

# Performance and meat quality traits of slow-growing chickens stimulated in ovo with galactooligosaccharides and exposed to heat stress

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**ABSTRACT** In vivo performance, carcass and meat quality traits of slow-growing chickens stimulated in ovo with trans galactooligosaccharides (GOS) and exposed to heat stress were evaluated. On d 12 of egg incubation, 3,000 fertilized eggs (Hubbard JA57) were divided into prebiotic group (GOS) injected with 3.5 mg GOS/egg, saline group (S) injected with physiological saline (only to assess the hatchability rate) and an uninjected control group (C). After hatching, 600 male chicks (300 from GOS and 300 from C) were housed on floor pens (6 pens/treatment, 25 birds/pen) and reared under neutral (TN) or heat stress conditions (HS, 30°C from 36 to 50 d). BW, daily feed intake (DFI), daily weight gain (DWG), feed conversion rate (FCR), and mortality were measured. At 50 d of age, 15 randomly selected birds/treatment/environmental conditions were slaughtered and the pectoral muscle (PM) was collected for analyses. Hatchability was similar among groups. BW of the

newly hatched chicks was lower ( $P < 0.01$ ) in GOS compared to C. Final BW, DWG, DFI, and FCR were not affected ( $P > 0.05$ ) by GOS. HS reduced final BW ( $-12.93\%$ ,  $P < 0.001$ ). During finisher phase, DFI and DWG were lower ( $P < 0.001$ ) and FCR was higher ( $P < 0.01$ ) in HS compared to TN. Mortality was not affected ( $P > 0.05$ ) by GOS and HS. Meat from GOS chickens had a higher ( $P < 0.01$ ) pH and was darker ( $P < 0.05$ ) compared to C. Proximate composition, cholesterol content, fatty acid profile, and intramuscular collagen properties of PM were not affected by GOS. The HS group showed a lower ( $P < 0.05$ ) content of both collagen and monounsaturated fatty acids than TN group. Significant interactions between GOS and temperature were found for FA composition. In conclusion, the differences in performance have had an impact on the responses to HS in Hubbard chickens, but not on mortality rate. GOS did not relieve the negative effect of HS on chickens' performance.

**Key words:** slow-growing broiler, in ovo stimulation, heat stress, performance, meat quality

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## INTRODUCTION

Poultry provides the main protein resources, meat and eggs, for humans in most parts of the world. However, high environmental temperature (heat stress, HS), one of the most relevant stressors, is affecting poultry production worldwide (Wasti et al., 2020). Also, in temperate regions of the world the high ambient temperature during the summer season often proves disastrous

for poultry farming as thermal stress induced by extremely high temperatures is responsible for massive economic losses to poultry industry (Nawaz et al., 2021).

Due to an impressive improvement in the genetic background over the past decades, modern hybrid chickens have a higher metabolism rate and production performance, and are more prone to heat stress (Tallentire et al., 2016). Furthermore, the high stocking density of birds also increases the propensity for thermal stress (Goo et al., 2019). The HS results from a negative balance between the net amount of energy flowing from the animal to its surrounding ambient and the amount of heat energy produced by the animal. Intense and prolonged HS could induce unfavorable changes in indigenous bacterial microbiota, which puts direct pressure on the integrity of the intestine; furthermore, HS causes

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adverse consequences for the whole body leading to high mortality rates (Slawinska et al., 2020). HS is associated with a higher production of reactive oxygen species with consequent damage to DNA, proteins and lipids (Wasti et al., 2020). Recent studies on commercial hybrid broiler chickens reared in intensive systems under heat stress condition have shown that HS can cause a faster pH drop and a pale color of breast meat (Baghban Kanani et al., 2017). Other studies found that meat from heat-stressed birds have higher pH values (Goo et al., 2019), lower protein content, higher fat deposits and altered fatty acid composition (Imik et al., 2012; Tavaniello et al., 2020). It has been estimated that the U.S. livestock production industry suffers severe losses, ranging from \$1.69 to \$2.36 billion/yr, because of high environmental temperature, out of which the poultry industry accounts for \$128 to 165 million/yr (St-Pierre et al., 2003).

There are several approaches to mitigate heat stress exposure of poultry, including the adaptation of the ventilation system, genetic breeding and dietary interventions (Wasti et al., 2020; Goel, 2021). The latter can help poultry to cope with heat stress, with 2 objectives: 1) reducing diet-induced thermogenesis by selecting nutrients with low heat-increment potential; 2) provide birds with specific bioactive compounds that correct the physiological dysfunctions induced by heat stress. Feed additives, such as probiotics, prebiotics and synbiotics, have been proposed as a nutritional strategy to improve the resilience of animals against heat stress. In our recent studies, conducted on modern commercial hybrids, which are more sensitive to stress conditions, we demonstrated that prebiotics (Galactooligosaccharides, **GOS**) delivered in ovo mitigated the negative effects of heat stress on production performance and welfare, as shown by improved feed conversion rate and growth efficiency, reduced body temperature and slightly improved survival rate; as well as GOS mitigated the detrimental effect of heat stress on some meat quality traits (Slawinska et al., 2020; Tavaniello et al., 2020). GOS is one of the three prebiotics that meets the criteria of the European Union and with a wide range of uses nowadays (Kolida and Gibson, 2011). In ovo is one of the precise animal husbandry tools in poultry production. The injection of prebiotics or synbiotics into the egg's air chamber can provide chicks with a beneficial bacterial profile at hatch (Bednarczyk et al., 2016), triggering the development of lifelong phenotypes during the embryonic growth (such as immunity, gut microbiota, performance, adaptation) (Siwek et al., 2018; Stefaniak et al., 2020). We believe that different chicken genotypes as fast-growing and slow-growing broilers could respond differently to GOS treatment and heat stress condition (Slawinska et al., 2019a,b; Pietrzak et al., 2020). Moreover, to the best of our knowledge, there is no prior study that has examined the effect of in ovo stimulation with GOS prebiotic on performance and meat quality traits of slow-growing chickens under heat stress condition reared indoor. Therefore, the present study aims to analyze the effects

of GOS delivered in ovo on in vivo performance, carcass traits and meat quality in slow-growing chickens exposed to heat stress.

## MATERIALS AND METHODS

### *In Ovo Treatment*

Fertilized eggs of slow-growing crossbred Hubbard chickens were incubated in a commercial hatchery. Before the start of incubation, eggs were warmed linearly in 14 h from storage temperature (20°C) to an egg-shell temperature of 37.8°C. The moment the eggs reached this temperature was considered to be the start of incubation. At incubation d 0, the temperature in the incubator was set to 37.9 ± 0.1°C and decreased continuously over 18 d to 37.1 ± 0.1°C, while relative humidity was maintained between 55 and 65%, and CO<sub>2</sub> level was 3,500 ppm. In the hatcher, the temperature on day 19 was 36.8 ± 0.1°C and it dropped to 36.4 ± 0.1°C at hatch, while relative humidity was set at 70 to 75% and CO<sub>2</sub> level at 4,000 ppm. Eggs were turned every hour by an angle of 90° from the start of incubation until d 18.

On d 12 of incubation, prior to injection, the eggs were candled and those unfertilized or with dead embryos were discarded. A total of 3,000 eggs were randomly and equally divided into 3 experimental groups: control group (**C**) uninjected; saline group (**S**) injected with 0.2 mL of physiological saline (0.9% NaCl); prebiotic group (**GOS**) injected with a single dose of 3.5 mg GOS/egg dissolved in 0.2 mL of physiological saline. It was proven that prebiotics stimulate native microflora from d 12 to 18 of egg incubation (Siwek et al., 2018), for this reason the injection was performed at d 12 of incubation according to the procedure described by Slawinska et al. (2019a). The composition and the injection dose of GOS (trade name: Bi2tos, Clasado Biosciences Ltd., Jersey, UK) is described by Tavaniello et al. (2020). Hatchability was measured as proportion of hatched chicks to the number of fertile eggs, candled at 12th d of incubation. Saline group was considered only for the assessment of the hatchability rate.

### *Animal Management*

After hatching, all the chicks were sexed and vaccinated according to the current commercial practice (coccidiosis, infectious bronchitis, Marek's, Newcastle, and Gumboro diseases). A total of 600 males were randomly chosen, 300 from C group and 300 from GOS group and allocated in 24 concrete floor pens (3.3 m<sup>2</sup> each), equally divided in 4 rooms (6 pens/room) presenting identical features (e.g., pens disposition and characteristics, artificial lighting, ventilation systems, etc.). The only difference between the 2 rooms was the presence of an electrical heating system, which was used to increase the environmental temperature during the thermal challenge. Each pen was equipped with one circular pan feeder, able to ensure a minimum of 2 cm of front space/

bird, and 5 nipples. Wood shaving was used as litter material (3–4 kg/m<sup>2</sup>). Stocking density (maximum 33 kg/m<sup>2</sup>) and photoperiod (23L:1D during 0–7 and 48–50 d; and 18L:6D from 8 to 47 d) were in compliance with the EU legislation, specifically with the Directive 2007/43/EC for the protection of chickens kept for meat production. Birds allocated in the 2 rooms were raised in thermoneutral conditions for the entire rearing cycle (0–50 d) (TN), with environmental temperature defined according to the age of the birds following the recommendations of the breeding company (i.e., 0 d: 30°C; 3 d: 28°C; 6 d: 27°C; 9 d: 26°C; 12 d: 25°C; 15 d: 24°C; 18 d: 23°C; 21 d: 22°C; 24 d: 21°C; 27 d-onwards: 20°C). The birds housed in the other 2 rooms were raised in similar environmental conditions to those belonging to the TN group until 35 d and then exposed to chronic heat stress conditions (HS). Chickens were randomly split in 4 groups according to treatment (C and GOS) and environmental temperature (TN and HS) of 6 replicate pens/group, 25 birds/pen, 10 birds/m<sup>2</sup>. Heat stress was induced on d 36 by increasing environmental temperature to 30°C and maintained for 14 consecutive days to mimic a constant chronic HS. Animals were fed ad libitum with commercial diets according to the age: starter (d 1–14), grower (d 15–36), and finisher (d 37–50). The basal diet was formulated to meet the dietary requirements of the broiler genotype used for the trial according to the nutritional recommendations provided by the breeding company (Aviagen Group, Huntsville, AL). The chemical composition and the ingredient profile of the diets is reported in Table 1. Animals had free

access to water. Performance parameters (BW; feed intake) were recorded on pen basis on d 0, 14, 36, and 50 of age. Daily weight gain (DWG), daily feed intake (DFI), and feed conversion rate corrected for mortality (FCR) were calculated. FCR was estimated as the ratio of FI to DWG. Mortality was recorded daily on d 0 to 50 and expressed as percentage. All the aspects related to handling, processing, and raising of the birds strictly accomplished with the European legislation (European Commission, 2007, 2009, 2010). The experimental procedures were approved by the Ministry of Health in Rome, Italy (no. 503/2016-PR).

## Slaughter Surveys

After a night of fasting, at 50 d of age, 15 birds/treatment/environmental condition randomly chosen were individually weighed, labeled, and slaughtered in a commercial slaughterhouse. Pectoral muscle (PM), including the pectoralis major and pectoralis minor, was removed from the carcasses and weighed. The pH was measured 24-h postmortem 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. Twenty-four hours postmortem, tristimulus color coordinates (lightness, L\*; redness, a\*; yellowness, b\*) were measured on the bone-side surface of left-side breast fillet using a Chroma Meter CR-300 (Konica Minolta B. S. Italia Spa, Milan, Italy). Water holding capacity (WHC) and shear force of meat were measured on the right PM 24 h after chilling. Part of the right PM was vacuum packaged and stored frozen (–20°C) until chemical analysis (proximate composition, fatty acids, cholesterol, and collagen content).

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

Item (%)	Period		
	Starter (0–14 d)	Grower (15–36 d)	Finisher (37–50 d)
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			
Vit-min premix <sup>1</sup>	0.50	0.50	0.50
Dry matter			
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

<sup>1</sup>Provided the following per kg of diet: vitamin A (retinyl acetate), 13,000 IU; vitamin D3 (cholecalciferol), 4,000 IU; vitamin E (DL- $\alpha$  tocopheryl acetate), 80 IU; vitamin K (menadione sodium bisulfite), 3 mg; riboflavin, 6 mg; panthothenic acid, 6 mg; niacin, 20 mg; pyridoxine, 2 mg; folic acid, 0.5 mg; biotin, 0.10 mg; thiamine, 2.5 mg; vitamin B12 20  $\mu$ g; Mn, 100 mg; Zn, 85 mg; Fe, 30 mg; Cu, 10 mg; I, 1.5 mg; Se, 0.2 mg; ethoxyquin, 100 mg.

## Water Holding Capacity and Warner-Bratzler Shear Force

WHC was measured using the press method (Grau and Hamm, 1953) and was expressed as expressible juice. As for the determination of cooking loss, the PM samples were weighed, placed in a metal tray, and then oven cooked until reaching a core temperature of 75°C. All cooked samples 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 the uncooked and cooked samples. For the determination of 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.



## Proximate Composition

Proximate composition (moisture, crude protein, total fat, and crude ash) of PM was determined following standard methods. The moisture content was calculated as the weight percentage lost after drying a 5 g sample in oven ( $103 \pm 2^\circ\text{C}$  for 16 h) (AOAC, 1990). The crude protein content was evaluated according to the Kjeldahl method (AOAC, 1990), lipids were extracted according to a chloroform: methanol extraction procedure (Folch et al., 1957). Crude ash was evaluated by weighing samples after incineration at  $525^\circ\text{C}$  (AOAC, 1990).

## Fatty Acids Profile

Fatty acids (FA) were quantified as methyl esters (FAME) using a gas chromatograph GC Trace 2000 (ThermoQuest EC Instruments, Milan, Italy) equipped with a flame ionization detector ( $260^\circ\text{C}$ ) and a fused silica capillary column (Zebron ZB-88, Phenomenex, Torrance, CA)  $100\text{ m} \times 0.25\text{ mm} \times 0.20\ \mu\text{m}$  film thickness. Helium was used as a carrier gas. The column temperature was held at  $100^\circ\text{C}$  for 5 min, then raised  $4^\circ\text{C}/\text{min}$  up to  $240^\circ\text{C}$  and maintained for 20 min. The individual fatty acids peaks were identified by comparison of retention times with those of known mixtures of standard fatty acids (37 Component FAME MIX and docosapentaenoic acid (cis-7,10,13,16,19), Supelco, Bellefonte, PA) 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 PUFA to n-3 PUFA (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 Content

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  $5\ \mu\text{m}$  C18 reverse-phase column ( $150\text{ cm} \times 4.6\text{ mm} \times 5\ \mu\text{m}$ ; Phenomenex), 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 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

Lyophilized PM samples were hydrolysed in 6N HCl at  $110^\circ\text{C}$  for 18 to 20 h for determination of hydroxyproline (Woessner, 1961). Intramuscular collagen

concentration was calculated, assuming that collagen weighed 7.25 times the measured hydroxyproline weight and expressed as micrograms of hydroxyproline per milligram of lyophilized tissue. The concentration of hydroxylysylpyridinoline (HLP), which is the major non-reducible cross-link of muscle collagen and highly correlated with the thermal stability of collagen (McCormick, 1999), was determined by the method described by (Eyre et al., 1984). A 535 Kontron HPLC (Kontron Instruments) equipped with a Luna C18 column ( $250 \times 4.6\text{ mm} \times 5\ \mu\text{m}$ ; Phenomenex) was used. The concentration of HLP residues in the sample was calculated based on the concentration of collagen in each hydrolysate, assuming that the molecular weight of collagen is 300,000 and the molar fluorescence yield of pyridoxamine (internal standard) is 3.1 times that of HLP (Eyre et al., 1984). Crosslinking concentration was expressed as moles of HLP per mole of collagen.

## Statistical Analyses

Data were analyzed by ANOVA in a  $2 \times 2$  factorial design (SPSS, 2010). The model included in ovo injection (C, GOS) and ambient temperature condition (TN, HS) as factors. Pen was considered as a biological replicate ( $n = 6$ ). For meat analyses each individual bird was considered as an experimental unit.

## RESULTS AND DISCUSSION

### Hatchability

Hatchability resulted similar among experimental groups, ranging from 92.6 to 91.8%, respectively for C and GOS, with intermediate values for S group (92.1%). Similar results were obtained by in ovo injection of GOS on Ross 308 eggs (Slawinska et al., 2020).

### In Vivo Performance

The effects of prebiotic in ovo stimulation and ambient temperature on overall performance results of the slow-growing broiler chickens are presented in Table 2. BW of the newly hatched chicks were significantly lower ( $P < 0.01$ ) in GOS compared to C. However, such negative effect of in ovo treatment was temporary and did not influence the subsequent stages of growth ( $P > 0.05$ ). Final BW, DWG, DFI, FCR and cumulative mortality were not affected ( $P > 0.05$ ) by prebiotic treatment. Similar results were found in our recent study (Slawinska et al., 2020), carried out with Ross 308 broiler chickens, in starter and grower phases, while in the finisher phase, birds in ovo injected with GOS were heavier and showed a lower FCR (1.65 %) compared to the control. The authors suggested that the improvement of growth performance can be explained with a better digestive ability due to the increased activity of the pancreatic digestive enzymes (amylase, lipase, trypsin) in birds in ovo injected with GOS.

**Table 2.** Productive performance of slow-growing broiler chickens injected in ovo with GOS in response to heat stress.

	Treatment (Tr) <sup>1</sup>		Temperature (T) <sup>2</sup>		SEM	Significance		
	C	GOS	TN	HS		Tr	T	TrxT
Chick body weight (g, d 0)	40.7	39.8	39.9	40.6	0.1	**	*	NS
Final body weight (g, d 50)	2,041	1,996	2,158	1,879	37.9	NS	***	NS
Daily weight gain (g/bird/d)								
d 0–14	16.4	16.9	16.8	16.6	0.15	NS	NS	NS
d 15–36	46.8	45.0	46.2	45.5	0.8	NS	NS	NS
d 37–50	52.8	52.4	62.0	43.2	1.7	NS	***	NS
d 0–50	40.0	39.1	42.4	36.8	0.8	NS	**	NS
Daily feed intake (g/bird/d)								
d 0–14	26.7	26.9	26.4	27.3	0.3	NS	NS	NS
d 15–36	88.3	86.8	88.1	87.0	0.6	NS	NS	NS
d 37–50	140.0	137.2	154.6	122.6	2.2	NS	***	NS
d 0–50	82.8	81.5	86.4	77.9	0.8	NS	***	NS
FCR <sup>3</sup>								
d 0–14	1.63	1.59	1.57	1.65	0.01	NS	**	NS
d 15–36	1.90	1.94	1.91	1.92	0.03	NS	NS	NS
d 37–50	2.72	2.69	2.52	2.89	0.06	NS	**	NS
d 0–50	2.28	2.30	2.25	2.33	0.04	NS	NS	NS
Mortality (%)								
d 0–14	0.58	0.86	0.29	1.15	0.30	NS	NS	NS
d 15–36	0.63	0.62	0.93	0.32	0.30	NS	NS	NS
d 37–50	0.62	0.00	0.31	0.31	0.22	NS	NS	NS
d 0–50	1.73	1.44	1.44	1.73	0.43	NS	NS	NS

<sup>1</sup>C = Control (untreated); GOS = in ovo injected with GOS.

<sup>2</sup>TN = thermoneutral conditions; HS = heat stress conditions (on d 36–50).

<sup>3</sup>Abbreviation: FCR, feed conversion ratio. Significance: NS =  $P > 0.05$ ; \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ .

Thermal challenge was applied for the last 14 d of rearing, during the finisher feeding phase. Heat stress reduced final BW ( $-12.93\%$   $P < 0.001$ ) and increased FCR in finisher phases ( $+12.8\%$ ;  $P < 0.01$ ). The DWG in HS conditions was reduced by as much as 18.8 g compared to the TN group ( $P < 0.001$ ). Loss in growth efficiency during HS could be also explained by reduction ( $P < 0.001$ ) in DFI in HS group ( $-32$  g) vs. TN group. We did not find statistical evidence whether GOS injected in ovo relieved the negative effect of HS on BW of chickens (interaction:  $P > 0.05$ ), but there was a minor numerical reduction in BW on d 50 in HS conditions in GOS vs. C (40.0g = 2% in GOS and 49.32g = 2.3% in C). Lower growth performance of chickens in hot conditions are well documented in literature (Lara and Rostagno, 2013; Goo et al., 2019). The growth rate in broilers mainly depends on the amount of feed intake (Awad et al., 2018). In the present work, feed consumption and efficiency decreased in response to HS. In HS conditions animals reduce feed ingestion but also increase energy expenditure for thermoregulation, this latter due to physiological and anatomical mechanisms (Wasti et al., 2020). Both mechanisms of metabolic heat reduction and energy expenditure by birds could be the cause of the decreased growth performance in Hubbard chickens under chronic HS. The lower feeding efficiency observed in the early stage (1–14 d) in birds of the HS group compared to those of the TN group ( $P < 0.01$ ) may be due to a higher starting body weight (40.6 g vs. 39.9 g, respectively;  $P < 0.05$ ) and slightly higher DFI ( $P = 0.08$ ). Mortality was not affected ( $P > 0.05$ ) by HS, indicating that Hubbard chickens are resilient to handle higher temperature. Differently, fast-growing broiler chickens are not adjusted to handle high temperatures due to inadequacy of

internal organs at the body dimension that can be twice as large as slow-growing ones. However, the resistance to heat stress in relation to different breeds has been widely investigated (Soleimani et al. 2011; Awad et al., 2020).

### Postmortem Performance

Results regarding the effects of in ovo injection of GOS in response to HS on BW, carcass traits and physico-chemical properties of breast muscle are reported in Table 3. Carcass traits, such as breast weight and breast yield, are of economic importance in meat production. GOS had no influence ( $P > 0.05$ ) on BW of slaughtered birds. In addition, GOS did not affect ( $P > 0.05$ ) carcass and PM yields. These results are consistent with those reported by Tavaniello et al. (2020) in Ross 308 broiler chickens treated with GOS in ovo injected. Conversely, in our previous works we found an improvement of carcass and breast yield in fast-growing chickens treated with different prebiotics in ovo injected (Maiorano et al., 2017; Tavaniello et al., 2018). As expected, chickens from HS groups were lighter ( $P < 0.01$ ) with a lower ( $P < 0.05$ ) carcass yield ( $-1.3\%$ ) and PM yield ( $-1.4\%$ ) compared to those reared under TN conditions (Table 3). Similar results were found by Goo et al. (2019) and Ma et al. (2021). In contrast with our results, in a study conducted on China local slow-growing chickens (Beijing You chicken), Lu et al. (2007) didn't find any detrimental effect of constant high ambient temperature (34°C, from 5 to 8 wk of age) on carcass traits (weights of carcass, breast, and leg). Other studies showed that chronic heat stress markedly reduced the proportion of pectoral muscles in broilers (Cramer et al.,

**Table 3.** Carcass traits and physico-chemical properties of breast muscle of slow-growing broiler chickens injected in ovo with GOS in response to heat stress.

	Treatment (Tr) <sup>1</sup>		Temperature (T) <sup>2</sup>		SEM	Significance		
	C	GOS	TN	HS		Tr	T	TrxT
Final body weight (g)	2,035	2,010	2,160	1,890	44.0	NS	**	NS
Carcass yield (%)	74.6	74.2	75.2	73.9	0.34	NS	*	NS
Breast yield (%)	20.9	20.7	21.4	20.4	0.23	NS	*	NS
pH <sub>24</sub>	5.75	5.83	5.81	5.77	0.01	**	NS	NS
Color 24 h								
L*	54.03	52.34	52.62	53.75	0.39	*	NS	NS
a*	1.56	2.09	2.14	1.51	0.12	NS	*	NS
b*	16.80	16.22	16.91	16.11	0.34	NS	NS	NS
WHC (%)	11.98	12.02	12.16	11.86	0.11	NS	NS	NS
Cooking loss (%)	22.22	22.31	22.27	22.27	0.12	NS	NS	NS
WBSF <sup>3</sup> (Kg)	1.57	1.65	1.62	1.57	0.05	NS	NS	NS

<sup>1</sup>C = Control (untreated); GOS = in ovo injected with GOS.

<sup>2</sup>TN = thermoneutral conditions; HS = heat stress conditions (on d 36–50).

<sup>3</sup>Abbreviation: WBSF, Warner-Bratzler shear force. Significance: NS =  $P > 0.05$ ; \*  $P < 0.05$ ; \*\*  $P < 0.01$ .

2018; Tavaniello et al., 2020). On the other hand, heat stress stimulates the hypothalamic–pituitary–adrenal axis in poultry and increases in circulating corticosterone hormone (Sapolsky et al., 2000), this would likely increase catabolism of skeletal muscle contributing to reduced body growth (Scanes, 2016; Beckford et al., 2020).

### Meat Physico-chemical Properties

Differently from our previous works (Maiorano et al., 2012; Tavaniello et al., 2018, 2020), in ovo delivery of GOS affected ultimate pH (pH<sub>24</sub>) of PM, while HS had no significant influence on it (Table 3). Meat from GOS chickens had a higher ( $P < 0.01$ ) pH compared to C chickens. In contrast to our results, some works found a higher meat pH in birds exposed to high ambient temperature (Lu et al., 2007; Goo et al., 2019; Tavaniello et al., 2020), while Awad et al. (2020) reported lower meat pH in chickens reared under HS. However, the variability of the effects reported for GOS or HS may be explained by different genetic backgrounds, slaughtering age, and the duration of the heat stress treatments applied. The pH value is one of the most important physical parameters of meat. It has a central role in determining the protein behavior both in fresh and processed meat products, in fact it is used as a predictor of meat technological and sensory quality (Fletcher, 1999; Van Laack et al., 2000). However, the ultimate pH values found in the present study can be considered normal values for breast muscles in broiler chickens (Maiorano et al., 2012).

In fresh meat, color is the first sensory attribute for consumers to evaluate the quality of meat and it is used as a purchase criterion. Meat from GOS chickens was darker ( $P < 0.05$ ) than that from C group. The pH of the meat seems to have a strong influence on the color of the meat, with higher pH values resulting in a darker meat color (Fletcher, 1999). No significant effects of the treatment on a\* and b\* values were found. The observed color coordinates (L\*, a\*, b\*) fit within the range which

is accepted for good chicken meat appearance, even if the L\* value in the C group (54.03) was slightly higher than that reported for normal meat (Table 3). Meat with L\* values (degree of paleness) higher than 54 is considered light and tends to be pale, soft, exudative meat (Woelfel et al., 2002). However, we could exclude the presence of defects in meat of C group because pH value was in the range for normal meat (5.74). Temperature also had a slight effect on meat color. Literature reported that the acute heat stress can increase lightness and reduce redness and yellowness of breast, due to the denaturation of sarcoplasmic proteins (Zhang et al., 2012). Nevertheless, other studies on Cobb (Goo et al., 2019) and local slow-growing chickens (Lu et al., 2007) reported no effect of heat stress on meat color. In the present study, meat from HS chickens had a lower redness index ( $P < 0.05$ ) compared with TN chickens, indicating probably a more oxidized myoglobin in the heat-exposed birds' muscle. Lightness and yellowness were not affected by HS conditions ( $P > 0.05$ ).

WHC, cooking loss, and Warner–Bratzler shear force were not affected (Cramer et al., 2018;  $P < 0.05$ ) by GOS and HS (Table 3). These findings agree with those of our previous experiment on fast-growing chickens (Tavaniello et al., 2020). Cramer et al. (2018) reported no significant effect of probiotic feeding and heat stress on WHC and shear force of breast muscle, while found a lower cooking loss in meat from chickens reared under heat-stressed compared to those reared under thermoneutral conditions. Other previous works reported a decrease in the WHC of meat from broilers exposed to heat stress (Lu et al., 2007) or no significant effect of heat stress on drip loss (Goo et al., 2019; Awad et al., 2020), but a higher shear force of broiler breast muscle reared in HS condition (Awad et al., 2020).

### Chemical Composition

Proximate composition, cholesterol content and intramuscular collagen properties of PM were not affected by

**Table 4.** Proximate composition, cholesterol content and intramuscular collagen properties of breast muscle of slow-growing broiler chickens injected in ovo with GOS in response to heat stress.

	Treatment (Tr) <sup>1</sup>		Temperature (T) <sup>2</sup>		SEM	Significance		
	C	GOS	TN	HS		Tr	T	TrxT
Moisture (%)	72.43	72.51	72.29	72.76	0.07	NS	NS	NS
Protein (%)	24.74	24.51	24.73	24.40	0.07	NS	NS	NS
Lipid (%)	2.43	2.29	2.32	2.39	0.05	NS	NS	NS
Ash (%)	0.81	0.83	0.81	0.84	0.01	NS	NS	NS
Cholesterol (mg/100 g)	37.42	37.72	38.29	36.85	0.69	NS	NS	NS
Total collagen ( $\mu\text{g}/\text{mg}$ <sup>3</sup> )	17.56	17.02	18.42	16.18	0.24	NS	*	NS
HLP <sup>4</sup> (mol/mol of collagen)	0.070	0.069	0.071	0.067	0.001	NS	NS	NS

<sup>1</sup>C = Control (untreated); GOS = in ovo injected with GOS.

<sup>2</sup>TN = thermoneutral conditions; HS = heat stress conditions (on d 36–50).

<sup>3</sup>liophilized muscle tissue.

<sup>4</sup>Abbreviation: HLP, hydroxylysylpyridinoline. Significance: NS =  $P > 0.05$ ; \*  $P < 0.05$ .

GOS (Table 4). Our results are consistent with the findings by Tavaniello et al. (2020) on fast-growing chickens.

Muscle collagen amount and collagen maturation (mol of HLP/mol of collagen) found in the present study were higher with respect to those reported by Tavaniello et al. (2020) in breast muscle of Ross 308 chickens (collagen: ranging from 12.94 to 13.85  $\mu\text{g}/\text{mg}$ ; HLP: ranging from 0.039 to 0.042 mol of HLP/mol of collagen), probably due to the higher maturity of collagen related to the slow-growing rate of the chicken strain used in this trial (Hubbard) compared with fast-growing chickens (Ross 308) used in the work of Tavaniello and co-authors. Indeed, several studies (McCormick, 1994; Harper, 1999; Maiorano et al., 2001) documented growth rate-dependent shifts in muscle collagen amount and crosslinking. During rapid growth (e.g., in fast-growing genotypes), newly synthesized collagen dilutes older collagen and is less crosslinked than the pre-existing collagen, with a positive effect on meat tenderness (McCormick, 1994).

The HS group showed a lower collagen content ( $-12.2\%$ ;  $P < 0.05$ ) than the TN group (Table 4). The reduction of the collagen could be related to the heat-induced changes in protein metabolism. Several researches (reviewed by Zhang et al., 2012) suggested that a high ambient temperature can reduce body protein content, protein gain, protein retain, and intake, due to a reduction of muscle protein synthesis and an increase of protein catabolism. On the other hand, protein synthesis is more susceptible than proteolysis to high environmental temperature ( $32^\circ\text{C}$ ; Temim et al., 2000). Furthermore, HS increases the level of circulating corticosterone and consequently causes the breakdown of muscle proteins to provide amino acid substrates for hepatic gluconeogenesis responsible for energy supply (Ma et al., 2021). HS did not affect ( $P > 0.05$ ) muscle collagen maturation (mol of HLP/mol of collagen).

HS did not affect ( $P > 0.05$ ) meat proximate composition (Table 4). It has been observed in fast-growing broiler exposed to chronic heat an increase of abdominal, subcutaneous, intramuscular fat deposition, and as well as in the liver (De Antonio et al., 2017; Lu et al., 2019; Tavaniello et al., 2020). It has been suggested that the

increase in fat deposition could be related to the reduction of basal metabolism and physical activity, this mechanism allows to reduce the production of metabolic heat and to maintain homeothermia (Geraert et al., 1996). However, increasing evidence indicates that much of the variation in response to heat stress is apparently genetically based (Soleimani et al., 2011; Felver-Gant et al., 2012; Mack et al., 2013). By comparing the results of the present study with those conducted on Ross 308 broiler chickens (Tavaniello et al., 2020), we can assume that slow-growing chickens may not reduce basal metabolism and physical activity due to high temperatures, and thus they may be more able to adapt to changes caused by heat stress as compared to fast-growing chickens. These outcomes are consistent with those of Lu et al. (2007) who found higher resistance to high ambient temperature in slow-growing chickens.

### Fatty Acid Profile

Results of the effect of GOS in ovo delivered in response to heat stress on FA composition of PM from broiler chickens are presented in Table 5. Total SFA, PUFA and MUFA content and the concentration of the single acids were similar ( $P > 0.05$ ) among treatment groups. The same trend ( $P > 0.05$ ) was found for the total amount of n-3 PUFA and n-6 PUFA, as well as for calculated nutritional ratios (n-6/n-3, P/S, AI and TI). These results contrast with the findings of our previous works carried out on Ross 308 chickens. In particular, Tavaniello et al. (2018) found that GOS in ovo injected increased the content of SFA and PUFA and reduced the MUFA content in breast muscle of chickens; in addition, better nutritional indices were found in GOS group. In a later study, Tavaniello et al. (2020) found that only the total PUFA and n-6 PUFA contents were slightly lower in breast muscle from GOS group compared to control. A possible explanation of the different effect of GOS on FA profile could be due to the different bird genotype used, fast-growing bird in the researches of Tavaniello and co-authors and slow-growing bird in the present experiment. However, it must be taken into account that FA composition of meat greatly depends



**Table 5.** Fatty acid composition (% of total fatty acids) and nutritional indices in breast muscle of slow-growing broiler chickens injected in ovo with GOS in response to heat stress.

	Treatment (Tr) <sup>1</sup>		Temperature (T) <sup>2</sup>		SEM	Significance		
	C	GOS	TN	HS		Tr	T	TrxT
Fatty acids <sup>3</sup>								
C14:0	0.39	0.36	0.34	0.40	0.02	NS	NS	NS
C14:1	0.06	0.05	0.06	0.05	0.01	NS	NS	NS
C15:0	0.08	0.07	0.07	0.07	0.01	NS	NS	NS
C16:0	27.14	26.89	27.432	26.60	0.30	NS	NS	NS
C16:1	1.93	1.90	2.04	1.91	0.08	NS	NS	NS
C17:0	0.17	0.16	0.15	0.18	0.01	NS	NS	NS
C18:0	8.65	7.76	7.11	9.30	0.47	NS	*	NS
C18:1 n-9	25.11	24.92	26.38	23.65	0.54	NS	*	NS
C18:2 n-6	23.93	23.78	23.24	24.48	0.27	NS	*	**
C18:3 n-6	0.13	0.12	0.13	0.12	0.00	NS	NS	NS
C18:3 n-3	1.22	1.13	1.18	1.17	0.04	NS	NS	NS
C20:0	0.11	0.11	0.10	0.11	0.01	NS	NS	NS
C20:1	0.20	0.20	0.21	0.19	0.00	NS	NS	NS
C20:2n-6	0.64	0.68	0.62	0.69	0.03	NS	NS	NS
C20:3 n-6	0.92	1.00	0.94	0.98	0.03	NS	NS	*
C20:3 n-3	0.09	0.11	0.08	0.12	0.01	NS	NS	NS
C20:4 n-6	7.29	7.71	7.58	7.42	0.25	NS	NS	NS
C20:5 n-3	0.29	0.32	0.32	0.29	0.02	NS	NS	NS
C22:0	0.16	0.17	0.16	0.16	0.01	NS	NS	NS
C22:1	0.02	0.03	0.02	0.04	0.01	NS	*	NS
C22:2n-6	0.05	0.03	0.03	0.05	0.01	NS	NS	NS
C22:4n-6	0.41	0.47	0.43	0.45	0.02	NS	NS	NS
C22:5 n-3	1.01	1.14	1.08	1.07	0.04	NS	NS	*
C22:6 n-3	0.69	0.77	0.74	0.72	0.03	NS	NS	NS
C24:0	0.07	0.06	0.05	0.08	0.02	NS	NS	NS
ΣSFA	36.76	35.57	35.42	36.90	0.53	NS	NS	NS
ΣMUFA	27.31	27.10	28.70	25.72	0.60	NS	*	NS
ΣPUFA	36.67	37.25	36.37	37.56	0.40	NS	NS	NS
Σn-6	33.37	33.78	32.96	34.19	0.36	NS	NS	*
Σn-3	3.94	4.15	4.03	4.06	0.08	NS	NS	NS
Nutritional indices <sup>4</sup>								
n-6/n-3	8.53	8.22	8.31	8.45	0.11	NS	NS	***
P/S	1.01	1.05	1.03	1.03	0.02	NS	NS	NS
AI	0.44	0.44	0.44	0.44	0.01	NS	NS	NS
TI	0.86	0.82	0.81	0.86	0.02	NS	NS	NS

<sup>1</sup>C = Control (untreated); GOS = in ovo injected with GOS.

<sup>2</sup>TN = thermoneutral conditions; HS = heat stress conditions (on d 36–50).

<sup>3</sup>SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

<sup>4</sup>Abbreviations: P/S, PUFA/SFA ratio; AI, atherogenic index; TI, thrombogenic index. Significance: NS =  $P > 0.05$ ; \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ .

on diet composition, but also on the production of short-chain FAs and their amount.

Overall, the SFA (ranging from 35.42 to 36.90%) and PUFA (ranging from 36.37 to 37.56%) were the most abundant fatty acids followed by MUFA (ranging from 25.72 to 28.70%). Considering the composition of single FA, among SFA, palmitic (C16:0), and stearic (C18:0) acids were the most abundant, while other detected SFA (C14:0, C15:0, C17:0, C20:0, C22:0, and C24:0) were less than 0.4%. Palmitic acid is thought to increase cholesterol levels together with lauric and myristic acid, while stearic acid has little or no effect. In fact, stearic acid is generally considered to be a neutral fatty acid because it has been shown to have no net impact on the plasmatic level of either LDL or HDL cholesterol in humans.

MUFA were mainly represented by oleic acid (C18:1 n-9; from 23.65 to 26.38%) and to a lesser extent by palmitoleic acid (C16:1; about 2%). Lower content of oleic acid was found by Tavaniello et al. (2020) (ranging from 18.06 to 19.06%), which leads to lower MUFA content (ranging from 20.18 to 22.23%). Another study reported

a concentration of oleic acid of breast muscle ranged from 21.79 to 30.43% among fast-growing chickens (Cobb 700), medium-growing strains (Naked neck Kabir) and slow-growing strains (Brown Classic Lohman; Sirri et al., 2011). The reason for variation in the concentration could be related to the dietary compositions and genetic background. As well known, from the nutritional point of view, oleic acid plays a key role in human diet in reducing lipaemia and consequently the risk of stroke (D'Alessandro et al., 2012).

PUFA are essential components of biological membranes and are precursors of a wide range of lipid regulators of cellular metabolism. PUFA were mainly in the form of linoleic acid (C18:2 n-6; from 23.24 to 24.48%), the precursor of the n-6 family, followed by the arachidonic acid (C20:4 n-6; less than 8%). The trend of the percentage values for main fatty acid components and each single fatty acid, above presented, agreed with those previously described for breast meat of slow-growing birds (Sirri et al., 2011) and fast-growing birds (Tavaniello et al., 2020).



The values of the n-6/n-3 ratio observed in this study are little bit higher than those found for fast-growing chickens (Tavaniello et al., 2020) and are distant from the ideal value of 1 and the maximum value of 4 (Wood et al., 2004). Generally, poultry is characterized by the highest n-6/n-3 ratio compared to other types of meat, essentially due to the higher amount of n-6 FA than muscles of the other species (Rule et al., 2002; Wood et al., 2004). In fact, linoleic acid is the predominant essential FA in poultry and as a result the n-6 PUFA are the primary products found in tissue lipids. However, based on cardiovascular considerations, the day-to-day consumption of a healthy amount of essential fatty acids for the adult population should be 250 mg for eicosapentaenoic acid (EPA) plus docosahexaenoic acid (DHA) (EFSA, 2017). If we take into account the average lipid content observed in the PM of the present study which is about 2.4 g/100 g, and the average content of EPA+DHA of 1.04%, the intake of these long-chain PUFA n-3 per day (25 mg/100 g) is able to satisfy about 10% of the daily long-chain PUFA n-3 requirement. Compared to C group, GOS showed a numerical higher incidence of EPA+DHA calculated on total lipid content (2.5 vs. 2.38 mg/100 g, respectively).

Heat stress (Table 5) affected the total MUFA content that was higher in TN group compared to HS one ( $P < 0.05$ ), while total contents of SFA and PUFA were not affected ( $P > 0.05$ ) by temperature. The analysis of individual FA shows that HS affected some of them; in particular, stearic and linoleic acids were found higher ( $P < 0.05$ ) in HS group compared to TN group; differently oleic acid was higher in TN group than HS one ( $P < 0.05$ ), conditioning the higher value of MUFA found in the TN group. Another statistically significant difference ( $P < 0.05$ ) was found for C22:1 (erucic acid), which was present in very small amount (less than 0.1%). In agreement with the results of Tavaniello et al. (2020), temperature did not affect the nutritional ratios (n6/n3, P/S, AI, and TI). To our knowledge few information regarding the effect of heat stress on FA composition of chicken meat are reported in literature. 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. It should be stated that genotypes, diet composition and heat stress patterns may change the FA profile. In a study conducted on Cobb 500, Jahromi et al. (2016) found that meat from heat-exposed birds (35°C) showed a greater MUFA content but lower PUFA content and PUFA/SFA ratio compared to control chickens; while, when birds were fed with basal diet plus 0.1% probiotic mixture raised in 35°C meat showed lower total SFA and higher total MUFA compared with control one.

From a nutritional point of view, the P/S values observed in the present study are favorably high (ranging from 1.01 to 1.05), a higher P/S ratio is

recommended and should be increased to above 0.4 (Wood et al., 2004). The AI and TI indices, that represent criteria for evaluating the level and interrelation through which some FA may have atherogenic or thrombogenic properties, were low (AI = 0.44; TI ranging from 0.81 to 0.86) revealing a good nutritional quality of the meat.

Several significant interactions between GOS treatment and temperature were found for FA composition. In particular, GOS in HS birds increased linoleic (C: TN = 24.20%, HS = 23.67%; GOS: TN = 22.28%, HS = 25.29%;  $P < 0.01$ ) and dihomo- $\gamma$ -linolenic acid (C: TN = 0.97%, HS = 0.87%; GOS: TN = 0.91%, HS = 1.09%;  $P < 0.05$ ) acids content, total n-6 content (C: TN = 33.66%, HS = 33.08%; GOS: TN = 32.26%, HS = 35.31%;  $P < 0.05$ ) and n-6/n-3 ratio (C: TN = 8.89%, HS = 8.18%; GOS: TN = 7.26%, HS = 8.72%;  $P < 0.01$ ); while, it decreased docosapentaenoic content (C: TN = 0.93%, HS = 1.09%; GOS: TN = 1.22%, HS = 1.05%;  $P < 0.05$ ).

## CONCLUSIONS

The present study provides further insights on the effects of heat stress on the physiological response in avian species, with particular regards to slow-growing chicken genotypes. These findings can impact on both science and industry while implementing strategies aiming at counteracting the consequences of climate changes on chicken production. In ovo injection with GOS had no negative effects on in vivo performance and meat quality traits. As expected, thermal challenge applied for the last 14 d of the rearing period, had a dampening effect on growth performance. However, mortality was not affected by HS indicating that Hubbard chickens here tested are resilient to handle higher temperature.

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## DISCLOSURES

The authors declare that they have no conflict of interests.

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