyde (MDA) content of broilers' meat. A variety of factors, including β-carotene, astaxanthin, lutein, bioactive peptides, phenolic compounds, and phycocyanin, may contribute to the antioxidant activity of microalgae in broilers (Barkia et al., 2019). In addition, lycopene and phycobiliproteins in microalgae may function as antioxidants that can neutralize excessive free radicals and thus prevent oxidative harm.

Recently, probiotics are gaining interest for combating oxidative damage from heat stress in the poultry industry. Probiotics are live microbial feed supplements included in animal feeds that by improving gastrointestinal environment, and the gut microbial balance have a positive effect on animal health (Mountzouris et al., 2010). Abou-Kassem et al. (2021) suggested that probiotics can improve carcass yield and water holding capacity (WHC) and reduce the cook loss and MDA in the meat of growing Japanese quails. A recent study suggested that feeding probiotics can also improve meat quality including WHC and oxidative stability of broilers (Park & Kim, 2014). The mechanism of probiotics as protective agents against lipid oxidation come from their ability to generate bacteriocins that prevent the production of free fatty acids by lipolytic microbes (Mohammed et al., 2021).

Probiotics given to broilers may be inactivated within the gastrointestinal tract due to the low pH found in the gizzard and other environmental conditions decreasing the effectiveness of probiotics prior to entering the small intestine (Gbassi & Vandamme, 2012). Encapsulation with hydrocolloids has been utilized to prolong the survival of probiotics during exposure to harsh conditions (Zanjani et al., 2018). Alginate is the most common biomaterial utilized in the encapsulation of probiotics (Gbassi & Vandamme, 2012). Sodium alginate, a polysaccharide extracted from seaweeds, acts perfectly as a membrane material; it is a biodegradable, biocompatible, non-toxic, water-soluble, and gelling agent (Jayakumar et al., 2009). Therefore, the purpose of this study was to investigate the effects of supplementation with *Lactobacillus acidophilus* (LA), encapsulated or not, or/and SP on the relative weight of carcass and carcass parts, meat quality, and meat stability in broilers under heat stress condition.

Material and methods

Analysis of chemical compounds of *Spirulina platensis* microalgae

SP powder was analyzed to determine its chemical compounds. 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 Na_2CO_3 solution (20%), then 1.16 mL of distilled water and 100 µL of Folin–Ciocalteu reagent were 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 (Spectrophotometer Dynamica, XB-10 England). Gallic acid was used as a standard for the calibration curve (Arabshahi-Delouee & Urooj, 2007). Antioxidant activity was determined by DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging method (Anandjiwala et al., 2008). Briefly, 1 mL of a 1 mM methanolic solution of DPPH was mixed with 3 mL of extract solution (500 ppm concentration). The mixture was then homogenized vigorously and left for 30 min in a dark place (at room temperature). Its absorbance was measured at 517 nm (Spectrophotometer Dynamica, XB-10 England), and activity was expressed as percentage of DPPH scavenging relative to control. Moisture, crude protein, lipid, and total ash contents in SP were analyzed according to the AOAC international procedures (AOAC, 1995). The chemical analysis of SP showed that total phenolic compounds were 75.2 mg. The DPPH-scavenging activity of *Spirulina* extract was 69.4%. Moisture, crude protein, lipid, and total ash contents in SP were 2.5%, 59%, 3.33% and 4.5% respectively.

Birds, diets and management

This work was performed at the University of Jiroft and the protocols were approved by the Animal Care Committee of University of Jiroft, Iran (2812-30009). A total of 240-day-old male Ross 308 broiler chicks (average weight of 49.25±1.13 g) were obtained from Mahan (Kerman, Iran) commercial hatchery. Table 1 shows that during the starter (1-10 days), grower (11-25 days), and finisher (26-42 days) phases, the birds were fed according to the Ross 308 recommendations (Aviagen, 2014). Broilers were allocated to 24-floor pens (1×1.5 m) with 10 chickens each and reared to 42 days of age in environmentally controlled houses. For the first 10 days prior to the start of the research, broilers were raised with commercial feed. From 11 days of age, the birds were assigned to six diets which differed in the inclusion or not of SP and LA or encapsulated LA. The diets included: (i) corn-soybean as control diet (CON), (ii) 0.02% LA, (iii) 1% SP, (iv) 0.02% LA + 1% SP, (v) 0.02% of the encapsulated LA, and (vi) 0.02% encapsulated LA + 1% SP.

Each pen was equipped with a feeding trough, and nipple drinkers and birds had free access to feed and water. From 1 to 7 days of age, the birds were subjected to a 24-hour lighting period, followed by a 23-hour light: 1-hour darkness cycle until they were 42 days old. For the first 3 days, the temperature was first set to 32°C and lowered by 2.5°C per week. To induce chronic heat stress, from 25 to 42 days of age, birds were exposed to an ambient temperature of 34°C for 8 h daily (from 10:00 am to 18:00 pm). For the remaining hours, the temperature was constant at 24°C (Chang et al., 2020).

The probiotic used in this experiment was the LA® (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).

Microencapsulation process of *Lactobacillus acidophilus*

For this purpose, first, 20 g of sodium alginate (Sigma Aldrich, USA) was added to 200 mL of distilled water and then sterilized at 121°C for 15 min. To allow the alginate particles to absorb water well, the alginate solution

Table 1. Composition of starter, grower, and finisher diets

	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 and mineral premix [1]	0.50	0.50	0.50
Calculated analysis			
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. 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.

was refrigerated overnight. The next day, 0.02% LA was added to alginate solution and homogenized. Extrusion technique using an insulin syringe was performed for microencapsulation process. The mixture of cell suspension and Na–alginate were injected into a 0.1 M $CaCl_2$ (Merck, Germany) solution. The droplets formed gel spheres immediately. The distance between the syringe and $CaCl_2$ solution was 25 cm. Diameter of the resultant beads was 500-1000 µm (Pan-Utai & Iamtham, 2018). Then the $CaCl_2$ solution was drained and isolated and the resulting beads were collected.

Carcass characteristics

At the end of the trial, birds were fasted for 4 h to make sure the digestive system was empty. Then, two male birds from each replicate (the nearest to the mean weight of the pen) were killed by cervical dislocation and considered to determine the carcass characteristics after removal of the head, feathers and feet. Carcass yields were calculated relative to the live body weight. The weights of the carcass cut, including breast and thigh and the weights of internal organs such as liver, spleen and bursa, were recorded and its proportion to the whole body was then calculated using the following formula: Relative organ weight = Organ weight (g) / Carcass (g) * 100 (Mashayekhi et al., 2018).

Meat quality traits

After slaughter, the right thigh and breast of broiler chickens were separated and kept at 4°C for measuring meat quality traits and at -20°C for meat oxidative stability assessment. Meat quality traits including WHC, cook loss, drip loss, moisture and pH, were measured the day after slaughter.

To determine the WHC, 4 g of breast muscle were placed in a filter paper and centrifuged at 1500×g for 4 min (Hettich, EBA 200, Germany). Then the samples were placed in an oven at 70°C for 24 h to be dried. WHC was calculated by the following equation (Castellini et al., 2002):

WHC = [Weight after centrifugation (g) - Weight after drying (g)] / Initial weight (g) × 100 (1)

In the case of cook loss, a piece of 1 cm^3 of breast muscle was weighed and kept at 4°C for 24 h, then placed in a water bath (Fan Azma Gostar, WM22, Iran) at 85°C for 10 min and finally cleaned and re-weighed with a linen cloth (Bertram et al., 2003). The percentage of cook loss was calculated as:

Cook loss = [Initial weight (g) - Final weight (g)] / Initial weight (g) × 100 (2)

For the measurement of drip loss, a piece of breast muscle was weighed and placed in a plastic bag and kept at 4°C for 24 h. The meat was then gently rubbed into the cloth and weighed again (Christensen, 2003). Drip loss percentage was calculated as:

Drip loss = [Initial weight (g) - Final weight (g)] / Initial weight (g) × 100 (3)

For the total moisture measurement of breast muscle**,** 5 g piece of the muscle was placed in an oven (Fan Azma Gostar, BFS120, Iran) at 105°C for 16 h, then it was weighed (AOAC, 1995) and moisture was calculated as:

Moisture = [Initial weight (g) - Final weight (g) after oven drying] /Initial weight (g) × 100 (4)

To determine the pH, 10 g of breast sample was grinded and 50 mL of distilled water was added; pH was measured using a pH meter (Sartorius, Professional Meter PP-50, Germany) at room temperature (Jeacocke, 1997).

Thiobarbituric acid reactive substances (TBARS) test

The TBARS test was done three times after slaughter (30, 37 and 44 days after slaughter) to calculate the MDA (the aldehyde that results from lipid peroxidation in foods) content. At first, 5 g of minced meat (breast and thigh muscles, each sample has four repetitions) was mixed with 45 mL of 0.9% NaCl and then homogenized, and centrifuged (Centurion Scientific, C2 Series, England) at 2800×g at 4°C for 15 min to collect the supernatant for further analysis. The MDA content was assessed as described by Ahn et al. (1998). Briefly, 2 mL of supernatant was added to 4 mL of trichloroacetic thiobarbituric acid [15% TCA (w/v) and 0.375% TBA (w/v) in 0.25 M HCl] and 100 µL of butyrated hydroxyanisole (7.2%, w/v), homogenized with a vortex and incubated in a water bath (95°C) for 30 min in order to develop the color reaction. The sample was cooled at room temperature and centrifuged at 2,800×g for 15 min (Hettich, EBA 200, Germany). The absorbance of the resulting supernatant solution was measured at 532 nm (Spectrophotometer Dynamica, XB-10 England), against a blank containing 2 mL of 0.9% NaCl and 4 mL of TCA/TBA stock solution. The results of the samples were plotted against a standard curve prepared with a known concentration of 1,1,3,3-tetraethoxypropane. The MDA content was expressed as nanomole/mg of protein.

Statistical analysis

All data were analyzed by ANOVA utilizing GLM procedure of SAS (2005). Analysis of variance was performed utilizing a completely randomized design considering dietary treatment as the classificatory effect. Means were compared for significant differences utilizing the Tukey multiple range test ($p<0.05$).

Results

The effects of dietary supplementation with LA (encapsulated or not) and SP on the relative weight of

Table 2. The effects of *Lactobacillus acidophilus* (LA) and *Spirulina platensis* (SP) addition with or without encapsulation in feeds on relative organ weight (relative weight: g/100 g of live weight) of broilers under high ambient temperature at 42 days of age.

Treatments	Carcass[1]	Breast	Thigh	Liver	Bursa	Spleen
Control	84.0^{b}	21.7^{b}	20.4	2.28	0.10	0.070
LA	86.6^{a}	23.2^{ab}	20.2	2.14	0.12	0.085
SP	86.9^{a}	23.6^{ab}	20.9	2.24	0.11	0.090
LA+SP	87.2^{a}	23.7^{a}	20.6	2.13	0.11	0.090
Encapsulated LA	85.7^{ab}	22.7^{ab}	21.0	2.16	0.13	0.085
Encapsulated LA+SP	86.4^{a}	23.0^{ab}	21.0	2.18	0.11	0.090
SEM	0.49	0.44	0.37	0.11	0.008	0.009
p-value	0.029	0.052	0.488	0.904	0.348	0.647

[1] Whole carcass with viscera. $^{a-b}$ Means within the same column with uncommon superscript differ significantly ($p<0.05$).

carcass and internal organs of broilers are shown in Table 2. Compared with the CON group, the relative weight of the carcass was increased in all experimental groups except that including encapsulated LA ($p<0.05$). On the other hand, dietary supplementation of LA+SP increased the relative weight of breast compared with the CON group ($p<0.05$). The relative weight of the thigh, liver, bursa, and spleen were not affected by the dietary treatment ($p>0.05$).

Among the breast meat quality traits mentioned in Table 3, the indices of WHC, cook loss, and drip loss showed significant differences ($p<0.05$) among treatments compared with the CON group, birds fed with SP, LA+SP, and encapsulated LA+SP showed the highest and lowest WHC and cook loss, respectively ($p<0.05$). The lowest ($p<0.05$) drip loss was registered in broilers fed LA+SP and encapsulated LA+SP. However, moisture and pH were not significantly affected by the treatments ($p>0.05$).

Effects of the dietary treatments on TBARS test in breast and thigh meat at days 30, 37, and 44 after slaughter, are shown in Table 4. The MDA content in thigh meat showed significant ($p<0.05$) effect among treatments in the three times period measured. However, the MDA content of breast meat showed significant ($p<0.05$) effect at 30 days after slaughter. At 30 days after slaughter, the CON group showed the highest MDA content both in thigh and breast meat compared with treatments SP, LA+SP, and LA encapsulated + SP groups ($p<0.05$). At 37 days after slaughter, animals fed the CON and LA diets showed higher ($p<0.05$) MDA content in thigh meat compared with the SP, LA+SP, and LA encapsulated + SP groups. At 44 days after slaughter, birds that received SP, LA+SP, and LA encapsulated + SP diets showed lower ($p<0.05$) MDA content in thigh meat compared with the CON group. There were not significant differences among experimental treatments in the MDA content of breast meat at 37 and 44 days after slaughter.

Table 3. The effects of *Lactobacillus acidophilus* (LA) and *Spirulina platensis* (SP) addition with or without encapsulation in feeds on quality of breast meat of broilers under high ambient temperature at 42 days of age.

Treatments	WHC (%)	Cook loss (%)	Drip loss (%)	Moisture (%)	pH
Control	62.62^{b}	33.61^{a}	11.26^{a}	69.50	5.54
LA	64.74^{ab}	31.47^{ab}	10.70^{ab}	69.75	5.44
SP	65.05^{a}	30.60^{b}	10.47^{ab}	70.61	5.43
LA+SP	66.56^{a}	30.10^{b}	10.11^{b}	70.50	5.42
Encapsulated LA	64.80^{ab}	31.46^{ab}	10.63^{ab}	70.25	5.51
Encapsulated LA+SP	65.44^{a}	29.90^{b}	10.10^{b}	72.28	5.48
SEM	0.48	0.64	0.19	0.68	0.05
p-value	<0.001	0.008	0.004	0.115	0.414

WHC: Water holding capacity. $^{a-b}$ Means within the same column with uncommon superscript differ significantly ($p<0.05$).

Table 4. The effects of *Lactobacillus acidophilus* (LA) and *Spirulina platensis* (SP) addition with or without encapsulation in feeds on malondialdehyde content (nmol/mg of protein) in the meat of broilers under high ambient temperature.

Treatments	Thigh			Breast		
	30 d	37 d	44 d	30 d	37 d	44 d
Control	10.50^{a}	10.92^{a}	14.01^{a}	10.73^{a}	10.53	13.75
LA	10.17^{ab}	10.68^{a}	13.48^{abc}	10.21^{ab}	10.41	13.61
SP	9.78^{b}	10.04^{b}	13.23^{bc}	9.98^{b}	10.29	13.33
LA+SP	9.57^{b}	9.52^{b}	12.94^{bc}	9.88^{b}	10.35	13.31
Encapsulated LA	10.20^{ab}	10.43^{ab}	13.59^{ab}	10.17^{ab}	10.48	13.44
Encapsulated LA+SP	9.64^{b}	9.64^{b}	12.88^{c}	9.78^{b}	10.46	13.20
SEM	0.16	0.22	0.17	0.15	0.19	0.15
p-value	0.004	0.001	<0.001	0.004	0.960	0.145

$^{a-c}$ Means within the same column with uncommon superscript differ significantly ($p<0.05$).

Discussion

The carcass composition of birds is altered by exposure to high ambient temperatures (Toghyani et al., 2012). In the present study, high ambient temperatures reduced carcass and breast muscle yield, which was consistent with the results of Ebrahimzadeh et al. (2013). Our results showed that all dietary treatments including LA, SP, and their combination and also their encapsulated form, increased the relative weight of the carcass and breast in 42-day-old broilers compared to the control group under high ambient temperatures, but the changes in the relative weight of the thigh, liver, bursa, and spleen were not significant. Muscle growth is the combined effect of the synthesis and degradation of the protein. It has been reported that chronic heat stress decreased protein deposition mainly by restraining protein synthesis in the breast muscle of broilers (Zuo et al., 2015).

Our observations demonstrated that dietary inclusion of LA and SP alone, or in combination, and also their encapsulated form, improved the carcass yield. Feeding birds by LA + SP treatments improved the relative weight of the breast. Another study has shown improving carcass yield by probiotic supplementation in the diet of broilers (Ghasemi-Sadabadi et al., 2019). Improving carcass and breast percentage by LA addition might be related to the inhibition of colonization of intestinal pathogens and improved utilization of nutrients (protein and energy) in the diet as described by Toghyani et al. (2011). It has been observed that the administration of probiotics microorganisms enhanced protein availability, nutrient uptake and nitrogen stability, which can significantly improve the carcass yield (Falaki et al., 2011).

SP has a high amount of carotenoids, vitamins, minerals and high-quality protein including all essential amino acids (Kaoud, 2012). SP is also a good source of phenolic compounds, all these properties make SP an excellent supplement for improving growth which leads to higher weight gain and carcass yield. SP has about 70% antioxidant activity, which can enhance the antioxidant ability of birds to overcome heat stress and growth better during heat stress. It has been shown that SP can enhance immune function, modulate the microbial community of intestinal toward increasing lactobacillus population and increase nutrient absorption (Mariey et al., 2012).

Similar to the current findings, Kaoud (2012) showed that the addition of 0.1% *Spirulina* to the broiler diet increased carcass percentage. Also Ansari et al. (2018), in an experiment on broilers with three levels of *Spirulina* (1, 1.5 and 2 g/kg of diet), indicated that the highest carcass percentage was seen in birds fed with 2 g *Spirulina*. Voltarelli & de Mello (2008) reported that increasing the relative weight of the breast in treatments containing *Spirulina* may be related to the ability of *Spirulina* cellular contents (e.g., fatty acids and branched-chain amino acids) which stimulate muscle protein synthesis. The highest carcass and breast percentage was seen in birds fed by both LA and SP, which may indicate that these two supplements can act as symbiotic and enhance their effects on improving growth, digestion and absorption and finally lead to higher carcass yield.

Since certain nutrients are easily lost during exudation due to water loss, factors, including WHC, drip loss and cook loss, are critical in determining meat quality (Chen et al., 2012). WHC is one of the major characteristics of meat quality which impairs the juiciness of fresh meat. There is a relation between WHC and drip and cook loss, as meat with higher WHC has lower drip and cook loss. The increasing metabolic rate during heat stress resulted in pronounced protein denaturation (Deng et al., 2002). Researchers reported

the increased cook loss in the breast muscle of chickens subjected to chronic heat stress due to more pronounced protein denaturation, which decreased the ability of the protein to bind to water (Zhang et al., 2012). In this study, high ambient temperature significantly reduced the WHC of broiler breast meat, which is in agreement with a report indicating that heat stress decreases the WHC of broiler breast meat (Cramer et al., 2018). Similar to the results of this study, Hu et al. (2020) reported that heat stress increased cook and drip loss of broiler breast meat. The current outcomes revealed that the SP, LA + SP and also encapsulated LA+SP diets increased WHC, but decreased cook loss and drip loss in broiler breast meat. Several studies have revealed that dietary probiotic supplements containing *Bacillus subtilis* increased the WHC in broiler breast and thigh meat (Park & Kim, 2014; Mohammed et al., 2021). Also, it has been reported that dietary probiotic supplements decreased cook loss and drip loss in broiler meat, but had no effect on pH (Park & Kim, 2014). In our study, LA treatment did not cause any significant difference with control, but a combination of LA and SP showed a significant difference, which may imply the effective role of SP in improving meat quality. El-Bahr et al. (2020) reported that using 0.1% *Spirulina* in broiler diets significantly decreased drip loss. Park et al. (2018) showed that the addition of *Spirulina* (0.25-1%) in broilers diets resulted in a significant reduction of drip loss after seven days of storage. These results are related to the delayed oxidation of the cell membrane caused by antioxidant compounds of SP, because SP has 70% antioxidant activity which can decrease the oxidation reactions of meat and maintain its quality during storage.

During normal cellular metabolism, sufficient levels of ROS are essential for animals. However, oxidative damage to the tissues could be caused by its overproduction. To defend cells from oxidative damage by quenching excessive ROS, antioxidant enzyme systems, including glutathione peroxidase and superoxide dismutase, play a vital function. Adverse factors, like high ambient temperatures, could break the balance between the antioxidant system and the production of free radicals (Cheng et al., 2018). It has been shown that heat stress could increment the development of hydrogen peroxide and superoxide ions, and also cause the accumulation of MDA concentration in the breast and thigh muscle of broilers (Cramer et al., 2018); this means that high ambient temperatures can impair muscle oxidative status. Similar to the results of the present study, Cheng et al. (2018) found that heat stress increased the levels of breast muscle MDA in broilers. In this study the birds fed by SP, LA + SP and encapsulated LA + SP had the lowest amount of MDA in the breast and thigh muscle, which shows the antioxidant capacity of SP in lowering MDA as a product of lipid oxidation during meat storage. Ansari et al. (2018) showed that using 0.2% *Spirulina* in broiler diet reduced the MDA content in the blood. El-Bahr et al. (2020) studied the effect of three microalgae, including *Chlorella vulgaris*, SP and *Amphora coffaformis,* and their results showed that all treatment groups decreased the cook loos and MDA content in the breast muscle of broilers.

Phycocyanin, xanthophyll, carotene, tocopherols and phenolic compounds are active components in *Spirulina,* which make this microalga act as a strong antioxidant with scavenging activities against ROS like superoxide and hydrogen peroxide radicals (Farag et al., 2016).

In conclusion, exposing broiler to high ambient temperature in this experiment impaired carcass characteristics, meat quality and oxidative stability, while dietary inclusion of LA*, Spirulina platensis*, LA + SP and encapsulated LA + SP had beneficial effects on carcass characteristics since they lead to increasing in the relative weight of the carcass. The relative weight of breast was also increased by LA + SP treatment. SP, LA + SP and encapsulated LA + SP treatments lead to improving meat quality (increased WHC, and decreased cook loss and drip loss), and meat oxidative stability (decreased MDA content). Therefore, we suggest using *Spirulina platensis* or a combination of 1% *Spirulina platensis* and 0.02% *Lactobacillus acidophilus* in simple form or encapsulated form in broiler diets under high ambient temperature for improving carcass yield and meat quality and stability.

Data availability: Not applicable

Competing interests: The authors have declared that no competing interests exist.

Authors' contributions: Zahra Ranjbarinasab: Conceptualization, Investigation, Methodology, Project administration, Writing – original draft. **Mozhgan Mazhari**: Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Supervision, Writing – review & editing. **Omidali Esmaeilipour**: Conceptualization, Investigation, Methodology, Project administration, Supervision,Writing – review & editing. **Fatemeh Shahdadi**: Conceptualization, Methodology, Project administration, Writing – review & editing. **Arsalan Barazandeh:** Conceptualization, Project administration, Writing – review & editing.

Funding: The authors received no specific funding for this work.

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