

Effects of posthatch feed deprivation on residual yolk absorption, macronutrients synthesis, and organ development in broiler chicks

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ABSTRACT The aim of the research was to evaluate the dynamic changes of early posthatch starvation on residual yolk absorption, synthesis of macronutrients (protein, lipid, and glycogen), and organ development in broiler chicks. A total of 720 1-day-old chicks (Lingnan Yellow) were randomly assigned to 3 treatments: group A (nonfasted), group B (fasting for 24 h after placement), and group C (fasting for 48 h after placement). The trial lasted for 168 h, and water was provided ad libitum all the time. Sampling was performed at 0, 24, 48, 72, 120, and 168 h. Nonfasting (group A) promoted ($P < 0.05$) the absorption of amino acids, fatty acids, mineral elements, protein, and maternal antibody in the residual

yolk of broiler chicks. The concentration of insulin-like growth factor 1 in plasma and the liver was higher ($P < 0.05$) in group A. Nonfasting enhanced ($P < 0.05$) the synthesis of protein and glycogen in the breast muscle and liver; the relative weights of the liver, pancreas, and spleen; and body weight, but retarded ($P < 0.05$) the synthesis of triglyceride in the liver. The results indicated that nonfasting (group A) after placement promoted the absorption of residual yolk and synthesis of protein and glycogen in the breast muscle and liver, whereas early feed deprivation promoted the synthesis of lipid in the liver. Thereby, nonfasting after placement promoted organ development and body growth of broiler chicks.

Key words: feed deprivation, residual yolk absorption, macronutrient synthesis, organ development, broiler chick

2020 Poultry Science 99:5587–5597

<https://doi.org/10.1016/j.psj.2020.08.032>

INTRODUCTION

Chicks hatch (with 472–510 h of incubation) within a time window of approximately 24 to 48 h and will be removed from the hatcheries only after the majority of the chicks have hatched, resulting in early feed intake and water deprivation after hatching (Careghi et al., 2005). In addition, newly hatched chicks are usually delayed of feed for an average of 48 h during transport to the farm in commercial poultry industry (Velleman and Mozdziaik, 2005). During the fasting period, the residual yolk is essential to meet the nutritional requirements of developing embryos, which supplies more than 90% of total energy requirement through utilization of yolk lipids (Yalcin et al., 2008). The yolk sac, constituting approximately 15 to 25% of the chick's body weight (BW) at

hatch, began to be withdrawn into the abdomen of the embryo from day 19 of incubation (Oliverira et al., 2015). The yolk contents at hatch contain about 35 to 40% lipids, mainly triglycerides (Ding and Lilburn, 1996). During embryonic development, nutrients are transferred directly from the yolk to the circulatory system through endocytosis (Lambson, 1970). However, the yolk is also transported to the intestine through the yolk stalk when it is close to hatch (Esteban et al., 1991). It was reported that feed intake stimulated the secretion of yolk to the small intestines after hatch and triggered the uptake of hydrophilic compounds (Noy and Sklan, 2001). Compared with delayed feeding, the liver, pancreas, and jejunum recorded significantly higher weights in chicks that were fed during the initial 24-hour period (Bhanja et al., 2009). As the time it takes for broilers to reach market size decreases, the period of embryonic development accounts for an increasing proportion of the life span. As a result, hatching and embryonic development are more important than ever to successful rearing of meat poultry (Hulet, 2007).

Previous research has been carried out to determine the effects of posthatch starvation on yolk absorption

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Received March 3, 2020.

Accepted August 6, 2020.

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and body growth (Bhanja et al., 2009; Lamot et al., 2014). However, information is lacking on the alterations in residual yolk nutrient composition and its impact on protein, lipid, and glycogen synthesis in tissues of chicks under early feeding or starvation. The main objective of this experiment was to identify the effects of early feeding on yolk contents and organ development during the first week after hatch in broilers. In this experiment, we investigated the effects of feed deprivation on composition of amino acids (AA), fatty acids (FA), mineral elements, protein, and immunoglobulin Y (IgY) in residual yolk. Furthermore, hormone levels, organ development, and macronutrient synthesis were evaluated within 168 h after hatch in broilers under feed deprivation or not.

MATERIALS AND METHODS

The experimental procedures followed Chinese guidelines for animal welfare and were approved by the Animal Welfare Committee of the College of Animal Sciences of Zhejiang University (no. ZJU2013105002) (Hangzhou, China).

Birds and Experimental Design

Hatching eggs were collected from breeders of 31-week-old Lingnan Yellow broilers (Lingnan Yellow broiler, a Chinese quality meat-type chicken, market age: 60 to 70 d, obtained from the Institute of Animal Science, Guangdong Academy of Agricultural Sciences, Guangzhou, China) at a local hatchery (Qunda Breeder Company, Jiaxing, China). The hatching eggs were incubated for 510 h under regular conditions (37.8°C, 55% relative humidity). Within 2 h after hatch (489–491 h), 720 newborn chicks with similar BW were collected and allotted randomly to 3 groups with 6 replicates of 40 birds each. Completely random design was adopted in the present study. After placement at the farm (Xingjian Culture-Farm, Jiaxing, China), group A was fed ad libitum immediately, and the first feed intake time of group A was defined as 0 h (corresponding to 4 h after hatch). Groups B and C were delayed access to feed for 24 and 48 h, respectively, after placement, corresponding to 28 and 52 h after hatch. All chicks were reared at floor pens with 5-cm-deep fresh wood shavings and were provided with water ad libitum after placement. Diets (Table 1) were formulated to meet the nutrient requirements as suggested by the NRC (1994). Birds were fed ad libitum once they had access to feed. The whole experiment lasted for 168 h with a light schedule of 23-hour light and 1-hour darkness. The temperature of the chicken house was maintained in the range of 32°C to 35°C.

Sample Collection

At 0, 24, 48, 72, 120, and 168 h after placement, 4 chicks from each replicate (a total of 24 chicks from each group) were weighted respectively. Blood samples

Table 1. Composition and nutrient levels of the basal diets for broilers.

Items	1–8 d
Ingredients (%)	
Corn	54.70
Wheat	5.00
Soybean meal	29.00
CGM	6.00
Soybean oil	1.00
NaCl	0.30
CaHPO ₄	1.70
Limestone	1.30
Premix ¹	1.00
Total	100.00
Nutrient levels² (%)	
ME (kcal/kg)	2,908
CP	20.96
Lys	1.10
Met	0.50
Met + Cys	0.85
Ca	0.99
TP	0.66

Abbreviations: CGM, corn gluten meal; CP, crude protein; ME, metabolizable energy; TP, total phosphorus.

¹Supplied per kilogram of diet: vitamin A, 9,600 IU; vitamin D₃, 2,700 IU; vitamin E, 36 mg; vitamin K₃, 3.0 mg; vitamin B₁, 3.0 mg; vitamin B₂, 10.5 mg; vitamin B₆, 4.2 mg; vitamin B₁₂, 0.03 mg; folic acid, 1.5 mg; nicotinamide, 60 mg; D-calcium pantothenate, 18 mg; biotin, 0.225 mg; choline chloride, 1,000 mg; Fe, 80 mg; Cu, 8.0 mg; Mn, 80 mg; Zn, 60 mg; I, 0.35 mg; Se, 0.15 mg.

²ME is the calculated value, and other nutrient levels are the measured values.

were obtained from the heart. Whole blood samples were collected in anticoagulant tubes coated with ethylenediaminetetraacetic acid. Plasma was then separated by centrifugation of blood samples at 4,000 *g* for 15 min at 4°C and stored at –80°C until subsequent analysis. After blood collection, the birds were slaughtered by cervical dislocation (Al-Marzooqi and Leeson, 2000). The residual yolk sac, liver, pancreas, spleen, and small intestine (including the duodenum, jejunum, and ileum) of each bird were weighed; after that, all the aforementioned samples, except the pancreas and spleen, were collected and frozen in liquid N₂ and then stored at –80°C. Besides, about 1 cm³ of the left chest muscle was collected and stored at –80°C for subsequent analysis. The sampling schedule has been described in detail (Table 2).

Measurement of Contents in Residual Yolk

The concentration of protein in the residual yolk sac was measured by using a total protein assay kit (A045-4-2, Wu et al., 2019) (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). An enzyme-linked immunosorbent assay kit (Shanghai Tongwei Bioengineering Institute, Shanghai, China) was used to detect IgY concentration in the residual yolk. Amino acid concentration (Agilent 1260 Infinity, Waldbronn, Germany) was quantified by high-performance liquid chromatography. Long-chain FA were analyzed by gas chromatography–mass spectrometry. Gas chromatography was performed using an Agilent GC 7890B equipped with an Agilent autosampler and injector 7693 (Agilent Technologies,

Table 2. Effects of feed deprivation on concentration of minerals in residual yolk (mg/kg).

Minerals	Time (h)	Group			SEM	P-value
		A	B	C		
Ca	0	462.6	456.2	455.7	78.75	0.995
	24	268.8 ^b	413.1 ^a	408.1 ^a	31.91	0.003
	48	204.9 ^c	452.7 ^b	715.8 ^a	14.42	0.000
	72	1,321 ^b	1,459 ^b	2,602 ^a	66.15	0.000
P	0	17.73	17.98	17.00	2.697	0.922
	24	13.33 ^b	24.37 ^a	30.30 ^a	3.141	0.005
	48	12.83 ^b	18.90 ^a	19.98 ^a	2.126	0.023
	72	16.98 ^b	25.18 ^{a,b}	29.28 ^a	3.828	0.029
K	0	19.43	19.23	20.40	3.053	0.906
	24	36.48	25.10	21.63	5.395	0.046
	48	29.20	33.23	28.33	3.537	0.338
	72	64.27	64.00	64.12	12.56	1.000
Na	0	4.39	4.67	4.34	0.704	0.880
	24	8.23	6.43	6.33	0.923	0.127
	48	7.47 ^c	12.00 ^b	14.83 ^a	0.909	0.001
	72	6.98 ^b	14.90 ^a	15.43 ^a	2.240	0.007
Fe	0	1.63	1.76	1.67	0.281	0.925
	24	1.07 ^b	2.05 ^a	2.50 ^a	0.192	0.001
	48	0.43 ^b	1.47 ^a	1.80 ^a	0.204	0.001
	72	1.54	1.68	1.86	0.400	0.526

^{a,b}Mean values within a row with unlike letters are significantly different ($P < 0.05$).

Mean values with their SEM ($n = 6$).

Group A = nonfasted, group B = fasting for 24 h after placement, group C = fasting for 48 h after placement.

Waldbronn, Germany). Mass spectrometry was performed using an Agilent 5799B MSD (Agilent Technologies, Waldbronn, Germany). Four macroelements (Ca, K, Na, and P) and one microelement (Fe) were analyzed by inductively coupled plasma optical emission spectrometry.

Hormone Levels in Plasma and the Liver

The concentrations of triiodothyronine (T_3) and insulin-like growth factor 1 (**IGF-1**) in plasma or the liver were quantified using enzyme-linked immunosorbent assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing) based on the manufacturer's protocols.

Measurement of Macronutrients in Tissues

The concentration of protein (A045-4-2, Wu et al., 2019) and glycogen (A043-1-1, Hu et al., 2020) in both the liver and breast muscle and the concentration of triglyceride (A110-1-1, Zhao et al., 2020) in the liver were quantified using detection kits (Nanjing Jiancheng Bioengineering Institute, Nanjing).

Statistical Analysis

Data from the present study were analyzed by one-way ANOVA using SPSS statistical software (version 20.0 for windows; SPSS Inc., Chicago, III). Differences among treatments were examined using Tukey's multiple range tests, and a probability of $P < 0.05$ was considered to be significant. Data were presented as means with the SEMs. Figures were made by

GraphPad Prism 7.00 software (GraphPad Software, San Diego, CA).

RESULTS

Nonfasting Promoted the Absorption of Residual Yolk

The relative weight of residual yolk indicated that nonfasting (group A) after placement greatly promoted the absorption of yolk (Figure 1). In detail, the relative weight of the yolk sac in group A was the minimum ($P < 0.05$) among the three groups during 24 to 72 h after placement. At 120 h, the relative weight of yolk of groups A and C was lower ($P < 0.05$) than that of group B. And there was no difference among the three groups at 168 h. Nonfasting (group A) promoted ($P < 0.05$) the absorption of protein in residual yolk (Figure 1), and group C showed highest ($P < 0.05$) protein concentration among the groups at 72 and 120 h. In addition, nonfasting promoted ($P < 0.05$) the absorption of IgY in the residual yolk (Figure 1), and group C showed highest ($P < 0.05$) IgY concentration at 120 h. Chicks subjected to nonfasting (group A) had lower ($P < 0.05$) concentration of Ca, P, Fe, and Na than chicks that were delayed feed for 24 or 48 h (groups B and C) at 24, 48, and 72 h (Table 2). To varying degrees, nonfasting (group A) promoted ($P < 0.05$) the absorption of lysine, methionine, cystine, threonine, arginine, histidine, leucine, isoleucine, phenylalanine, tyrosine, and valine in residual yolk (Table 3). Similarly, feeding delay (groups B and C) retarded ($P < 0.05$) the absorption of oleic acid (C18:1) and linolenic acid (C18:3) in the residual yolk (Table 4). No significant difference ($P > 0.05$)

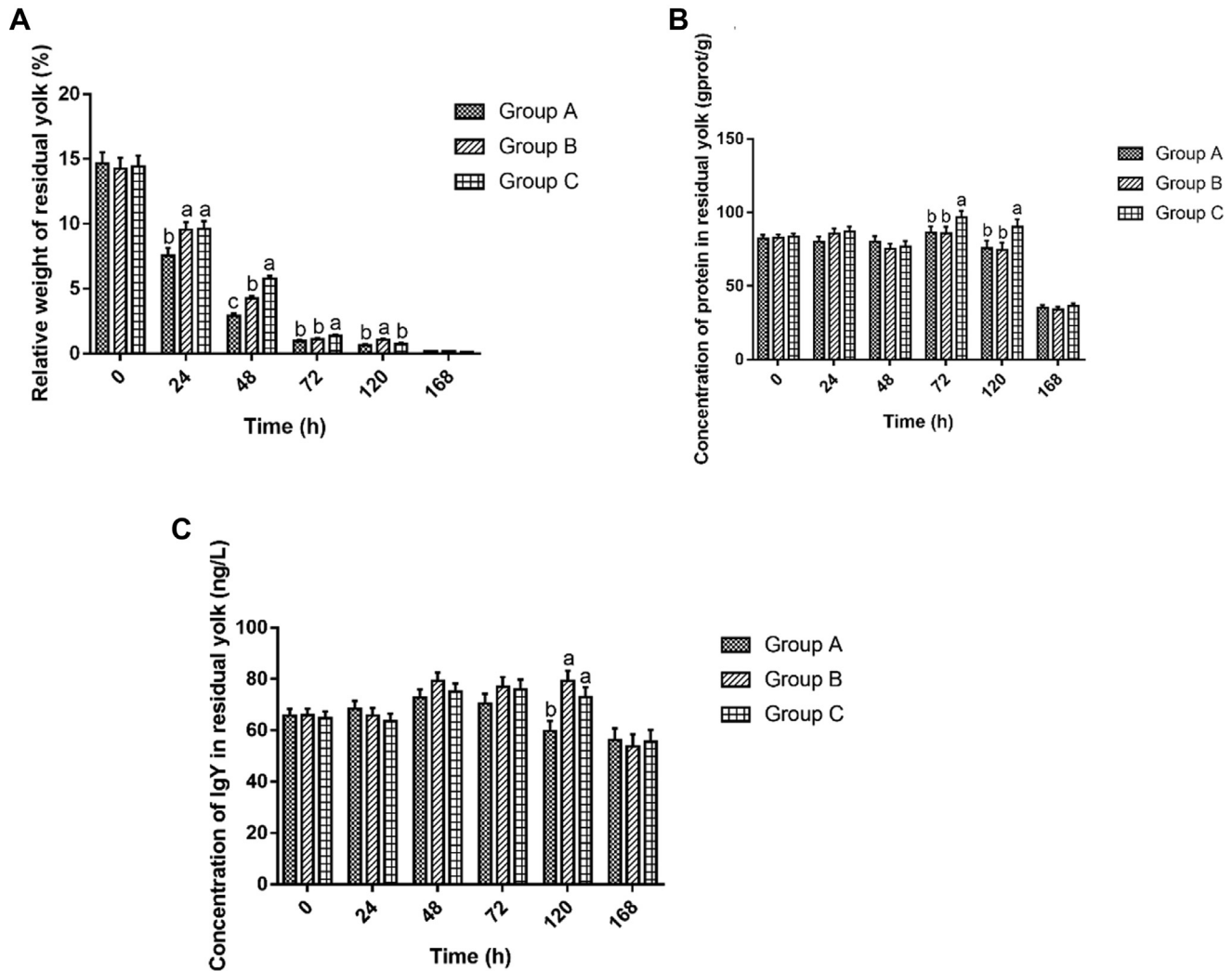


Figure 1. Effects of feed deprivation on (A) relative weight of residual yolk, (B) concentration of protein, and (C) concentration of IgY in residual yolk. Mean values with their SEM ($n = 6$). ^{a-c}Mean values within a row with unlike letters are significantly different ($P < 0.05$). Group A = nonfasted, group B = fasting for 24 h after placement, group C = fasting for 48 h after placement. Abbreviation: IgY, immunoglobulin Y.

was observed in the concentration of myristic acid (C14:0), palmitic acid (C16:0), heptadecanoic acid (C17:0), linoleic acid (C18:2), and arachidonic acid (C20:4) in the residual yolk.

Effects of Early Starvation on Hormone Levels

Regarding T_3 concentration in plasma (Figure 2), a significant difference was only observed at 72 h, with group C having a higher ($P < 0.05$) value than the other 2 groups. Group A showed higher ($P < 0.05$) plasma concentration of IGF-1 at 72, 120, and 168 h (Figure 2). And there was no significant difference ($P > 0.05$) between groups B and C. Group B showed the highest ($P < 0.05$) level of IGF-1 in the liver at 48 and 72 h (Figure 2), whereas at 120 and 168 h, group A had the highest ($P < 0.05$) level of IGF-1 in the liver, and there was no significant difference between groups A and B.

Early Feeding Promoted the Synthesis of Macronutrients in Tissues

As shown in Figure 3, group C showed higher ($P < 0.05$) concentration of triglyceride in the liver among the three groups at 48, 72, and 120 h. After feeding for 24 h, groups A, B, and C showed highest ($P < 0.05$) concentration of glycogen in the liver, respectively, at 24, 48, and 72 h. After that, group A showed the highest ($P < 0.05$) level of glycogen at 120 and 168 h in the liver. Regarding protein concentration in the liver, significant difference was only observed at 48 h such that group A had highest ($P < 0.05$) concentration of protein in the liver among the three groups. Significant difference of protein concentration in the breast muscle was observed at 24 and 120 h, in which group A showed a higher ($P < 0.05$) level than groups B and C (Figure 4). In addition, group A showed highest ($P < 0.05$) concentration of glycogen at 24, 48, 120, and 168 h.

Table 3. Effects of feed deprivation on concentration of amino acids in the residual yolk (g/100 g).

Amino acids	Time (h)	Group			SEM	P-value
		A	B	C		
Lysine	0	2.554	2.496	2.544	0.221	0.961
	24	1.645	1.888	1.891	0.162	0.314
	48	1.757 ^b	2.516 ^a	2.191 ^{a,b}	0.237	0.050
	72	1.145 ^b	0.345 ^c	1.900 ^a	0.051	0.000
Methionine	0	1.115	1.099	1.041	0.222	0.940
	24	0.912 ^b	2.869 ^a	3.065 ^a	0.313	0.001
	48	1.049	1.129	0.982	0.176	0.717
	72	0.683 ^b	1.467 ^a	1.630 ^a	0.182	0.004
Cystine	0	0.977	0.951	0.990	0.062	0.823
	24	0.850	0.924	0.933	0.125	0.773
	48	0.862 ^b	1.187 ^a	1.220 ^a	0.106	0.027
	72	0.775	1.096	0.964	0.106	0.062
Threonine	0	0.710	0.704	0.717	0.038	0.937
	24	1.519	1.429	1.442	0.229	0.916
	48	0.735 ^b	1.285 ^a	1.327 ^a	0.088	0.001
	72	0.414	0.454	0.496	0.092	0.688
Arginine	0	0.155	0.155	0.165	0.019	0.851
	24	0.155	0.153	0.155	0.019	0.994
	48	0.130 ^b	0.160 ^a	0.151 ^a	0.007	0.012
	72	0.053 ^b	0.157 ^a	0.134 ^a	0.022	0.008
Histidine	0	0.846	0.852	0.866	0.082	0.968
	24	0.556	0.834	0.884	0.146	0.129
	48	0.457 ^b	0.977 ^a	0.971 ^a	0.069	0.000
	72	0.551 ^b	0.518 ^b	0.804 ^a	0.074	0.016
Leucine	0	3.510	3.356	3.480	0.159	0.617
	24	3.627	4.043	4.013	0.513	0.680
	48	3.070 ^b	3.987 ^a	3.980 ^a	0.269	0.022
	72	2.125 ^b	2.728 ^{a,b}	3.178 ^a	0.343	0.039
Isoleucine	0	2.080	2.180	2.093	0.128	0.710
	24	1.437 ^b	2.942 ^a	3.180 ^a	0.460	0.018
	48	2.407	2.380	3.273	0.496	0.204
	72	2.617	2.560	1.927	0.434	0.285
Phenylalanine	0	2.179	2.256	2.279	0.129	0.728
	24	2.677	2.796	2.623	0.118	0.387
	48	2.162	2.657	2.509	0.163	0.055
	72	1.365 ^c	2.127 ^b	2.458 ^a	0.063	0.000
Tyrosine	0	0.934	0.912	0.996	0.074	0.540
	24	0.895	1.292	1.402	0.179	0.065
	48	0.837 ^b	1.063 ^a	1.040 ^a	0.056	0.012
	72	0.709	0.847	0.938	0.080	0.072
Valine	0	2.281	2.244	2.285	0.157	0.959
	24	2.869	3.165	3.251	0.358	0.567
	48	1.961 ^b	2.588 ^a	2.636 ^a	0.152	0.008
	72	1.502 ^b	2.183 ^{a,b}	2.452 ^a	0.288	0.040

^{a,b}Mean values within a row with unlike letters are significantly different ($P < 0.05$).

Mean values with their SEM (n = 6).

Group A = nonfasted, group B = fasting for 24 h after placement, group C = fasting for 48 h after placement.

Early Feeding Promoted Organ and Body Development

As shown in Figure 5, group A showed the largest ($P < 0.05$) relative weight of the liver at 24, 48, and 120 h, and there was no significant difference between groups A and B at 48 and 120 h. Conversely, group C showed largest ($P < 0.05$) relative weight of the liver among the three groups at 72 h. During 24 to 72 h, group A showed the largest ($P < 0.05$) relative weight ($P < 0.05$) of the pancreas among the three groups. The result of relative weight of the spleen was similar to that of pancreas except that group A still showed higher ($P < 0.05$) values than group C at 120 h. As shown in Figure 6, nonfasting after placement (group A) resulted in increase ($P < 0.05$) in BW during 24 to 168 h

significantly compared with feeding delay (groups B and C), and group C even showed the lowest ($P < 0.05$) performance of BW at 48 and 72 h, whereas no difference was observed between groups A and B at 120 and 168 h.

DISCUSSION

Owing to prolonged incubation time, manipulations, and transport to the farm, feed deprivation time for hatched chicks is delayed by up to 72 h in commercial poultry practice (Willemsen et al., 2010). Despite the residual yolk being sufficient to maintain chicks, however, it cannot provide the required level of nutrients to fully support the potential growth and development of the gastrointestinal tract or the immune system during the

Table 4. Effects of feed deprivation on contents of fatty acids in the residual yolk (%).

Fatty acids	Time (h)	Group			SEM	P-value
		A	B	C		
Myristic acid (C14:0)	0	3.108	3.018	3.165	0.325	0.902
	24	2.840	2.761	2.812	0.404	0.981
	48	2.533	2.787	2.550	0.125	0.158
	72	2.073	2.553	2.053	0.259	0.173
Palmitic acid (C16:0)	0	39.52	39.27	39.50	1.998	0.990
	24	38.83	36.85	37.75	1.933	0.601
	48	38.01	37.82	39.63	2.940	0.802
	72	32.22	32.98	33.64	2.625	0.866
Heptadecanoic acid (C17:0)	0	1.433	1.368	1.336	0.335	0.957
	24	1.704	1.751	1.732	0.229	0.979
	48	1.354	1.606	1.275	0.162	0.185
	72	1.534	1.161	1.448	0.159	0.124
Oleic acid (C18:1)	0	16.76	16.90	16.01	3.439	0.962
	24	19.82	20.23	19.89	1.173	0.932
	48	24.92	28.75	30.93	3.024	0.213
	72	25.43 ^b	29.39 ^{a,b}	32.53 ^a	1.813	0.022
Linoleic acid (C18:2)	0	17.44	17.48	17.04	0.889	0.863
	24	19.18	18.46	19.15	1.993	0.919
	48	19.00	17.88	18.89	0.573	0.178
	72	20.98	18.75	19.49	1.069	0.186
Linolenic acid (C18:3)	0	2.949	2.927	2.941	0.283	0.997
	24	1.721	1.624	1.786	0.145	0.563
	48	2.205	1.985	1.558	0.374	0.288
	72	1.287 ^b	1.966 ^a	1.545 ^{a,b}	0.180	0.025
Arachidonic acid (C20:4)	0	5.129	5.275	5.181	0.251	0.844
	24	5.007	5.214	5.267	0.217	0.494
	48	5.913	5.666	6.013	0.329	0.584
	72	9.536	8.187	7.589	0.689	0.073

^{a,b}Mean values within a row with unlike letters are significantly different ($P < 0.05$).

Mean values with their SEM (n = 6). Group A = nonfasted, group B = fasting for 24 h after placement, group C = fasting for 48 h after placement.

first 3 to 4 d of life (Panda et al., 2015). The exogenous nutrients are complementary to the yolk nutrients when chicks had access to feed after hatch (Murakami et al., 1992). In our experiment, compared with group C, early feeding (groups A and B) promoted the absorption of residual yolk in broiler chicks. The reason may be that intestinal motility of fed chicks facilitated the transfer of yolk (Bhanja et al., 2009).

The immune system of hatchlings requires oral nutrients for rapid development, which depends on the supply of early nutrition before or immediately after hatching (Noy and Uni, 2010). Chicks' humoral immunity depends on maternal antibodies (IgY) received from the yolk before the immune system is mature enough to produce its own B lymphocytes (Ulmer-Franco et al., 2012). A large amount of the egg protein in hatchlings is composed of maternal antibodies (Dibner et al., 1998). Bigot et al. (2001) suggested that in chicks that fasted for more than 24 h after hatch, degradation of the immunoglobulins in the residual yolk sac would take place to produce proteins to maintain their survival. Early feeding is not only associated with the development of immune organs but also associated with immune system function in chicks (Panda et al., 2015). It was reported that delay in access to feed may limit the utilization of the yolk sac and lead to a decline in the immunity of the newborn chick (Bhanja, 2008). In our experiment, the lower content of IgY and protein in the yolk of the feed deprivation group (groups B and C) indicated that nonfasting after hatch (group A) was conducive

to strengthening the chick's humoral immunity. It was indicated that feed deprivation after hatch depressed development of the spleen (Panda et al., 2010), which is consistent with our results. This could be attributed to an early antigen stimulus for early feeding chicks, further facilitating rapid differentiation of the immune organs (Rao et al., 1978).

There is still a high demand for minerals during the initial stages after hatching because of incompletely formed bones in chicks at hatch (Oliveira et al., 2015). In addition, mineral deficiency can cause skeletal, immune, and cardiovascular system disorders (Yair and Uni, 2011). Yair and Uni (2011) reported a very low concentration of microminerals (Zn, Cu, Mn) in the yolk at hatch. This may be the reason that we did not precisely detect the concentration of Zn, Cu, and Mn in the residual yolk. Embryo and chicken growth and development rely on mineral nutrition (Yair and Uni, 2011). In our experiment, nonfasting (group A) promoted the absorption of Ca, P, Na, and Fe overall in the residual yolk, suggesting that early feeding was beneficial to tissue development in chicks.

Many researchers aimed to increase chick weight at hatch through AA administration in ovo (Bhanja and Mandal, 2005). In this experiment, nonfasting (group A) promoted AA absorption in the residual yolk. Fatty acids are stored in egg yolk in the form of triacylglycerol and phospholipids (De Oliveira et al., 2008). Feed-deprived poult had a greater use of FA owing to increased plasma concentrations of nonesterified FA

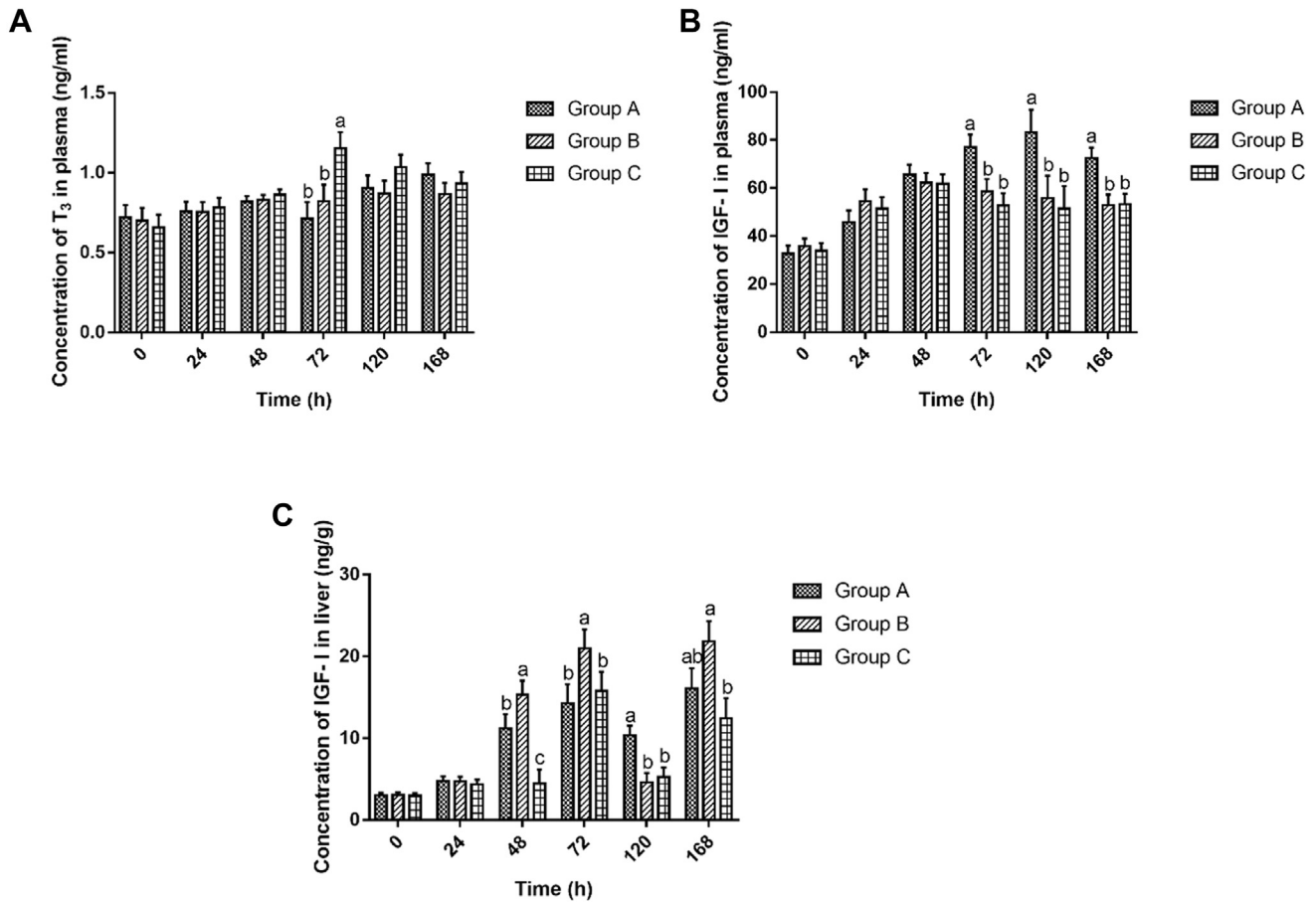


Figure 2. Effects of feed deprivation on the concentration of (A) T₃ in plasma, (B) IGF-1 in plasma, and (C) IGF-1 in the liver. Mean values with their SEM (n = 6). ^{a,b,c}Mean values within a row with unlike letters are significantly different ($P < 0.05$). Group A = nonfasted, group B = fasting for 24 h after placement, group C = fasting for 48 h after placement. Abbreviations: IGF-1, insulin-like growth factor 1; T₃, triiodothyronine.

(Noy et al., 2001). Increased lipid intake reduced the percentage of oleic acid absorption (Noy and Sklan, 2001). Preferentially selective absorption of some FA by the embryo during incubation was presented by Sahan et al., 2014. There is nearly no research about changes of FA absorption and lipid synthesis under feed deprivation. In our results, feed deprivation (groups B and C) showed no significant difference on the absorption of FA except oleic acid (C18:1) and linolenic acid (C18:3), but promoted the synthesis of lipid in the liver. The reason may be that chicks with delayed access to feed used FA more efficiently than chicks fed immediately. We need more further research studies to explore the mechanism of lipid synthesis under feed deprivation.

Insulin-like growth factor 1 can stimulate the growth of the skeletal muscle by enhancing the rate of protein synthesis, and the concentration of IGF-1 is usually positively related to the BW of broiler chickens (Wen et al., 2014). Kita et al. (2002) demonstrated that feed deprivation reduces plasma IGF-1 levels compared with conventional chicks. In our findings, higher concentration of IGF-1 both in plasma and the liver was observed in chicks subjected to nonfasting (group A). Feed deprivation depressed the plasma T₃ concentrations in chicks and poults (Reyns et al., 2002), which indicating a lower metabolic rate. However, after refeeding, T₃

concentration increased significantly to levels found in the fed groups (Careghi et al., 2005). In our results, there was no decrease observed in T₃ concentration in plasma among the three groups. Conversely, the concentration of T₃ concentration in group C increased compared with the other 2 groups at 72 h. This only suggested a higher metabolic rate in chicks when refeeding for 24 h after fasting for 48 h.

It was concluded that muscle ribosomal S6 kinase 1 (a key element in the control of protein synthesis) is activated only in the presence of feed (Bigot et al., 2003). Delayed feeding exacerbated the poor nutritional status and had further negative effects on protein synthesis of the muscle and finally the growth and development of chicks (Kadam et al., 2013). Our results found negative effects on protein synthesis in the breast muscle caused by feed deprivation (groups B and C). Our research also found that posthatch starvation (groups B and C) decreased the glycogen concentration in the breast muscle at 24, 48, 120, and 168 h after placement. This is supported by Kornasio et al. (2011) who found that birds in the early feeding group exhibited 3-fold increase in muscle glycogen. The starvation significantly decreased the blood glucose level and liver glycogen content in chicks after hatch (Wang et al., 2014). When the chicks had access to feed, glycogen reserves begin to be replenished

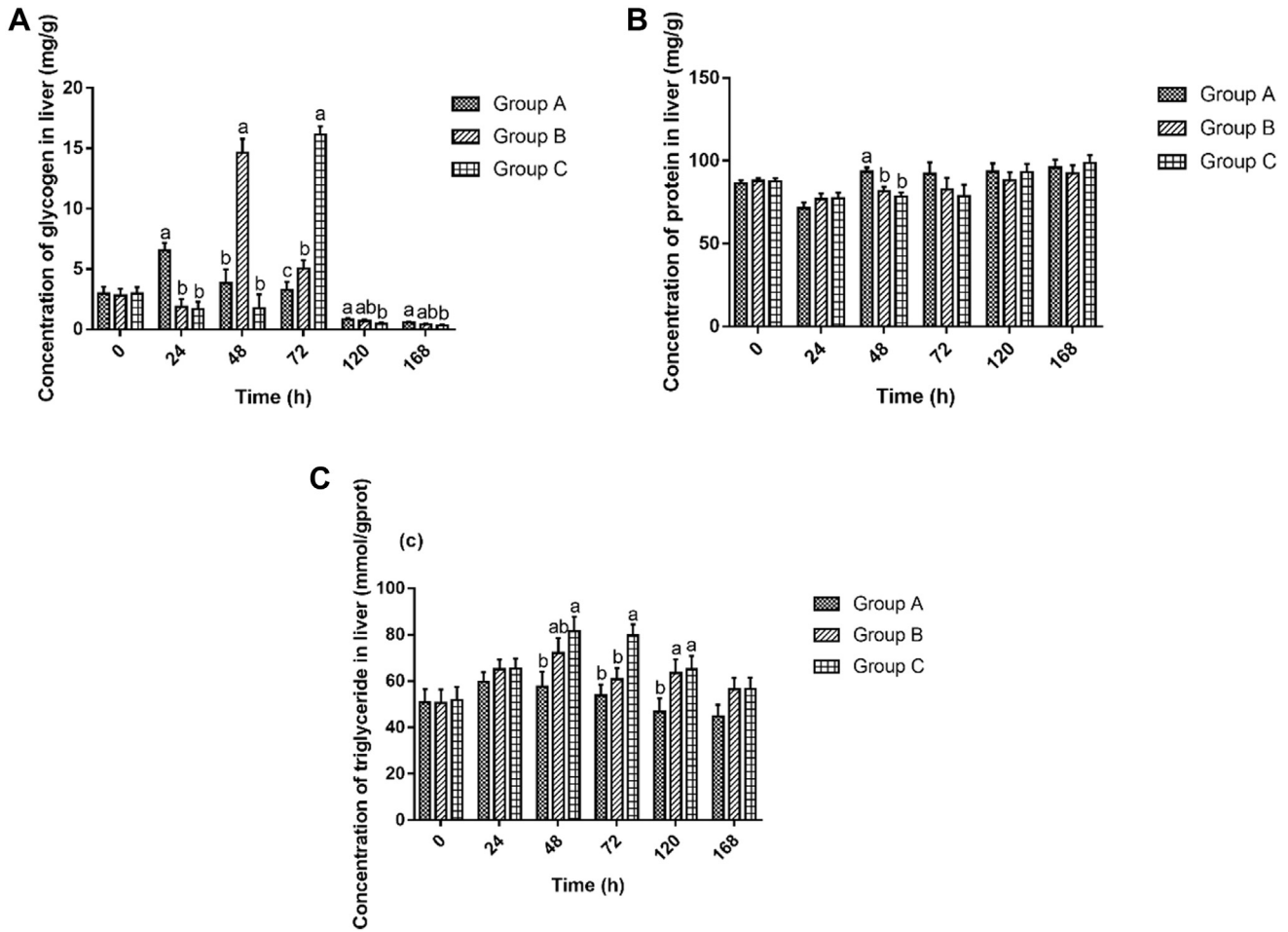


Figure 3. Effects of feed deprivation on the concentration of (A) glycogen, (B) protein, and (C) triglyceride in the liver. Mean values with their SEM ($n = 6$). ^{a,b,c}Mean values within a row with unlike letters are significantly different ($P < 0.05$). Group A = nonfasted, group B = fasting for 24 h after placement, group C = fasting for 48 h after placement.

(Moran, 2007). Compared with the direct-fed chicks, chicks without feed access exhibited a very low level of glycogen in the liver (Kornasio et al., 2011). This is in

agreement with our results, in which nonfasting (group A) increased the liver glycogen concentration significantly.

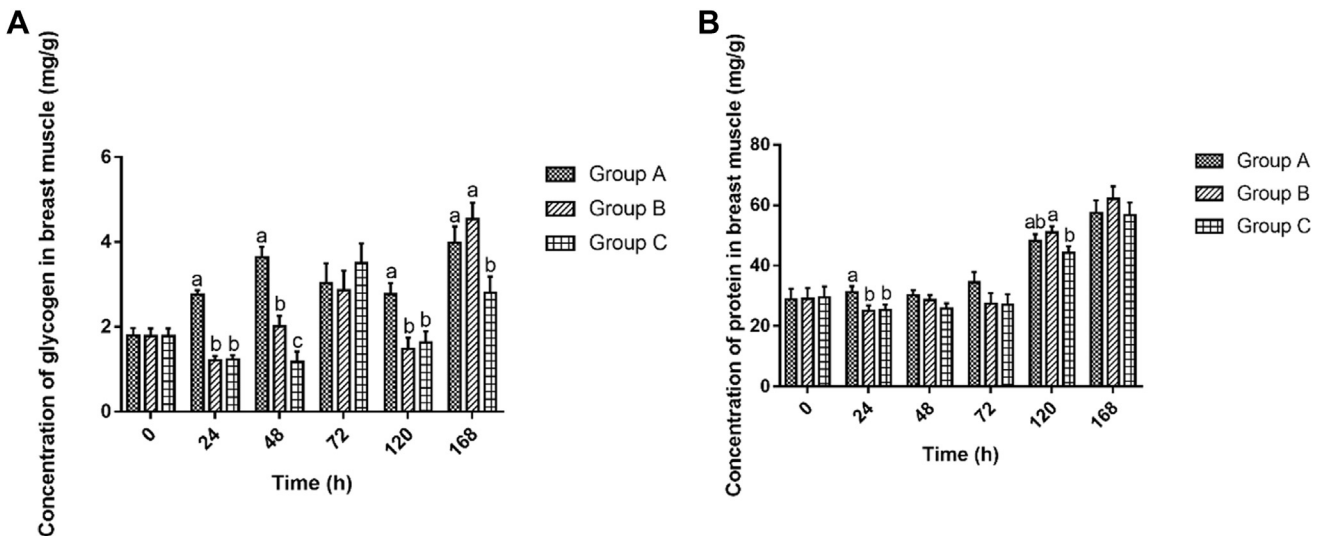


Figure 4. Effects of feed deprivation on the concentration of (A) glycogen and (B) protein in the breast muscle. Mean values with their SEM ($n = 6$). ^{a,b,c}Mean values within a row with unlike letters are significantly different ($P < 0.05$). Group A = nonfasted, group B = fasting for 24 h after placement, group C = fasting for 48 h after placement.

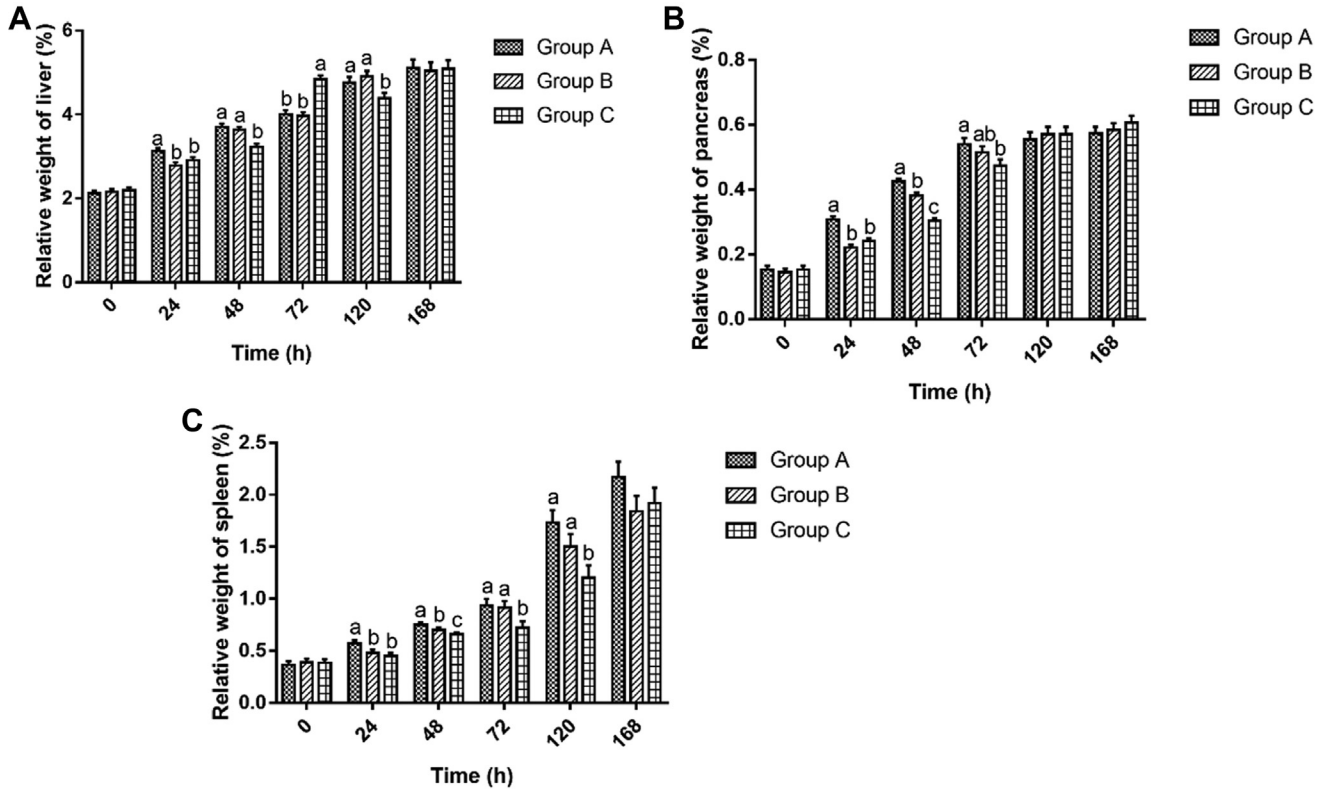


Figure 5. Effects of feed deprivation on relative weight of the (A) liver, (B) pancreas, and (C) spleen. Mean values with their SEM (n = 6). ^{a,b,c}Mean values within a row with unlike letters are significantly different ($P < 0.05$). Group A = nonfasted, group B = fasting for 24 h after placement, group C = fasting for 48 h after placement.

The relative weight of the liver, pancreas, heart, and jejunum was higher for direct-fed chicks than for delayed fed chicks (de Jong et al., 2017). In our research, we also found higher relative liver weight in nonfasted chicks (group A), which is concomitant with higher relative weight of the pancreas, spleen, and small intestines (Wang et al., unpublished data). Through meta-analysis, it was indicated that posthatch feed and water deprivation resulted in significantly lower BW in chicks than in chickens fed immediately up to 6 wk of age (de Jong et al., 2017). Lamot et al. (2014) also reported that direct feed intake resulted in higher BW gain and feed intake than delayed feed access up to day 7. Bhuiyan et al.

(2011) found feeding immediately after hatch increased BW of both chicks. Fasting for 48 to 72 h after hatching resulted in weight loss, delayed intestinal development, and lower pectoral muscle weight (Kornasio et al., 2011). Our findings were in accordance with these previous studies, in which feed deprivation for 24 or 48 h (groups B and C) decreased BW of hatchlings until 168 h after hatch.

To conclude, our results indicated that nonfasting (group A) promote the absorption of the yolk sac and its internal contents including AA, FA, minerals, protein, and IgY. In addition, nonfasting (group A) promoted the synthesis of protein and glycogen in tissues. Conversely, feed deprivation (groups B and C) promoted the synthesis of triglycerides in the liver. In addition, nonfasting (group A) promoted the development of the liver, pancreas, and spleen and finally body growth. It is not clear how the body absorbs nutrients in the yolk and whether there is a selective absorption of nutrients. Further studies are needed to explore the mechanism of lipid synthesis of chicks under feed deprivation. It is recommended that early feeding could promote residual yolk utilization and body growth because the first week is a vital part of the whole life span in broiler chicks.

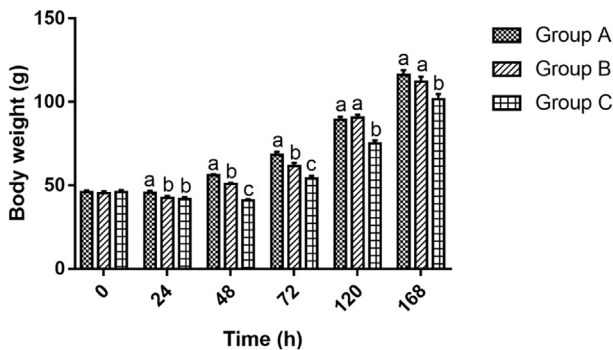


Figure 6. Effects of feed deprivation on body weight. Mean values with their SEM (n = 6). ^{a,b,c}Mean values within a row with unlike letters are significantly different ($P < 0.05$). Group A = nonfasted, group B = fasting for 24 h after placement, group C = fasting for 48 h after placement.

ACKNOWLEDGMENTS

This study was supported by China Agriculture Research System (CARS-41-G20, Beijing, China) and

Zhejiang Province Key R&D Program (2013C02010; 2018C02035, Hangzhou, China).

Conflict of Interest Statement: The authors did not provide a conflict of interest statement.

SUPPLEMENTARY DATA

Supplementary data associated with this article can be found in the online version at <https://doi.org/10.1016/j.psj.2020.08.032>.

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