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Marco Zampiga, Giorgio Brugaletta, Filiberto Ceccaroni, Alessio Bonaldo, Stefano Pignata, Federico Sirri

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1 **Performance response of broiler chickens fed diets containing dehydrated**
2 **microalgae meal as partial replacement for soybean until 22 days of age**

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16 European Union.

17 **Abstract**

18 Microalgae meal (**MM**) could represent a sustainable alternative to soybean meal as
19 protein ingredient for broiler diets. The aim of this study was to investigate the effects
20 of the dietary substitution of soybean with MM (*Arthrospira spp.*) during the first stages
21 of the rearing cycle on the growth performance of broiler chickens. A total of 1 000 one-
22 day-old Ross 308 male chicks were divided into 4 experimental groups (10 replicate
23 pens/group with 25 birds each) receiving, during the starter (0-12 days) and grower
24 (13-22 days) phases, either a conventional soybean-based diet (**CON** group) or the
25 same diet including MM at low (**LM** group: 50 g/kg in both phases), intermediate (**IM**
26 group: 100 and 90 g/kg, respectively), or high dosages (**HM** group: 150 and 140 g/kg,
27 respectively). From 23 d onwards, all groups received the same conventional soybean-
28 based diet up to slaughter age (47 days). All diets were formulated to be iso-energetic
29 and with a similar amino acid profile. Productive parameters were recorded on a pen
30 basis at housing (0 day), at 22 days, and at slaughter. At 22 days, body weight was
31 linearly reduced and feed conversion ratio significantly worsened as the dietary
32 inclusion of MM increased (931 vs. 850 vs. 709 vs. 462 g, and 1.539 vs. 1.656 vs.
33 1.783 vs. 2.312 for CON, LM, IM and HM groups, respectively; $P < 0.001$). CON and
34 LM groups presented similar feed intake from 0 to 22 days, which was significantly
35 higher if compared to IM and HM (1.367 vs. 1.333 vs. 1.184 vs. 0.964 kg/bird,
36 respectively; $P < 0.001$). At 47 days, CON and LM groups exhibited comparable body
37 weight, while IM and HM showed lower values (3,455 vs. 3,446 vs. 3,221 vs. 2,802 g,
38 respectively; $P < 0.001$). No significant difference in FCR was observed in the overall
39 period of trial (0-47 days). Similarly, liveability was not substantially affected by the
40 treatments. Overall, these results indicate that the dietary administration of MM during
41 the first 22 days of life significantly impaired broiler growth performance regardless of

42 the dosage. However, by re-feeding a conventional soybean-based diet up to slaughter
43 (47 days), broilers receiving 50 g/kg of MM up to 22 days achieved similar growth
44 performance and productive efficiency to those fed a conventional soybean-based diet
45 in all feeding phases.

46 **Keywords:** broiler chicken, nutrition, microalgae, alternative protein source, soybean,
47 productive performance

48 **Abbreviations:** SBM, soybean meal; MM, microalgae meal; CON, control; LM, 50 g/kg
49 of microalgae meal during starter and grower phases; IM, 100 and 90 g/kg of
50 microalgae meal during starter and grower phases; HM, 150 and 140 g/kg of
51 microalgae meal during starter and grower phases; BW, body weight; DWG, daily
52 weight gain; DFI, daily feed intake, FCR, feed conversion ratio; EPEF, European
53 Production Efficiency Factor; EBI, European Broiler Index.

54 **Introduction**

55 The growth of world population and the concomitant increase in animal products
56 demand are leading to a remarkable increment in annual world feed supply (Kim et al.,
57 2019). The poultry sector accounts for approximately 600 million tons of dry matter
58 feed per year with relatively high concentrations of feed protein raw materials (Mottet
59 and Tempio, 2017), which are considered as one of the most expensive and limiting
60 ingredients (Beski et al., 2015). Soybean meal (**SBM**) is the most important and widely
61 used protein source in commercial poultry feeding, primarily because of its well-
62 balanced amino acid profile (Beski et al., 2015). The forecasted increase in feed
63 production is exacerbating the environmental, economic and social issues related to
64 the production, processing and transportation of soybean (Kim et al., 2019; Zalles et

65 al., 2019; Song et al., 2021). These sustainability concerns are giving momentum to
66 the identification of alternative protein sources that might replace SBM in poultry diets
67 without compromising animal growth performance and health status.

68 Microalgae are a heterogeneous group of photosynthetic aquatic plants that utilize
69 atmospheric CO₂ and light energy for their metabolic activities, producing a variety of
70 essential nutrients and bioactive compounds such as proteins, amino acids, long-chain
71 polyunsaturated fatty acids, vitamins and carotenoids (Świątkiewicz, 2015; Saadaoui
72 et al., 2021). Microalgae could be safely included in poultry diets (Świątkiewicz, 2015),
73 with inclusion levels of around 20 g/kg that have been identified by Coudert et al. (2020)
74 as suitable to provide benefits on growth performance, health status and product
75 quality of broilers. However, the same authors also highlighted that most of the studies
76 conducted so far have considered microalgae meal (**MM**) mainly as feed supplement,
77 which could be included in standard formulations at low dosages to enhance animal
78 health and product quality traits, rather than a major source of macronutrients such as
79 protein and amino acids. Indeed, some microalgae strains such as the widely known
80 *Arthrospira spp.* (Spirulina) are characterized by relevant amounts of crude protein (up
81 to 700 g/kg) with a balanced essential amino acid profile (Saadaoui et al., 2021).
82 Nevertheless, the large-scale use of MM as protein source is still limited, mostly
83 because of its high cost and the scarce knowledge regarding digestibility and optimal
84 dietary inclusion rates (Saadaoui et al., 2021). A potential strategy to promote an
85 economically sustainable use of MM in broiler nutrition might be administering it during
86 the first phases of the rearing cycle, when diets with high crude protein concentration
87 should be provided to meet the elevated protein and amino acid requirements of
88 animals with still limited feed ingestion capacity. However, the information regarding
89 the animal growth response to this nutritional approach is scant and inconsistent,

90 especially to as concern the potential implications in the entire rearing cycle. Therefore,
91 the aim of the present study was to evaluate the growth performance of broiler chickens
92 fed diets with increasing dosages of MM (*Arthrospira spp.*) up to 22 days of age and
93 then a conventional soybean-based diet until slaughtering.

94 **Material and methods**

95 ***Ethic statement***

96 Birds were raised, handled and processed according to the Directive 2007/43/EC for
97 the protection of chickens kept for meat production, the Regulation 1099/2009/EC for
98 the protection of animals at the time of killing, and the Directive 2010/63/EU for the
99 protection of animals used for scientific purposes. The Ethical Committee of the
100 University of Bologna approved the experimental protocol (ID: 1145/2020).

101 ***Animals and housing***

102 One thousand day-old male Ross 308 chicks were obtained from the same breeder
103 flock and hatching session. The chicks were vaccinated at the hatchery and then
104 transported to an environmentally-controlled poultry facility, where they were randomly
105 distributed in 40 concrete floor pens arranged in randomized blocks to minimize any
106 environmental effect. Each pen was equipped with one circular pan feeder and 5
107 nipple-type waterers. Wood shaving was utilized as bedding material (3-4 kg/m²). The
108 stocking density did not exceed 33 kg/m² and the photoperiod was 23 h light – 1 h dark
109 during 0-7 and 45-47 days, and 18 h light – 6 h dark from 8-44 days. The environmental
110 temperature within the barn was defined according to the age of the birds in line with
111 the current recommendations.

112 **Experimental diets**

113 The analysed chemical composition and amino acid profile of the MM (*Arthrospira* spp.;
114 VAXA Impact Nutrition, Reykjavík, Iceland) is reported in Table 1. The most relevant
115 fatty acids in the MM were palmitic acid (C16:0), palmitoleic acid (C16:1), linoleic acid
116 (C18:2, n-6) and γ -linolenic acid (C18:3, n-6) (419.4, 102.4, 186.5 and 271.8 g/kg of
117 total fat, respectively). A 3-phase feeding program was adopted: starter (0–12 days),
118 grower (13–22 days) and finisher (23–47 days). The ingredients as well as the
119 chemical composition of the diets (either analysed or calculated values) in the different
120 feeding phases are shown in Table 2. Briefly, the control diet (**CON**) was a conventional
121 corn-wheat-soybean basal diet formulated to meet the nutritional requirements of Ross
122 308 (Aviagen, 2019). The experimental diets containing MM were obtained using the
123 same ingredients of the CON diet with the inclusion, during the starter and grower
124 phases, of MM at either low (**LM**: 50 g/kg in both phases), intermediate (**IM**: 100 and
125 90 g/kg, respectively), or high dosages (**HM**: 150 and 140 g/kg, respectively). Each
126 experimental group was constituted by 10 replicate pens of 25 birds each. The
127 inclusion of MM was performed by reducing the dietary concentration of both soybean
128 meal and full-fat soybean in respect to CON diet. All diets were isoenergetic and with
129 a similar amino acid profile, which was optimized maintaining the same ratio of total
130 essential amino acids to total lysine (Table 2). From 23 days to slaughter (47 days), all
131 groups received the CON diet formulated according to the nutritional specifications for
132 the finisher phase (Table 2). All diets were administered in mash form and feed and
133 water provided *ad-libitum*.

134 **Productive performance**

135 Birds were weighed on a pen basis at housing (0 day), at 22 days and at slaughter (47
136 days). Similarly, feed consumption was determined at 22 and 47 days. Mortality was
137 monitored daily. Dead birds were recorded and weighed to calculate the liveability rate
138 and to adjust the productive performance data. Body weight (**BW**), daily weight gain
139 (**DWG**), daily feed intake (**DFI**) and feed conversion ratio (**FCR**) were obtained
140 accordingly. The results were reported for the following periods: 0-22 days, 23-47 days
141 and 0-47 days. For the overall period of trial (0-47 days), production efficiency
142 indicators such as the European Production Efficiency Factor (**EPEF** = [liveability (%)
143 × BW (kg) / age (days) × FCR (kg feed/kg gain)] × 100) and the European Broiler Index
144 (**EBI** = [liveability (%) × DWG (g/bird/day) / FCR (kg feed/kg gain) × 10] were calculated
145 on a pen basis. At 47 d, all birds were processed in a commercial slaughterhouse.

146 ***Statistical analysis***

147 Data were analysed by means of one-way ANOVA and Tukey post-hoc test
148 considering the diet as experimental factor and the pen as experimental unit.
149 Polynomial contrasts were used to assess linear and quadratic responses to increasing
150 dietary dosages of MM. Prior to analysis, liveability data were submitted to arcsine
151 transformation. Differences were considered as statistically significant when $P < 0.05$.

152 **Results and Discussion**

153 At placement, chick BW was similar among experimental groups with group values
154 ranging from 42.2 to 42.6 g (Table 4). After 22 days, the dietary inclusion of MM
155 determined a significant reduction in BW and DWG compared to CON (931 vs. 850 vs.
156 709 vs. 462 g, and 40.3 vs. 36.6 vs 30.1 vs. 18.9 g/bird/day, respectively for CON, LM,
157 IM and HM; both linear and quadratic, $P < 0.001$). However, CON and LM groups

158 presented comparable DFI and FI, which were significantly higher than those of IM and
159 HM (62.0 vs. 60.5 vs. 53.7 vs. 43.7 g/bird/day, and 1.364 vs. 1.330 vs. 1.181 vs. 0.961
160 kg/bird, respectively for CON, LM, IM and HM; both linear and quadratic, $P < 0.001$).
161 FCR from 0 to 22 days was significantly affected by MM administration, with the lowest
162 value observed in CON and the highest one in HM (1.539 vs. 1.656 vs. 1.783 vs. 2.312,
163 respectively for CON, LM, IM and HM; both linear and quadratic, $P < 0.001$). The dietary
164 treatments did not significantly influence liveability rate. Overall, the dietary inclusion
165 of MM up to 22 days of age dramatically impaired the growth performance of broiler
166 chickens. Early studies on this topic reported a significant reduction of weight gain in
167 Single Comb White Leghorn chickens fed diets containing 100 or 200 g/kg of
168 dehydrated Spirulina as a substitute for SBM during the first three weeks of life (Ross
169 and Dominy, 1990). However, the same authors reported that FCR was not
170 significantly affected by the dietary treatment. Similarly, the dietary provision of 75 g/kg
171 of defatted diatom *Staurosira sp.* biomass as a replacement for SBM during the first 3
172 weeks negatively affected body weight gain and tended to reduce FI and gain:feed
173 ratio (Austic et al., 2013). Our results do not support those reported by Evans et al.
174 (2015), who stated that up to 160 g of dehydrated full-fat Spirulina meal per kg feed
175 can be included into starter diets without negative consequences on growth
176 performance or amino acid digestibility of Hubbard x Cobb 500 broilers. However,
177 higher dosages (i.e., 210 g/kg) were associated with a significant reduction in BW, FI
178 and amino acid digestibility. Furthermore, the authors reported that neither FCR nor
179 mortality were substantially influenced by the dietary dosage of Spirulina. In the
180 present study, even the lowest dosage (50 g/kg) significantly worsened DWG and
181 FCR, although the birds belonging to LM group consumed a comparable amount of
182 feed compared to CON. These outcomes could suggest that the negative effects on

183 growth performance exerted by MM inclusion are not exclusively a direct consequence
184 of FI reduction, but also other factors such as poor digestibility rate and unbalanced
185 composition could have played a role. This hypothesis finds confirmation in the work
186 of Coudert et al. (2020). In particular, digestibility could be affected by the high fiber
187 and polysaccharides content of algae as well as by the presence of phenolic
188 compounds that can react with amino acids forming insoluble complexes (Saadaoui et
189 al. 2021). Further studies are warranted to evaluate the digestibility rate of the MM
190 used in the present study.

191 At slaughtering (47 days; Table 4), CON and LM achieved similar BW, whereas IM and
192 HM birds were significantly lighter (3,455 vs. 3,446 vs. 3,221 vs. 2,802 g, respectively
193 for CON, LM, IM and HM; both linear and quadratic, $P < 0.001$). HM group presented
194 lower DWG than the other groups from 23 to 47 days (101.0 vs. 104.0 vs. 100.3 vs.
195 93.6 g/bird/day, respectively for CON, LM, IM and HM; both linear and quadratic,
196 $P < 0.001$). DFI was similar between CON and LM, which consumed more feed than IM
197 and HM (193.7 vs. 189.3 vs. 176.2 vs. 159.5 g/bird/day, and 4.843 vs. 4.733 vs. 4.406
198 vs. 3.988 kg/bird, respectively for CON, LM, IM and HM; linear, $P < 0.001$; quadratic:
199 $P < 0.01$). FCR was linearly affected by the dietary treatments with the highest value
200 observed for CON group, followed by LM, IM and HM (1.921 vs. 1.822 vs. 1.757 vs.
201 1.704 g feed/g bird, respectively; $P < 0.001$). Once again, liveability showed comparable
202 values among groups. In this feeding phase, the growth performance of LM birds was
203 comparable to that of CON ones, coupled also with a better FCR. This allowed to cover
204 the BW gap between CON and LM accumulated during the first 22 days of trial,
205 resulting in similar BW at slaughter. On the other hand, final BW and DFI of IM and HM
206 broilers were still significantly lower than those of CON and LM. It should be considered
207 that MM administration during the first 22 days generated large differences in BW

208 among the experimental groups, particularly for IM and HM (i.e., approximately -25 and
209 -50% compared to CON), which could have hindered feed ingestion capacity during
210 the last part of the rearing cycle although all groups received the same basal diet.
211 Considering the results in the overall period of trial (0-47 days; Table 4), CON and LM
212 showed comparable DWG and DFI, followed by IM and then by HM (71.9 vs. 71.6 vs.
213 66.6 vs. 57.7 g/bird/day, and 130.5 vs. 127.4 vs. 117.3 vs. 103.9 g/bird/day,
214 respectively; both linear and quadratic, $P < 0.001$). FCR as well as liveability exhibited
215 no substantial variations among experimental groups. Furthermore, EPEF and EBI
216 were similar among CON, LM and IM groups, but significantly lower in HM (Table 4).
217 These results indicate that, in the overall period of trial (0-47 days), broiler chickens
218 receiving 50 g/kg MM from 0 to 22 days and then a conventional soybean-based diet
219 performed similarly to those fed the soybean-based diet in all feeding phases.
220 However, the negative impact exerted by higher dietary dosages of MM was not
221 completely reversed by this feeding strategy. Overall, it can be concluded that the
222 dietary administration of MM during the first 22 days of life significantly impaired the
223 growth performance of fast-growing broiler chickens regardless of the inclusion
224 dosage. However, by re-feeding a conventional soybean-based diet up to slaughter
225 (23-47 days), broilers receiving 50 g/kg of MM up to 22 days achieved similar growth
226 performance and productive efficiency to those fed a conventional soybean-based diet
227 in all feeding phases.

228 **Author contributions**

229 **Marco Zampiga:** Conceptualization; Data curation; Formal analysis; Investigation,
230 Methodology; Visualization; Writing - original draft; Writing – review & editing.

231 **Giorgio Brugaletta:** Data curation; Investigation; Writing – review & editing.

232 **Filiberto Ceccaroni:** Conceptualization; Methodology.

233 **Alessio Bonaldo:** Conceptualization; Funding acquisition; Project administration;
234 Resources.

235 **Stefano Pignata:** Data curation; Investigation; Methodology.

236 **Federico Sirri:** Conceptualization; Funding acquisition; Methodology; Project
237 administration; Resources; Supervision; Writing – review & editing.

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248 of data, writing of the report, and decision to submit the article for publication.

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289

290 **Table 1.** Analysed chemical composition and amino acid profile of the microalgae
291 meal.

Composition	g/kg
Dry matter	940.0
Crude protein	702.4
Crude fiber	46.8
Ash	73.3
Total fat	116.0
Calcium	3.10
Phosphorous	11.3
Sodium	6.30
Chlorine	1.30
Lysine	31.7
Methionine	15.9
Cysteine	6.30
Methionine + Cysteine	22.3
Threonine	32.3
Arginine	49.9
Isoleucine	37.3
Leucine	57.7
Valine	41.1
Histidine	10.4
Serine	31.6
Glycine	33.3
Proline	24.1
Alanine	50.5
Phenylalanine	31.5
Glutamic acid	94.4
Aspartic acid	68.7
AME (MJ/kg) ¹	12.6

292 Abbreviations: AME = Apparent Metabolizable Energy

293 ¹Estimated value.

294

295 **Table 2.** Composition of the experimental diets.

<i>Ingredients (g/kg)</i>	Starter (0-12 d)				Grower (13-22 d)				Finisher (23-47 d)
	CON	LM	IM	HM	CON	LM	IM	HM	CON
Microalgae meal	0.00	50.0	100.0	150.0	0.00	50.0	90.0	140.0	0.00
Corn	358.0	392.0	428.0	462.0	389.0	432.0	488.0	531.0	386.0
Wheat	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0	200.0
Vegetable oil	24.9	18.4	11.6	5.10	27.6	20.2	12.5	5.1	40.4
Wheat bran	20.0	29.9	40.1	50.0	20.0	23.9	28.0	31.9	20.0
Soybean meal	219.0	159.0	97.5	37.6	173.0	116.0	57.1	0.00	117.0
Full-fat soybean	99.9	73.5	46.4	20.0	150.0	118.0	85.1	53.2	150.0
Sunflower meal	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Corn gluten	30.0	30.0	30.0	30.0	0.00	0.00	0.00	0.00	0.00
Pea	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Calcium carbonate	3.90	4.30	4.70	5.00	5.50	5.80	6.10	6.40	9.60
Dicalcium phosphate	10.9	9.50	8.00	6.60	5.60	4.30	3.00	1.80	1.10
Sodium chloride	3.50	2.80	2.00	1.30	3.00	2.40	1.70	1.00	2.40
Sodium bicarbonate	0.70	0.60	0.60	0.50	0.70	0.60	0.60	0.50	1.70
Choline	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Lysine sulphate	5.60	6.80	8.00	9.20	3.70	5.20	6.70	8.20	3.40
DL-Methionine	2.90	2.70	2.40	2.20	3.00	2.80	2.60	2.40	2.50
L-Threonine	1.20	0.90	0.60	0.30	0.90	0.70	0.50	0.40	0.70
Phytase	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	1.50
NSP enzyme	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Mix amino acids (Arg+Val)	0.70	0.70	0.60	0.50	0.50	0.50	0.50	0.50	0.50
Mycotoxin binder	1.00	1.00	1.00	1.00	0.00	0.00	0.00	0.00	0.00
Vitamin-mineral premix ¹	5.00	5.00	5.00	5.00	4.50	4.50	4.50	4.50	2.50
Composition (g/kg)									
Dry Matter ²	912.0	903.0	905.0	903.0	903.0	905.0	904.0	903.0	904.0
Crude protein ²	229.0	221.0	224.0	226.0	200.0	204.0	208.0	205.0	183.0
Total lipid ²	63.0	56.0	49.0	44.0	73.0	65.0	55.0	47.0	90.0
Crude fibre ²	33.0	28.0	23.0	27.0	32.0	28.0	28.0	28.0	30.0
Ash ²	51.2	45.0	46.7	47.7	50.1	48.6	44.5	42.6	35.8
Calcium (total)	7.30	7.30	7.30	7.30	6.40	6.40	6.40	6.40	6.10
Phosphorous (total)	6.00	6.00	6.00	6.00	4.90	4.90	4.90	4.90	4.00
Lysine (total)	14.0	14.0	14.0	14.0	12.6	12.6	12.6	12.6	11.0
Meth + Cys (total)	10.5	10.5	10.5	10.5	9.70	9.70	9.70	9.70	8.70
Threonine (total)	9.50	9.50	9.50	9.50	8.50	8.50	8.50	8.50	7.50
AME (MJ/kg)	12.6	12.6	12.6	12.6	13.0	13.0	13.0	13.0	13.5

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Abbreviations: AME = Apparent Metabolizable Energy. NSP: Non-starch polysaccharides.

¹ Provided 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.

² Analyzed values.

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305 **Table 4.** Growth performance of broiler chickens fed a conventional soybean-based
 306 diet (CON) or diets with different dosages of microalgae meal (LM, IM and HM) up to
 307 22 days of age.
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Parameter	Experimental groups				SEM	P-value	Response	
	CON	LM	IM	HM			Linear	Quadratic
0-22 d								
Chick BW (g)	42.6	42.2	42.4	42.3	0.10	0.61	0.51	0.60
BW (g)	931 A	850 B	709 C	462 D	29.5	<0.001	<0.001	<0.001
DWG (g/bird/day)*	40.3 A	36.6 B	30.1 C	18.9 D	1.34	<0.001	<0.001	<0.001
DFI (g/bird/day)*	62.0 A	60.5 A	53.7 B	43.7 C	1.22	<0.001	<0.001	<0.001
FI (kg/bird)*	1.364 A	1.330 A	1.181 B	0.961 C	0.03	<0.001	<0.001	<0.001
FCR (g feed/g gain)*	1.539 D	1.656 C	1.783 B	2.312 A	0.05	<0.001	<0.001	<0.001
Livability (%)	99.2	99.6	98.2	98.2	0.02	0.36	0.16	0.75
23-47 d								
BW (g/bird)	3,455 A	3,446 A	3,221 B	2,802 C	45.6	<0.001	<0.001	<0.001
DWG (g/bird/day)*	101.0 A	104.0 A	100.3 A	93.6 B	0.77	<0.001	<0.001	<0.001
DFI (g/bird/day)*	193.7 A	189.3 A	176.2 B	159.5 C	2.38	<0.001	<0.001	<0.01
FI (kg/bird)*	4.843 A	4.733 A	4.406 B	3.988 C	0.06	<0.001	<0.001	<0.01
FCR (g feed/g gain)*	1.921 A	1.822 B	1.757 BC	1.704 C	0.02	<0.001	<0.001	0.31
Livability (%)	99.6	98.3	99.5	100.0	0.01	0.09	0.27	0.07
0-47 d								
BW (g/bird)	3,455 A	3,446 A	3,221 B	2,802 C	45.6	<0.001	<0.001	<0.001
DWG (g/bird/day)*	71.9 A	71.6 A	66.6 B	57.7 C	0.98	<0.001	<0.001	<0.001
DFI (g/bird/day)*	130.5 A	127.4 A	117.3 B	103.9 C	1.80	<0.001	<0.001	<0.001
FI (kg/bird)*	6.209 A	6.066 A	5.591 B	4.951 C	0.08	<0.001	<0.001	<0.001
FCR (g feed/g gain)*	1.818	1.781	1.762	1.799	0.01	0.27	0.41	0.08
Livability (%)	98.8	98.0	97.8	98.2	0.02	0.78	0.56	0.41
EPEF [†]	401 A	404 A	380 A	326 B	6.23	<0.001	<0.001	<0.001
EBI [#]	392 A	394 A	370 A	316 B	6.23	<0.001	<0.001	<0.001

309 Abbreviations: BW = body weight; DWG = daily weight gain; DFI = daily feed intake; FI = feed intake; FCR = feed conversion
 310 ratio; EPEF = European Production Efficiency Factor; EBI = European Broiler Index; SEM = standard error of the mean.
 311 *: corrected for mortality.

312 †: EPEF = [livability (%) × BW (kg) / age (days) × FCR (kg feed/kg gain)] × 100
 313 #: EBI = [livability (%) × DWG (g/bird/day) / FCR (kg feed/kg gain) × 10].

314 A, B: P<0.01