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

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Abstract

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

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

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

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

Introduction

The growth of world population and the concomitant increase in animal products demand are leading to a remarkable increment in annual world feed supply (Kim et al., 2019). The poultry sector accounts for approximately 600 million tons of dry matter feed per year with relatively high concentrations of feed protein raw materials (Mottet and Tempio, 2017), which are considered as one of the most expensive and limiting ingredients (Beski et al., 2015). Soybean meal (**SBM**) is the most important and widely used protein source in commercial poultry feeding, primarily because of its well-balanced amino acid profile (Beski et al., 2015). The forecasted increase in feed production is exacerbating the environmental, economic and social issues related to the production, processing and transportation of soybean (Kim et al., 2019; Zalles et al., 2019; Song et al., 2021). These sustainability concerns are giving momentum to the identification of alternative protein sources that might replace SBM in poultry diets without compromising animal growth performance and health status.

Microalgae are a heterogeneous group of photosynthetic aquatic plants that utilize atmospheric CO_2 and light energy for their metabolic activities, producing a variety of essential nutrients and bioactive compounds such as proteins, amino acids, long-chain polyunsaturated fatty acids, vitamins and carotenoids (Świątkiewicz, 2015; Saadaoui et al., 2021). Microalgae could be safely included in poultry diets (Świątkiewicz, 2015), with inclusion levels of around 20 g/kg that have been identified by Coudert et al. (2020) as suitable to provide benefits on growth performance, health status and product quality of broilers. However, the same authors also highlighted that most of the studies conducted so far have considered microalgae meal (**MM**) mainly as feed supplement, which could be included in standard formulations at low dosages to enhance animal health and product quality traits, rather than a major source of macronutrients such as protein and amino acids. Indeed, some microalgae strains such as the widely known *Arthrospira spp.* (Spirulina) are characterized by relevant amounts of crude protein (up to 700 g/kg) with a balanced essential amino acid profile (Saadaoui et al., 2021). Nevertheless, the large-scale use of MM as protein source is still limited, mostly because of its high cost and the scarce knowledge regarding digestibility and optimal dietary inclusion rates (Saadaoui et al., 2021). A potential strategy to promote an economically sustainable use of MM in broiler nutrition might be administering it during the first phases of the rearing cycle, when diets with high crude protein concentration should be provided to meet the elevated protein and amino acid requirements of animals with still limited feed ingestion capacity. However, the information regarding the animal growth response to this nutritional approach is scant and inconsistent, especially to as concern the potential implications in the entire rearing cycle. Therefore, the aim of the present study was to evaluate the growth performance of broiler chickens fed diets with increasing dosages of MM (*Arthrospira spp*.) up to 22 days of age and then a conventional soybean-based diet until slaughtering.

Material and methods

Ethic statement

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

Animals and housing

One thousand day-old male Ross 308 chicks were obtained from the same breeder flock and hatching session. The chicks were vaccinated at the hatchery and then transported to an environmentally-controlled poultry facility, where they were randomly distributed in 40 concrete floor pens arranged in randomized blocks to minimize any environmental effect. Each pen was equipped with one circular pan feeder and 5 nipple-type waterers. Wood shaving was utilized as bedding material (3-4 kg/m^2). The stocking density did not exceed 33 kg/m^2 and the photoperiod was 23 h light – 1 h dark during 0-7 and 45-47 days, and 18 h light – 6 h dark from 8-44 days. The environmental temperature within the barn was defined according to the age of the birds in line with the current recommendations.

Experimental diets

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

Productive performance

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

Statistical analysis

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

Results and Discussion

At placement, chick BW was similar among experimental groups with group values ranging from 42.2 to 42.6 g (Table 4). After 22 days, the dietary inclusion of MM determined a significant reduction in BW and DWG compared to CON (931 vs. 850 vs. 709 vs. 462 g, and 40.3 vs. 36.6 vs 30.1 vs. 18.9 g/bird/day, respectively for CON, LM, IM and HM; both linear and quadratic, $P<0.001$). However, CON and LM groups presented comparable DFI and FI, which were significantly higher than those of IM and 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 kg/bird, respectively for CON, LM, IM and HM; both linear and quadratic, $P<0.001$). FCR from 0 to 22 days was significantly affected by MM administration, with the lowest value observed in CON and the highest one in HM (1.539 vs. 1.656 vs. 1.783 vs. 2.312, respectively for CON, LM, IM and HM; both linear and quadratic, $P<0.001$). The dietary treatments did not significantly influence liveability rate. Overall, the dietary inclusion of MM up to 22 days of age dramatically impaired the growth performance of broiler chickens. Early studies on this topic reported a significant reduction of weight gain in Single Comb White Leghorn chickens fed diets containing 100 or 200 g/kg of dehydrated Spirulina as a substitute for SBM during the first three weeks of life (Ross and Dominy, 1990). However, the same authors reported that FCR was not significantly affected by the dietary treatment. Similarly, the dietary provision of 75 g/kg of defatted diatom *Staurosira sp.* biomass as a replacement for SBM during the first 3 weeks negatively affected body weight gain and tended to reduce FI and gain:feed ratio (Austic et al., 2013). Our results do not support those reported by Evans et al. (2015), who stated that up to 160 g of dehydrated full-fat Spirulina meal per kg feed can be included into starter diets without negative consequences on growth performance or amino acid digestibility of Hubbard x Cobb 500 broilers. However, higher dosages (i.e., 210 g/kg) were associated with a significant reduction in BW, FI and amino acid digestibility. Furthermore, the authors reported that neither FCR nor mortality were substantially influenced by the dietary dosage of Spirulina. In the present study, even the lowest dosage (50 g/kg) significantly worsened DWG and FCR, although the birds belonging to LM group consumed a comparable amount of feed compared to CON. These outcomes could suggest that the negative effects on growth performance exerted by MM inclusion are not exclusively a direct consequence of FI reduction, but also other factors such as poor digestibility rate and unbalanced composition could have played a role. This hypothesis finds confirmation in the work of Coudert et al. (2020). In particular, digestibility could be affected by the high fiber and polysaccharides content of algae as well as by the presence of phenolic compounds that can react with amino acids forming insoluble complexes (Saadaoui et al. 2021). Further studies are warranted to evaluate the digestibility rate of the MM used in the present study.

At slaughtering (47 days; Table 4), CON and LM achieved similar BW, whereas IM and HM birds were significantly lighter (3,455 vs. 3,446 vs. 3,221 vs. 2,802 g, respectively for CON, LM, IM and HM; both linear and quadratic, $P<0.001$). HM group presented lower DWG than the other groups from 23 to 47 days (101.0 vs. 104.0 vs. 100.3 vs. 93.6 g/bird/day, respectively for CON, LM, IM and HM; both linear and quadratic, $P<0.001$). DFI was similar between CON and LM, which consumed more feed than IM 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 vs. 3.988 kg/bird, respectively for CON, LM, IM and HM; linear, $P<0.001$; quadratic: $P<0.01$). FCR was linearly affected by the dietary treatments with the highest value observed for CON group, followed by LM, IM and HM (1.921 vs. 1.822 vs. 1.757 vs. 1.704 g feed/g bird, respectively; $P<0.001$). Once again, liveability showed comparable values among groups. In this feeding phase, the growth performance of LM birds was comparable to that of CON ones, coupled also with a better FCR. This allowed to cover the BW gap between CON and LM accumulated during the first 22 days of trial, resulting in similar BW at slaughter. On the other hand, final BW and DFI of IM and HM broilers were still significantly lower than those of CON and LM. It should be considered that MM administration during the first 22 days generated large differences in BW among the experimental groups, particularly for IM and HM (i.e., approximately -25 and -50% compared to CON), which could have hindered feed ingestion capacity during the last part of the rearing cycle although all groups received the same basal diet. Considering the results in the overall period of trial (0-47 days; Table 4), CON and LM showed comparable DWG and DFI, followed by IM and then by HM (71.9 vs. 71.6 vs. 66.6 vs. 57.7 g/bird/day, and 130.5 vs. 127.4 vs. 117.3 vs. 103.9 g/bird/day, respectively; both linear and quadratic, $P<0.001$). FCR as well as liveability exhibited no substantial variations among experimental groups. Furthermore, EPEF and EBI were similar among CON, LM and IM groups, but significantly lower in HM (Table 4). These results indicate that, in the overall period of trial (0-47 days), broiler chickens receiving 50 g/kg MM from 0 to 22 days and then a conventional soybean-based diet performed similarly to those fed the soybean-based diet in all feeding phases. However, the negative impact exerted by higher dietary dosages of MM was not completely reversed by this feeding strategy. Overall, it can be concluded that the dietary administration of MM during the first 22 days of life significantly impaired the growth performance of fast-growing broiler chickens regardless of the inclusion dosage. However, by re-feeding a conventional soybean-based diet up to slaughter (23-47 days), broilers receiving 50 g/kg of MM up to 22 days achieved similar growth performance and productive efficiency to those fed a conventional soybean-based diet in all feeding phases.

Author contributions

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

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

Filiberto Ceccaroni: Conceptualization; Methodology.

Alessio Bonaldo: Conceptualization; Funding acquisition; Project administration; Resources.

Stefano Pignata: Data curation; Investigation; Methodology.

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

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Table 1. Analysed chemical composition and amino acid profile of the microalgae 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

Abbreviations: AME = Apparent Metabolizable Energy
[1]Estimated value.

Table 2. Composition of the experimental diets.

	Starter (0-12 d)				Grower (13-22 d)				Finisher (23-47 d)
Ingredients (g/kg)	**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

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 μg; 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.

Table 4. Growth performance of broiler chickens fed a conventional soybean-based diet (CON) or diets with different dosages of microalgae meal (LM, IM and HM) up to 22 days of age.

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

Abbreviations: BW = body weight; DWG = daily weight gain; DFI = daily feed intake; FI = feed intake; FCR = feed conversion ratio; EPEF = European Production Efficiency Factor; EBI = European Broiler Index; SEM = standard error of the mean.
*: corrected for mortality.
[†]: EPEF = [liveability (%) × BW (kg) / age (days) × FCR (kg feed/kg gain)] × 100
[#]: EBI = [liveability (%) × DWG (g/bird/day) / FCR (kg feed/kg gain) × 10].
A, B: P<0.01