

Effect of galactooligosaccharides delivered *in ovo* on meat quality traits of broiler chickens exposed to heat stress

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ABSTRACT A study was carried out to evaluate meat quality traits in fast-growing chickens stimulated *in ovo* with trans-galactooligosaccharides (GOS) and exposed to heat stress. On day 12 of egg incubation, 3,000 fertilized eggs (Ross 308) were divided into prebiotic group (GOS) injected with 3.5 mg GOS/egg, saline group (S) injected with physiological saline, and control group (C) uninjected. After hatching, 900 male chicks (300 chicks/treatment) were reared in floor pens in either thermoneutral (TN; 6 pens/group, 25 birds/pen) or heat stress conditions (HS, 30°C from 32 to 42 D; 6 pens/group, 25 birds/pen). At 42 D of age, 15 randomly chosen birds/treatment/temperature were slaughtered and the pectoral muscle (PM) was removed for analyses. Data were analyzed by GLM in a 3 × 2 factorial design. *In ovo* treatment had no effect on PM weight, pH, water-holding capacity, and shear force. GOS and S birds had lighter (L*, $P < 0.01$) PM than C group, whereas the latter showed a higher ($P <$

0.05) yellowness index (b*) compared to S group. Proximate composition, cholesterol, and intramuscular collagen properties were not affected by treatment. As for fatty acid composition, only total polyunsaturated fatty acids (PUFA) content and n-6 PUFA were slightly lower in GOS group compared to S. Heat stress had a detrimental effect on PM weight ($P < 0.01$) and increased meat pH ($P < 0.01$). PM from HS chickens was darker with a higher b* index ($P < 0.05$) and had a higher ($P < 0.01$) lipid content and a lower ($P < 0.05$) total collagen amount. Total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and PUFA were similar among groups. Significant interactions between factors were found for fatty acid composition: GOS decreased ($P < 0.01$) SFA and increased ($P < 0.05$) MUFA contents in HS birds. In conclusion, *in ovo* injection of GOS could mitigate the detrimental effect of heat stress on some meat quality traits.

Key words: galactooligosaccharides, *in ovo* injection, heat stress, meat quality, fatty acids

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INTRODUCTION

Continuous selection for fast growth and improved feed efficiency has made modern poultry genotypes more susceptible to heat stress than ever before (reviewed by Deeb and Cahaner, 2002). Heat stress is one of the major environmental stressors in poultry production since it adversely affects behavior, immune

response, intestinal integrity, productivity, and meat quality of chickens (Lara and Rostagno, 2013). Either acute or chronic heat stress could lead to meat quality issues due to an increased ante/postmortem glycolytic metabolisms coupled with a reduced protein synthesis and turnover, enhanced fat deposition, and overproduction of reactive oxygen species (Temim et al., 2000; Lu et al., 2007; Zhang et al., 2012; Zaboli et al., 2019). Exposure of broilers to high temperatures can induce a lower ultimate pH with variation in meat color, water-holding capacity (WHC), and tenderness of meat (Berri et al., 2005; Aksit et al., 2006; Zhang et al., 2012; Wang et al., 2017), resulting in a lower consumer's acceptability.

In recent years, poultry industry has made effort to mitigate the negative effects of heat stress on poultry production to reduce economic losses. Feed additives, such as probiotics, prebiotics, and synbiotics,

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have been proposed as a nutritional strategy to improve the resilience of animals against heat stress. The rationale for the administration of such bioactives is to improve intestinal health, which is one of the main factors influencing the vulnerability of the chickens to heat (Ashraf et al., 2013; Varasteh et al., 2015; Sugiharto et al., 2017; Cramer et al., 2018). Currently, there is growing evidence that the supplementation with prebiotics can be effective in alleviating the detrimental effects of heat stress in chickens. Prebiotics such as fructooligosaccharides, galactooligosaccharides (GOS), and mannanoligosaccharides are considered preventative agents since they can select for a gastrointestinal microbiota which not only benefits the host but can serve as a barrier to pathogen colonization (Ricke, 2018). In the poultry practice, prebiotics and probiotics are conventionally added to feed and/or water at first hours/days post-hatching. The main concern about the use of these bioactives is their efficient administration under fully controlled conditions. Moreover, during early post-hatching period, the possible infection of chicks by harmful bacteria cannot be overlooked. Thus, to ensure that the chicks' intestine is protected over that time, the natural promoters of the beneficial microflora such as prebiotics, probiotics, and synbiotics should be applied before hatching. The main concept of *in ovo* technology is to apply bioactives long before the bird hatches (day 12 of egg incubation) to stimulate native egg microflora and helps to program lifelong phenotypes (e.g., immunity, gut microbiome, performance, adaptive) already during the embryonic phase (Siwek et al., 2018).

Several studies suggest the gut health-promoting effect of dietary GOS. Varasteh et al. (2015) found that GOS could not mitigate the alterations in the ileum, but successfully prevented all heat-stress induced changes in the jejunum. Recently, Slawinska et al. (2019a) found that GOS delivered *in ovo* had a bifidogenic effect in adult chickens. It also increased the expression of the cytokine genes, barrier function genes, and free fatty acid receptors, and varied the expression of the glucose transporter genes in the intestinal mucosa. Moreover, in a subsequent study, Slawinska et al. (2019b) found that stimulation *in ovo* with GOS prebiotic dampened heat-induced immune- and stress-related gene expression signatures in spleen of chickens exposed to acute heat stress. Promotion of the intestinal health through embryonic stimulation of the intestinal microflora with GOS has not only a beneficial effect on host systems but also on growth performance. In fact, GOS delivered *in ovo* significantly improved the resilience of birds exposed to heat stress and improved feed and growth efficiency (Slawinska et al., 2019c). As for welfare traits, Slawinska et al. (2019c) also found that *in ovo* stimulation with GOS dampened body temperature in both thermoneutral and heat stress conditions and numerically improved survivability during heat stress; in addition, it was found that GOS delivered *in ovo* decreased the prevalence of foodpad dermatitis in thermoneutral

conditions by 20% (no lesions in 81% in GOS vs. 60% in C). Considering all this evidence, the aim of this study was to evaluate the effect of *in ovo* GOS prebiotic injection and chronic heat stress on meat quality traits of fast-growing broiler chickens.

MATERIALS AND METHODS

Birds and Experimental Design

Fertilized eggs obtained from the same breeder flock (Ross 308) were incubated in a commercial hatchery. On day 12 of incubation, after candling, 3,000 eggs with viable embryos were randomly divided into 3 experimental groups: prebiotic group (GOS) injected with a single dose of 3.5 mg GOS/egg suspended in 0.2 mL of physiological saline; saline group (S) injected with 0.2 mL of physiological saline (0.9% NaCl); control group (C) uninjected. Saline and GOS solution were injected into the air chamber, and the hole was sealed with organic glue. GOS prebiotic used in this study (trade name: Bi²tos, Clasado Biosciences Ltd., Jersey, UK) is manufactured by enzymatic transgalactosylation of the milk lactose by the whole cells of *Bifidobacterium bifidum* 41171 (Tzortzis et al., 2005). The GOS product obtained this way is a dry powder containing a mixture (wt: wt) of the following oligosaccharides: 45% lactose, 9.9% disaccharides [Gal (β 1-3)-Glc; Gal (β 1-3)- Gal; Gal (β 1-6)-Gal; Gal (α 1-6)- Gal], 23.1% trisaccharides [Gal (β 1-6)-Gal (β 1-4)- Glc; Gal (β 1-3)- Gal (β 1-4)- Glc], 11.55% tetrasaccharides [Gal (β 1-6)- Gal (β 1-6)-Gal (β 1-4)- Glc], and 10.45% pentasaccharides [Gal (β 1-6)- Gal (β 1-6)- Gal (β 1-6)- Gal (β 1-4)-Glc]. The injection dose used was already tested by Bednarczyk et al. (2016) based on the criterion of egg hatchability and intestinal bacteria abundance in 1-day-old chicks. After hatching, 900 male chicks (300 chicks/treatment) were reared in floor pens and exposed either to thermoneutral (TN, 6 pens/group, 25 birds/pen) or chronic heat stress conditions (HS, 30°C for 8 h; 6 pens/group, 25 birds/pen) from 32 D till the end of the experiment (42 D). Birds were fed the same commercial diet according to the age. The basal diet was formulated to meet the dietary requirements of the selected broiler genotype according to the nutritional recommendations provided by the breeding company (Aviagen Group, Huntsville, AL). The basal diet is representative of the current commercial practices in Europe as it is manufactured and used by one of the major Italian poultry producers. The chemical composition and the ingredient profile were reported in Table 1.

In the same trial, along the rearing period, *in vivo* performance and welfare traits (rectal temperature, mortality, and incidences of foot pad dermatitis) were recorded as described by Slawinska et al. (2019c). The experimental procedures were approved by the Ministry of Health in Rome, Italy (no. 503/2016-PR).

Table 1. Composition of the diet supplied to the birds of all the experimental groups.

Item (%)	Starter (0–13 D)	Grower (14–27 D)	Finisher (28–42 D)
Corn	42.17	34.96	12.73
White corn	0.00	0.00	15.00
Wheat	10.00	20.00	25.01
Sorghum	0.00	0.00	5.00
Soybean meal	23.11	20.63	17.60
Expanded soybean	10.00	10.00	13.00
Sunflower	3.00	3.00	3.00
Corn gluten	4.00	3.00	0.00
Soybean oil	3.08	4.43	5.48
Dicalcium phosphate	1.52	1.20	0.57
Calcium carbonate	0.91	0.65	0.52
Sodium bicarbonate	0.15	0.10	0.15
Salt	0.27	0.27	0.25
Coline chloride	0.10	0.10	0.10
Lysine sulfate	0.59	0.55	0.46
DL-Methionine	0.27	0.29	0.30
Threonine	0.15	0.14	0.14
Enzyme—Roxazyme G2g	0.08	0.08	0.08
Phytase 0.1%	0.10	0.10	0.10
Coccidiostat	0	0	0
Vitamin-mineral premix ¹	0.50	0.50	0.50
DM,%	88.57	88.65	88.64
CP,%	22.70	21.49	19.74
Lipid,%	7.06	8.24	9.74
Fiber,%	3.08	3.04	3.07
Ash,%	5.85	5.17	4.49
Lysine,%	1.38	1.29	1.21
Methionine,%	0.67	0.62	0.59
Methionine+cysteine,%	1.03	0.97	0.91
Calcium,%	0.91	0.80	0.59
Phosphate,%	0.63	0.57	0.46
ME, Kcal/Kg	3.076	3.168	3.264

¹Provided the following per kg of diet: vitamin A (retinyl acetate), 13,000 IU; vitamin D3 (cholecalciferol), 4,000 IU; vitamin E (DL- α -tocopheryl acetate), 80 IU; vitamin K (menadione sodium bisulfite), 3 mg; riboflavin, 6.0 mg; pantothenic acid, 6.0 mg; niacin, 20 mg; pyridoxine, 2 mg; folic acid, 0.5 mg; biotin, 0.10 mg; thiamine, 2.5 mg; vitamin B12 20 μ g; Mn, 100 mg; Zn, 85 mg; Fe, 30 mg; Cu, 10 mg; I, 1.5 mg; Se, 0.2 mg; ethoxyquin, 100 mg.

Slaughter Surveys

At 42 D, 15 randomly chosen birds/treatment/environmental condition were individually weighed and slaughtered. Pectoral muscle (**PM**), including pectoralis major and pectoralis minor, was removed from the carcass and weighed. The pH was measured 24 h post-mortem on the upper part of the left-side breast fillet using a portable pH meter (FiveGo, Mettler-Toledo, Switzerland) equipped with a penetrating glass electrode. Tri-stimulus color coordinates (lightness, **L***; redness, **a***; yellowness, **b***) were measured 24 h post-mortem on the bone-side surface of left-side breast fillet using a Chroma Meter CR-300 (Minolta Corporation, Italia s.r.l., Milano).

Water-Holding Capacity, Cooking Loss, and Warner–Bratzler Shear Force Analyses

Water-holding capacity, expressed as expressible juice, was measured on PM 24 h after chilling using the press method (Grau and Hamm, 1953). As for cooking loss determination, PM samples were individually weighed, placed in metallic trays, and introduced in the

oven. All cooked samples (internal temperature 75°C) were drained from the excess liquid in a plastic net, then again individually weighed. Cooking loss was expressed as g/100 g by weight difference between uncooked and cooked samples. For the determination of meat tenderness, meat samples were cut into 6 cores with similar sizes; each core was sheared perpendicular to the longitudinal orientation of the muscle fiber using a Warner–Bratzler shear blade with the triangular slot cutting edge mounted on Salter model 235 (Warner–Bratzler meat shear, G-R manufacturing Co. 1317 Collins LN, Manhattan, KS) to determine the peak force (kg) when the samples were sheared. Shear force was determined as the average of the maximum force of the 6 replicates from each sample.

Nutrients Content, Fatty Acid Profile, and Cholesterol Content

Proximate composition (moisture, crude protein, total fat, and crude ash) of PM was determined following standard methods. In particular, moisture content was calculated as the percentage of weight lost after drying 5 g of sample in oven ($103 \pm 2^\circ\text{C}$ for 16 h) (AOAC, 1990). Crude protein content was assessed according to the Kjeldahl method by using copper sulfate as catalyst (AOAC, 1990), whereas lipids were extracted following the chloroform: methanol extraction procedure (Folch et al., 1957). Crude ash content was assessed by weighing samples after incineration at 525°C (AOAC, 1990). Following lipid extraction, fatty acids (**FA**) were quantified as methyl esters (**FAME**) using a gas chromatograph GC Trace 2000 (ThermoQuest EC Instruments) equipped with a flame ionization detector (260°C) and a fused silica capillary column (SGE Forte BP \times 90, Phenomenex, Torrance, CA) 100 m \times 0.25 mm \times 0.25 μm film thickness. Helium was used as carrier gas. The oven temperature program was 100°C for 5 min then increasing at 4°C/min up to 240°C where it was maintained for 20 min. The individual FA peaks were identified by comparison of retention times with those of FAME authentic standards run under the same operating conditions. Results were expressed as percentage of the total FA identified. To assess the nutritional implications, the ratio of n-6 to n-3 FA (**n-6/n-3**) and the ratio of polyunsaturated fatty acids (**PUFA**) to saturated fatty acids (**SFA**) (**P/S**) were calculated. Moreover, to evaluate the risk of atherosclerosis and the potential aggregation of blood platelets, the atherogenic index (**AI**) and the thrombogenic index (**TI**) were calculated according to the formulas suggested by Ulbricht and Southgate (1991).

Cholesterol was extracted using the method of Maraschiello et al. (1996) and then quantified by HPLC. A Kontron HPLC (Kontron Instruments, Milan, Italy) model 535, equipped with a Kinetex 5 μ C18 reverse-phase column (150 \times 4.6 mm \times 5 μm ; Phenomenex, Torrance, CA), was used. The HPLC mobile phase consisted of acetonitrile: 2-propanol (55:45, vol/vol) at a flow rate of 1.0 mL/min. The detection

Table 2. Weight and physico-chemical traits of breast muscle of broiler chickens injected in ovo with GOS in response to heat stress.

Traits	Treatment (Tr) ¹			Temperature (T) ²		SEM	Significance		
	C	S	GOS	TN	HS		Tr	T	Tr × T
Breast muscle weight, g	615.7	651.8	675.5	687.9	607.4	11.9	ns	**	ns
pH ₂₄	6.03	6.08	6.04	5.97	6.12	0.01	ns	**	ns
<i>Color 24 h</i>									
L*	52.42 ^B	55.07 ^A	54.48 ^A	54.55	53.43	0.22	**	*	**
a*	3.07	3.28	3.07	3.26	3.01	0.10	Ns	ns	**
b*	5.84 ^a	4.72 ^b	5.46 ^{a,b}	4.92	5.76	0.18	*	*	ns
WHC, %	12.57	12.70	13.24	13.11	12.57	0.15	ns	ns	ns
Cooking loss, %	19.70	19.77	19.67	19.70	19.72	0.12	ns	ns	ns
WBSF, ³ kg/cm ²	1.19	1.23	1.13	1.19	1.19	0.04	ns	ns	ns

¹C = control (untreated); S = in ovo injected with physiological saline; GOS = in ovo injected with GOS.

²TN = thermoneutral conditions; HS = heat stress conditions (on day 32 to 42).

³WBSF = Warner–Bratzler shear force.

SEM = standard error means.

Significance: ns = $P > 0.05$; * $P < 0.05$; ** $P < 0.01$.

^{A,B}Means within a row lacking a common superscript differ ($P < 0.01$).

^{a,b}Means within a row lacking a common superscript differ ($P < 0.05$).

wavelength was 210 nm. The quantitation of muscle cholesterol content was based on the external standard method using a pure cholesterol standard (Sigma, St. Louis, MO).

Intramuscular Collagen Properties

At analysis, muscle samples were thawed, at room temperature, trimmed of fat and epimysium, lyophilized for 48 h, and hydrolyzed in Duran tubes (Schott AG, Mainz, Germany) in 5 mL of 6 N HCl at 110°C for 18 to 20 h (Etherington and Sims, 1981) for determination of hydroxyproline (Woessner, 1961) and crosslinking. The analyses were carried out in duplicate. Intramuscular collagen concentration was calculated assuming that collagen weighed 7.25 times the measured hydroxyproline weight (Eastoe and Leach, 1958) and expressed as micrograms of hydroxyproline per milligram of lyophilized tissue. Hydroxylysylpyridinoline (HLP) concentration, the principal nonreducible crosslink of muscle collagen and highly correlated with the thermal stability of collagen (McCormick, 1999), was determined using the procedure described by Eyre et al. (1984). A Kontron HPLC (Kontron Instruments, Milan, Italy) model 535, equipped with a Luna C18 column (250 × 4.6 mm × 5 μm; Phenomenex, Torrance, CA), was used. The concentration of HLP residues in the samples was calculated based on the concentration of collagen in each hydrolyzate, assuming that the molecular weight of collagen was 300,000 and the molar fluorescence yield of pyridoxamine (internal standard) was 3.1 times that of HLP (Eyre et al., 1984). Crosslink concentration was expressed as moles of HLP per mole of collagen.

Statistical Analyses

Data were analyzed by GLM procedure using the SPSS statistical package (SPSS, 2010), where treatment

(GOS, S, C) and temperature (TN, HS) were the main factors. Differences among the means were determined with Scheffé's test.

RESULTS AND DISCUSSION

Breast Muscle Weight, pH, and Color

Results of the effect of GOS in ovo injected in response to heat stress on weight and physico-chemical traits of PM from broiler chickens are presented in Table 2. The PM weight was not influenced ($P > 0.05$) by GOS delivered in ovo. The heat stress that was applied for the last 10 D of the rearing cycle significantly reduced PM weight ($P < 0.01$). These results match those observed in earlier studies (Lu et al., 2007; Zhang et al., 2012; Cramer et al., 2018) which found a lower weight of the breast muscle in response to heat stress, due to heat-induced suppression of growth. On the other hand, heat stress stimulates the hypothalamic–pituitary–adrenal axis in poultry and increases the concentration of circulating corticosterone hormone (Sapolsky et al., 2000), which increments protein degradation and breakdown of skeletal muscle (Yunianto et al., 1997; Scanes, 2016).

No significant interaction ($P > 0.05$) was found between in ovo treatment and heat stress for PM weight. In accordance with our previous works (Maiorano et al., 2012; Tavaniello et al., 2018), in ovo delivery of GOS did not affect ($P > 0.05$) ultimate pH (pH₂₄) of PM, while heat stress had a significant influence on it. Meat from HS chickens had a higher ($P < 0.01$) pH compared to chickens reared under thermoneutral conditions. Similarly, Lu et al. (2007) found a higher meat pH in both fast- and slow-growing chicken strains kept at constant high ambient temperature (34°C, from 5 to 8 wk of age) compared to pair-fed chickens kept at constant optimal ambient temperature. However, several studies suggest

Table 3. Proximate composition, cholesterol content, and intramuscular collagen properties of breast muscle of broiler chickens injected in ovo with GOS in response to heat stress.

	Treatment (Tr) ¹			Temperature (T) ²		SEM	Significance		
	C	S	GOS	TN	HS		Tr	T	Tr × T
Moisture, %	74.47	74.66	74.24	74.59	74.32	0.11	ns	ns	ns
Crude protein, %	22.32	21.80	22.09	22.16	21.98	0.12	ns	ns	ns
Total lipid, %	2.70	2.85	3.09	2.66	3.10	0.08	ns	**	ns
Crude ash, %	0.90	0.93	0.90	0.90	0.92	0.01	ns	Ns	ns
Cholesterol, mg/100 g	38.25	36.98	37.61	36.86	38.87	0.54	ns	Ns	ns
Total collagen, $\mu\text{g}/\text{mg}$ ³	13.85	13.15	12.94	14.14	12.49	0.31	ns	*	ns
HLP, ⁴ mol/mol of collagen	0.040	0.039	0.042	0.040	0.041	0.001	ns	ns	ns

¹C = control (untreated); S = in ovo injected with physiological saline; GOS = in ovo injected with GOS.

²TN = thermoneutral conditions; HS = heat stress conditions (on day 32 to 42).

³Liophylized muscle tissue.

⁴HLP = hydroxylsilylpyridinoline.

SEM = standard error mean.

Significance: ns = $P > 0.05$; * $P < 0.05$; ** $P < 0.01$.

that heat stress could increase the rate of glycolysis in skeletal muscles causing a built-up of lactic acid within the muscle tissue (Zhang et al., 2012), which induces a faster pH decline with a lower ultimate pH (Zhang et al., 2012; Cramer et al., 2018; Zaboli et al., 2019). Color parameters were affected by both factors (Table 2). Meat from GOS and S chickens were lighter ($P < 0.01$) than that from C group; whereas meat from the latter group showed a higher ($P < 0.05$) yellowness index (b^*) compared to S one. No significant effect of the treatment on redness (a^*) was found. The observed color coordinates fit within the range which is accepted for good chicken meat appearance, even if lightness was slightly higher than that reported for normal meat ($46 < L^* < 53$; Bianchi et al., 2005). Temperature had also an evident effect on meat color. It has been reported that the acute heat stress can increase lightness (L^*) and reduce redness (a^*) and yellowness (b^*) of breast meat. This could be due to the denaturation of sarcoplasmic proteins which results in scattering of light (reviewed in Zhang et al., 2012). However, in the present study, meat from HS chickens was darker ($P < 0.05$) with a higher yellowness index ($P < 0.05$), compared with meat of the TN chickens. Redness index (a^*) was not affected ($P > 0.05$) by heat stress. Interactions ($P < 0.01$) between treatment and temperature were found for L^* and a^* indices: in ovo delivery of GOS increased L^* and decreased a^* indices of meat from HS animals. Neither HS nor GOS delivered in ovo had impact ($P > 0.05$) on WHC, cooking loss, and Warner–Bratzler shear force (Table 2). Our results are inconsistent with the findings by Lu et al. (2007), who observed an increase in L^* value and decrease in the WHC of meat from broilers exposed to heat stress. In a similar study, Cramer et al. (2018) did not find any significant effect of heat stress and probiotic feeding on color characteristics, WHC, and shear force of broiler breast muscle, but observed a lower cooking loss in heat-stressed chickens compared to those reared under thermoneutral conditions.

Nutrients Content, Cholesterol Content, Intramuscular Collagen Properties, and FA Profile

Regarding meat nutritional properties, there was no significant difference between treatment groups in proximate composition, cholesterol content, and intramuscular collagen properties (Table 3). To our knowledge, limited information is available in the literature on the effect of prebiotics on nutritional properties of chicken meat. Regarding collagen, Maiorano et al. (2012) found a lower intramuscular collagen content in broiler chickens in ovo injected with prebiotics and synbiotics compared to control group, probably due to a slightly greater PM weight and muscle fiber diameter. Muscle collagen maturation values found in the present study (ranging from 0.039 to 0.042 mol of HLP/mol of collagen) resulted half than those observed by Maiorano et al. (2012) for Ross 308 broiler chickens (ranging from 0.065 to 0.079 mol of HLP/mol of collagen, in control and prebiotic group, respectively), probably due to the immaturity of collagen related to the fast-growing rate of the modern chicken strain used in this trial. Lipid and cholesterol contents of meat have been of great interest for the researchers for decades. Cholesterol content has become an important component in composition studies on meat and poultry products (Dinh et al., 2011). In accordance with our findings, Tavaniello et al. (2018) did not find any significant effect of different prebiotics, in ovo injected, on muscle cholesterol content.

Heat stress only affected the breast muscle content of collagen and lipid (Table 3). Total collagen concentration was lower in HS group compared to TN one ($P < 0.05$). It can be assumed that the observed reduction could be related to the heat-induced changes in protein metabolism. In fact, it has been reported that high ambient temperature significantly decreased body protein content, protein gain, protein retain and intake, due to a decreased muscle protein synthesis and increased

Table 4. Fatty acid composition (% of total fatty acid) and nutritional indices in breast muscle of broiler chickens injected in ovo with GOS in response to heat stress.

	Treatment (Tr) ¹			Temperature (T) ²		SEM	Significance		
	C	S	GOS	TN	HS		Tr	T	Tr × T
<i>Fatty acids</i>									
C 14:0	0.40	0.45	0.45	0.37	0.50	0.26	ns	*	ns
C 14:1	0.21	0.13	0.30	0.21	0.22	0.06	ns	ns	*
C 15:0	0.46	0.48	0.53	0.36	0.63	0.08	ns	ns	***
C 16:0	24.59	24.34	27.81	26.49	24.60	1.00	ns	ns	**
C 16:1	1.69	1.72	2.53	2.00	1.95	0.17	ns	ns	***
C 17:0	0.38	0.28	0.22	0.18	0.41	0.05	ns	*	**
C 18:0	10.56	11.02	9.50	9.99	10.77	0.53	ns	ns	ns
C 18:1 n-9	19.05	18.06	19.06	19.38	18.02	0.70	ns	ns	*
C 18:2 n-6	23.34	23.97	24.40	24.94	22.78	0.74	ns	ns	**
C 18:3 n-6	0.22	0.17	0.27	0.26	0.18	0.03	ns	ns	Ns
C 18:3 n-3	1.72	1.70	1.79	1.78	1.69	0.17	ns	ns	**
C 20:0	0.20	0.13	0.16	0.15	0.18	0.03	ns	ns	**
C 20:1	0.16	0.13	0.24	0.27	0.07	0.05	ns	*	Ns
C 20:2n-6	1.28	1.48	1.17	1.27	1.36	0.07	ns	ns	**
C 20:3n-6	1.10	1.30	1.25	1.15	1.29	0.03	ns	ns	*
C 20:4 n-6	9.63 ^a	9.54 ^a	5.72 ^b	7.12	9.57	0.57	**	*	ns
C 20:5 n-3	0.51	0.51	0.48	0.39	0.62	0.04	ns	ns	ns
C 22:0	0.11 ^b	0.13 ^{a,b}	0.21 ^a	0.16	0.14	0.01	*	ns	**
C 22:1	0.09	0.14	0.10	0.06	0.16	0.03	ns	ns	**
C 22:2n-6	0.45	0.50	0.24	0.15	0.67	0.10	ns	*	**
C 22:4n-6	0.78	0.79	0.65	0.57	0.93	0.09	ns	*	***
C 22:5 n-3	1.75	1.71	1.65	1.61	1.80	0.11	ns	**	*
C 22:6 n-3	0.90	0.84	0.67	0.68	0.93	0.06	ns	*	ns
C 24:0	0.26	0.17	0.39	0.26	0.28	0.12	ns	Ns	ns
<i>Partial sum</i> ³									
ΣSFA	36.95	37.01	39.27	37.96	37.52	0.94	ns	ns	**
ΣMUFA	21.19	20.18	22.23	21.92	20.42	0.76	ns	ns	*
ΣPUFA	41.86	42.81	38.50	40.12	42.06	0.73	*	ns	ns
Σn-6	36.81	37.74	33.71	35.46	36.77	0.66	*	ns	ns
Σn-3	6.33	6.54	5.96	5.93	6.65	0.18	ns	*	ns
<i>Nutritional indices</i> ⁴									
n-6/n-3	5.94	5.88	5.69	6.02	5.63	0.14	ns	ns	*
P/S	1.17	1.17	1.03	1.09	1.15	0.04	ns	ns	*
AI	0.42	0.41	0.50	0.46	0.43	0.02	ns	ns	**
TI	0.75	0.74	0.84	0.80	0.75	0.03	ns	ns	*

¹C = control (untreated); S = in ovo injected with physiological saline; GOS = in ovo injected with GOS.

²TN = thermoneutral conditions; HS = heat stress conditions (on day 32 to 42).

³SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

⁴P/S = PUFA/SFA ratio; AI = atherogenic index; TI = thrombogenic index.

SEM = standard error mean.

Significance: ns = $P > 0.05$; * $P < 0.05$; ** $P < 0.01$.

^{a,b}Means within a row lacking a common superscript differ ($P < 0.05$).

protein catabolism (reviewed by Zhang et al., 2012). Temim et al. (2000) found that protein synthesis is more susceptible than proteolysis to high environmental temperature (32°C); furthermore, it was demonstrated that heat stress determines changes in ribosomal gene transcription lowering the protein synthesis (Jacob, 1995; Temim et al., 1998). Muscle collagen maturation (mol of HLP/mol of collagen) was not affected ($P > 0.05$) by temperature.

As for proximate composition, total lipid content was higher in HS chickens compared to C ones ($P < 0.01$). The obtained results are in accordance with those reported by Zhang et al. (2012). Exposure to high ambient temperature has been recognized as responsible of increased abdominal, subcutaneous, and intermuscular fat deposits (Ain Baziz et al., 1996; Geraert et al., 1996). The increased fat deposition could be related to

reduction in basal metabolism and physical activity in order to reduce metabolic heat production and maintain homeothermy (Geraert et al., 1996). Heat stress had no effect on muscle cholesterol content ($P > 0.05$).

Results of the effect of GOS in ovo injected in response to heat stress on FA composition of PM from broiler chickens are presented in Table 4. Taking into account the general FA profile, total PUFA were the most abundant FA (ranging from 38.50 to 42.81%), followed in descending order by SFA (ranging from 36.95 to 39.27%) and monounsaturated fatty acids (MUFA, ranging from 20.18 to 22.23%). Total SFA content was similar ($P > 0.05$) among treatment groups. Similarly, the concentration of the single SFA showed no significant difference among groups, except for C22:0 that was higher ($P < 0.05$) in GOS group compared with C group, with intermediate value in S group ($P > 0.05$).

Palmitic (C16:0) and stearic (C18:0) acids were the most abundant SFA, while other detected SFA (C14:0, C15:0, C17:0, C20:0, C22:0, and C24:0) were less than 1% each. Likewise, both the total and the single MUFA amounts were not affected ($P > 0.05$) by GOS in ovo injection. MUFA were mainly in the form of oleic acid (C18:1 n-9), ranging from 18.06 to 19.06%. Treatment significantly affected ($P < 0.05$) the total PUFA content, which was slightly lower ($P = 0.077$) in GOS group compared to S one, with intermediate values ($P > 0.05$) for C group. The same trend was found for n-6 PUFA ($P = 0.062$). The precursor of the n-6 family, the linoleic acid (C18:2), quantitatively the most concentrated n-6 PUFA (from 23.34 to 24.40%), was not affected ($P > 0.05$) by the prebiotic treatment; however, significant differences ($P < 0.01$) were observed for arachidonic acid (C20:4 n-6), which was higher ($P < 0.05$) in C and S groups as compared with GOS group. On the contrary, total n-3 PUFA and individual n-3 PUFA were not affected by GOS ($P > 0.05$). In our recent study (Tavaniello et al., 2018), we found that GOS in ovo injected increased the content of SFA and PUFA, and reduced MUFA content in breast muscle of chickens. However, it must be taken into account that FA composition of meat greatly depends on diet composition, but also on the production of short-chain FAs and their amount. The nutritional ratios (n6/n3, P/S, AI, and TI) were not affected ($P > 0.05$) by treatment. On the contrary, Tavaniello et al. (2019) found that treatment with GOS increased P/S ratio (0.88 vs. 0.83) and reduced both n-6/n-3 ratio (2.31 vs. 4.25, respectively) and AI (0.62 vs 0.73), as compared with control group. The n-6/n-3 ratio found in the present study is at a distance from the ideal value of 1 and above the recommended maximum of 4. However, based on cardiovascular considerations, the dietary advice for the adult population should be 250 mg for eicosapentaenoic acid (EPA) plus docosahexaenoic acid (DHA) (EFSA, 2017). Considering that the average lipid content of PM found in this study is about 2.9 g/100 g, and the average content of EPA+DHA is about 1.3%, the intake of these long-chain PUFA n-3 per day (38 mg/100 g) is able to satisfy 15% of the daily long-chain PUFA n-3 requirement. The P/S values observed in the present study are favorably high (ranging from 1.03 to 1.17). From a nutritional point of view, a higher P/S ratio is recommended; indeed, it should be increased to above 0.4 (Wood et al., 2004). The AI and TI represent criteria for evaluating the level and interrelation through which some FA may have atherogenic or thrombogenic properties, respectively. The low AI and TI values found in the current study revealed a good nutritional quality of the meat.

Heat stress had a marginal effect on FA composition (Table 4). Total contents of SFA, MUFA, and PUFA were similar ($P > 0.05$) between groups. Also, the composition of the single FA was slightly affected by temperature; in particular, arachidonic acid (C20:4 n-6) was higher ($P < 0.05$) in HS group compared to

TN one, as well as other long-chain PUFA of both n-3 (C22:5, C22:6) and n-6 (C22:2, C22:4) series were higher ($P < 0.01$ and 0.05) in HS group; consequently, the total content of n-3 PUFA was also higher ($P < 0.05$). Other statistically significant differences ($P < 0.05$) were found for C14:0, C17:0, C20:1, which were present in very small amount (less than 1%). The nutritional ratios (n6/n3, P/S, AI, and TI) were not affected ($P > 0.05$) by temperature. To our knowledge the information regarding the effect of heat stress on FA composition of chicken meat is limited. In a study conducted on French local broiler chicken, Ain Baziz et al. (1996) found that meat from heat-exposed birds (32°C from 4 to 7 wk old) had the same FA profile than that of control chickens with ad libitum feeding, while in pair-feeding conditions, heat-exposed birds showed a higher SFA and lower PUFA contents compared to control chickens. In general, the effect of heat exposure upon FA composition needs more deepen study. Several interactions between treatment and temperature were found for FA composition. In particular, it was found that in ovo delivery of GOS decreased ($P < 0.01$) SFA content (C: TN = 34.26%, HS = 40.33%; S: TN = 35.47%, HS = 38.94%; GOS: TN = 45.70%, HS = 34.13%) and increased ($P < 0.05$) MUFA content (C: TN = 23.95%, HS = 17.74%; S: TN = 21.73%, HS = 18.25%; GOS: TN = 19.26%, HS = 24.31%) in HS animals. Significant interactions ($P < 0.01$ and $P < 0.05$) were also found for all nutritional indices with a positive effect of GOS treatment.

In conclusion, the results of this investigation show that in ovo injection of GOS prebiotic had no negative effect on physico-chemical and nutritional properties of meat; furthermore, GOS could contrast the negative effect of heat exposure on FA composition, with positive effect from the nutritional point of view.

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