

# Effect of a Large-sized Cage with a Low Metabolizable Energy and Low Crude Protein Diet on Growth Performance, Feed Cost, and Blood Parameters of Growing Layers

Yi Wan, Ruiyu Ma, Yan Li, Wei Liu, Junying Li and Kai Zhan

Anhui Key Laboratory of Livestock and Poultry Product Safety Engineering, Institute of Animal Husbandry and Veterinary Medicine, Anhui Academy of Agriculture Science, NO. 40 Nongke West Road, Hefei 230031, P. R. China

Effects of a large-sized cage with a low metabolizable energy and low crude protein (LME-LCP) diet on growth performance, feed cost, blood biochemistry, and antibody response of growing layers were evaluated. A total of 668 one-day-old female Gushi chicks were randomly allocated into three different cages, namely, large, medium, and small cages, referred to as Group A, Group B, and Group C, respectively, and fed LME-LCP diets. A fourth group of birds raised in small cages and fed a standard diet (STD) was designated Group D i.e. the control. Equal stocking densities were maintained among the four groups throughout the experiment, from 50–20 birds/m<sup>2</sup>. Large cages with LME-LCP diet (Group A) increased the shank length and girth as chicks grew, improved the activities of serum creatine kinase, and reduced serum triglyceride and cholesterol concentrations. The total feed intake in birds from Group A was higher than those from the other groups at every stage. The total cost (rmb/bird) of feed was 6.70% lower in Group A than that in Group D, which indicated the cost-effectiveness of large cages. In conclusion, large cages with LME-LCP diets have positive effects on body weight, shank growth, and serum biochemical indices of growing Gushi chicks, and can reduce feed costs.

**Key words:** blood parameter, growth performance, immune response, large cage, layer

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## Introduction

Cage design is a component of a hen's environment and plays a critical role in determining its well-being (Widowski *et al.*, 2016). The effect of cage size on chickens has been studied in different ways. Decreased cage space reportedly decreases biological function, egg production, egg weight, body weight, and feed intake, and increases mortality (Sohail *et al.*, 2004; Hartcher and Jones, 2017). Chickens grow more slowly and jostle each other more at higher stocking densities, and a narrow living space can restrain their movements and activities, which leads to osteoporosis (Stamp *et al.*, 2004; Webster, 2004). In contrast, enough free space for movement can allow caged hens to perform most normal

patterns of behavior (Mench and Blatchford, 2014). It has been reported that foraging, wing stretching, leg stretching, and tail wagging all increase in frequency when chickens are housed in larger cages (Lay *et al.*, 2011), and that larger spaces for chickens are associated with improved bone mass and bone quality (Eric *et al.*, 2015). Similarly, Meng *et al.* (2016) found that large furnished cages allowed hens to have stronger or heavier tibias than small furnished cages. Our previous study (Li *et al.*, 2019) indicated that large and medium cages were superior to small cages and were beneficial for the growth and development of birds. Therefore, increasing the space in cages could be advantageous for poultry production.

In addition to the various reported effects of cage size on the performance of chickens, differing diets have also been implicated as having an effect on the growth, carcass traits, and blood serum parameters of hens. Low metabolizable energy (LME) and low crude protein (LCP) diets reportedly enhanced immune functions and feed conversion ratio (Nahashon *et al.*, 2007; Sigolo *et al.*, 2017). Moreover, Kidd *et al.* (2001) reported that LCP diets improved protein efficiency and increased feed intake. However, few studies have been conducted on the synergistic effect of cage size and diet

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Correspondence: Kai Zhan, Institute of Animal Husbandry and Veterinary Medicine, Anhui Academy of Agriculture Science, NO. 40 Nongke West Road, Hefei 230031, P. R. China. (E-mail: zhankai633@126.com)

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on chicken performance. Madrid *et al.* (1981) reported that maintenance energy requirements increased as cage space decreased, whereas Jalal *et al.* (2006) found that energy intake increased as cage space increased.

Based on the abovementioned findings, cage size plays an important role in enhancing the well-being of chickens and affects their energy needs, and larger cages are expected to improve chicken growth. In this study, we evaluated the effects of a large-sized cage with LME-LCP diets on the growth performance, blood parameters, and immune responses of growing layers.

### Material and Methods

The study protocol was approved by the Committee for the Care and Use of Experimental Animals at Anhui Academy of Agricultural Science under permit no. A11-CS06.

#### *Birds and Dietary Treatments*

Gushi chickens, originating from central China, are used as a dual-purpose breed. A total of 668 one-day-old female Gushi chicks were obtained from Anhui Wanxi Poultry Development Co., Ltd. (Luan, China) and assigned to two dietary treatments (Table 1): a standard diet (STD) and an LME-LCP diet. The STD contained 12.38 MJ of metabolizable energy (ME)/kg and 20.13% crude protein (CP) from days 1–28, 12.68 MJ of ME/kg and 18.20% CP from days 29–56 and 12.72 MJ of ME/kg and 16.03% CP from days 57–98. The LME-LCP diet contained 2% less CP than the STD at each growth stage and 6.7%, 6.0%, and 5.9% less ME than the STD at the three growth stages, respectively. The diets were provided in mash form, and the feeder space among the treatments was kept identical. Feed and water were provided *ad libitum*. Feeds were prepared once per

week to avoid mildew.

#### *Cages and Grouping*

This study was performed using three different-sized cages for birds: a large cage (1.6×1.6×0.42 m), medium cage (1.2×1.2×0.42 m), and small cage (0.8×0.7×0.37 m). Experimental birds raised in large, medium, and small cages and fed the LME-LCP diet were assigned to Group A, Group B, and Group C, respectively. A fourth group of birds raised in small cages and fed STD was assigned to Group D as a control. The experiment lasted for 14 weeks. The stocking densities of the four groups, which were identical and adjusted with age during the experimental period (Table 2), were 50 birds/m<sup>2</sup> (from 1–14 days), 40 birds/m<sup>2</sup> (from 15–28 days), 30 birds/m<sup>2</sup> (from 29–42 days), 25 birds/m<sup>2</sup> (from 43–56 days), 22 birds/m<sup>2</sup> (from 57–70 days), and 20 birds/m<sup>2</sup> (from 71–98 days).

All the birds were reared in the same experimental room. The room temperature was 32°C at the start of the trial and was reduced gradually to 20°C by 21 days of age and kept at that temperature for the remaining experimental period. The light regimen at 1 day old was 24 h light (L):0 h darkness (D), which was stepped down to 9L:15D by 50 days of age, and maintained until the end of the study. Birds were vaccinated in accordance with the standard vaccination schedule.

#### *Measurements and Sampling*

On day 1, the chicks were individually weighed, wing-tagged, and separated into different groups. Body weight, shank length, and shank girth were measured every 2 weeks (from 1–14 weeks). Body weight was measured using an electronic scale. Shank length was measured using a digital Vernier caliper while shank girth was measured using a tape rule. Feed intake and mortality per pen were recorded daily.

Table 1. **Ingredient composition and nutrient levels of experimental diets fed from days 1–98**

Age (days)	1–28		29–56		57–98	
	STD <sup>1</sup>	LME-LCP <sup>2</sup>	STD	LME-LCP	STD	LME-LCP
	Ingredients, g/kg					
Corn	503.2	618.1	549.0	660.1	617.2	728.2
Soybean meal	367.3	301.2	315.3	250.1	254.2	189.2
Soybean oil	55.0	6.2	61.2	15.3	54.1	8.1
Limestone	25.0	25.0	25.0	25.0	25.0	25.0
Salt	3.0	3.0	3.0	3.0	3.0	3.0
Premix <sup>3</sup>	46.5	46.5	46.5	46.5	46.5	46.5
	Calculated level					
ME, MJ/kg	12.38	11.55	12.68	11.92	12.72	11.97
CP, %	20.13	18.13	18.20	16.20	16.03	14.03
Lysine, %	1.10	1.00	1.00	0.83	0.83	0.68
Methionine, %	0.45	0.42	0.42	0.40	0.38	0.36
Methionine + cystine, %	0.82	0.75	0.74	0.71	0.68	0.62
Calcium, %	1.00	1.00	1.00	1.00	1.00	1.00
Available phosphorus, %	0.47	0.43	0.42	0.42	0.40	0.38

<sup>1</sup> STD: Standard diet.

<sup>2</sup> LME-LCP: low metabolizable energy and low crude protein diet.

<sup>3</sup> Premix provided the following per kg of diet: copper, 10.0 mg; iron, 30.0 mg; manganese, 60.0 mg; zinc, 65.0 mg; selenium, 0.3 mg; retinol, 2.7 mg; cholecalciferol, 77.5 mg; tocopheryl acetate, 16.7 mg; menadione, 0.5 mg; thiamine, 5 mg; riboflavin, 2 mg; cyanocobalamin, 0.02 mg; pyridoxine, 3.5 mg; biotin, 0.1 mg; folacin, 1 mg; pantothenic acid, 12 mg; and nicotinic acid, 38 mg.

Table 2. The stocking density of different groups of Gushi chickens during different growth periods

Group <sup>1</sup>	Age (days)	1 <sup>st</sup>	14 <sup>th</sup>	28 <sup>th</sup>	42 <sup>nd</sup>	56 <sup>th</sup>	70 <sup>th</sup> –98 <sup>th</sup>
<b>Group A,</b> large cages, 1.6 m×1.6 m	Number of cages	2	2	3	3	3	3
	Birds/cage	128	102	76	64	56	51
	Total number of birds	256	204	228	192	168	153
	Base area of cage (m <sup>2</sup> )				2.56		
<b>Group B,</b> medium cages, 1.2 m×1.2 m	Number of cages	3	3	5	5	5	5
	Birds/cage	72	58	43	36	32	29
	Total number	216	174	215	180	160	145
	Base area of cage (m <sup>2</sup> )				1.44		
<b>Groups C and D,</b> small cages, 0.8 m×0.7 m	Number of cages	7	8	11	13	12	13
	Birds/cage	28	22	17	14	12	11
	Total number	196	176	187	182	144	143
	Base area of cage (m <sup>2</sup> )				0.56		
	<b>Stocking density (birds/m<sup>2</sup>)</b>	50	40	30	25	22	20

<sup>1</sup> Groups A, B, and C were fed a low metabolizable energy and low crude protein (LME-LCP) diet; Group D was fed a standard (STD) diet.

For each group, 30 birds were randomly selected for blood sampling on days 56, 70, 84, and 98 days. A 4-mL blood sample was collected into two heparinized tubes (2 mL in each tube) from the chickens by wing vein puncture. The time between catching the bird and taking the blood sample did not exceed 45 s. Samples were placed in an ice bath immediately after collection, and then transported to the laboratory for processing. Blood serum was separated by centrifugation at 3000 rpm for 10 min and stored at  $-20^{\circ}\text{C}$  until analysis.

The collected serum was assayed for levels of corticosterone (CORT), creatine kinase (CK), triglyceride (TG), total cholesterol (T-CH), blood urea nitrogen (BUN), and glutathione (GSH). The concentrations of these parameters were determined with an automatic biochemical analyzer (Tecan, Männedorf, Switzerland) using commercial laboratory kits (Xinqidi Biotech Co., Wuhan, China). Antibody titers of avian influenza viruses, H5N1 (Re-5 strain) and H9N2 (Re-2 strain), and Newcastle disease virus (NDV) were determined using enzyme-linked immunosorbent assay (ELISA) kits (Mlbio Biotech Co., Shanghai, China) according to the manufacturer's protocol. Antibody titer data were logarithmically transformed (base 2) prior to analysis.

#### Statistical Analysis

Data were subjected to analysis of variance (ANOVA) using the General Linear Model (GLM) command in SAS version 9.3 statistical software (SAS Institute Inc., Cary, NC, USA). When differences among individual means were found in ANOVA tests ( $P < 0.05$ ), the means were compared using Tukey's multiple test.

## Results

### Growth Performance

The effects of large cages fed with LME-LCP diet on body

weight, shank length, and shank girth of chickens over the 14-week study period are presented in Table 3. The body weight of Group D (fed STD) was significantly higher ( $P < 0.05$ ) than those of Groups A, B, and C, which were fed LME and LCP diets during the early period (from 14–56 days). As the hens aged, the body weights of Groups A, B, and D were similar from days 70–98, while birds from Group C were significantly ( $P < 0.05$ ) lighter in weight than those raised in the other groups. No significant differences were found in shank length between Groups A and D from days 14–70 except on the 42<sup>nd</sup> day. Large cages (Group A) appeared to increase shank length from 8.08–8.47 cm between 84 and 98 days of age, which were significantly ( $P < 0.05$ ) greater than those observed in Groups C and D. A similar trend was found in the values of shank girth in which chickens from Group A had a greater shank girth than those in the other three groups from days 42–98, while the shank girth of Group C was significantly smaller than those of the other three groups.

### Feed Cost

The consumption and cost of feed of different groups are shown in Table 4. The total feed intake of Group A was 3775.76 g/bird, which was higher than that observed for the other groups at every stage, while Group C (small cages with LME-LCP diet) had the lowest total feed intake of 3392.04 g/bird. In terms of total feed cost per bird throughout the experiment (from birth to 98 days), the four groups ranked as follows: Group D > Group A > Group B > Group C, with values of 10.60 rmb, 9.89 rmb, 9.47 rmb, and 8.87 rmb, respectively.

### Blood Parameters

The effects of cage type on blood serum parameters are given in Fig. 1. Birds in large cages that received the LME-LCP diet (Group A) had higher ( $P < 0.05$ ) blood serum con-

Table 3. The body weight, shank length, and shank girth of different groups of Gushi chickens during different growth periods

Age (days)	LME-LCP <sup>1</sup>				STD <sup>2</sup>				P-value
	Group A	N <sup>3</sup>	Group B	N	Group C	N	Group D	N	
	Mean body weight, g								
14 <sup>th</sup>	91.56 <sup>a</sup>	256	89.08 <sup>a</sup>	216	91.94 <sup>a</sup>	196	97.80 <sup>b</sup>	196	<0.001
28 <sup>th</sup>	189.36 <sup>a</sup>	204	184.31 <sup>a</sup>	174	197.36 <sup>b</sup>	176	210.73 <sup>c</sup>	176	<0.001
42 <sup>nd</sup>	340.92 <sup>a</sup>	228	331.06 <sup>ab</sup>	215	322.36 <sup>b</sup>	187	356.94 <sup>c</sup>	187	<0.001
56 <sup>th</sup>	524.17 <sup>a</sup>	192	520.85 <sup>a</sup>	180	507.62 <sup>a</sup>	182	553.04 <sup>b</sup>	182	<0.001
70 <sup>th</sup>	727.95 <sup>a</sup>	168	706.77 <sup>a</sup>	160	675.62 <sup>b</sup>	144	728.59 <sup>a</sup>	144	<0.001
84 <sup>th</sup>	870.77 <sup>a</sup>	153	887.15 <sup>a</sup>	145	824.59 <sup>b</sup>	143	866.19 <sup>a</sup>	143	<0.001
98 <sup>th</sup>	1006.30 <sup>a</sup>	153	1009.99 <sup>a</sup>	145	945.29 <sup>b</sup>	143	1006.95 <sup>a</sup>	143	<0.001
	Shank length, cm								
14 <sup>th</sup>	3.25 <sup>a</sup>	256	3.06 <sup>b</sup>	216	3.10 <sup>b</sup>	196	3.21 <sup>a</sup>	196	<0.001
28 <sup>th</sup>	4.33 <sup>ab</sup>	204	4.26 <sup>a</sup>	174	4.37 <sup>b</sup>	176	4.26 <sup>a</sup>	176	0.012
42 <sup>nd</sup>	5.18 <sup>a</sup>	228	5.12 <sup>a</sup>	215	5.13 <sup>a</sup>	187	5.31 <sup>b</sup>	187	0.002
56 <sup>th</sup>	6.47 <sup>ac</sup>	192	6.37 <sup>ad</sup>	180	6.31 <sup>bd</sup>	182	6.57 <sup>c</sup>	182	<0.001
70 <sup>th</sup>	6.95 <sup>a</sup>	168	6.93 <sup>a</sup>	160	6.80 <sup>b</sup>	144	7.02 <sup>a</sup>	144	0.005
84 <sup>th</sup>	8.08 <sup>a</sup>	153	8.05 <sup>a</sup>	145	7.75 <sup>b</sup>	143	7.54 <sup>c</sup>	143	<0.001
98 <sup>th</sup>	8.47 <sup>a</sup>	153	8.26 <sup>b</sup>	145	8.16 <sup>b</sup>	143	8.26 <sup>b</sup>	143	<0.001
	Shank girth, cm								
14 <sup>th</sup>	1.71 <sup>a</sup>	256	1.73 <sup>ab</sup>	216	1.75 <sup>b</sup>	196	1.75 <sup>b</sup>	196	0.008
28 <sup>th</sup>	2.20 <sup>ac</sup>	204	2.17 <sup>bc</sup>	174	2.23 <sup>ad</sup>	176	2.27 <sup>d</sup>	176	<0.001
42 <sup>nd</sup>	2.70 <sup>a</sup>	228	2.66 <sup>ab</sup>	215	2.61 <sup>b</sup>	187	2.66 <sup>a</sup>	187	0.005
56 <sup>th</sup>	2.97	192	2.94	180	2.93	182	2.95	182	0.400
70 <sup>th</sup>	3.24 <sup>a</sup>	168	3.22 <sup>a</sup>	160	3.09 <sup>b</sup>	144	3.20 <sup>a</sup>	144	<0.001
84 <sup>th</sup>	3.42 <sup>a</sup>	153	3.41 <sup>a</sup>	145	3.34 <sup>b</sup>	143	3.41 <sup>a</sup>	143	<0.001
98 <sup>th</sup>	3.58 <sup>a</sup>	153	3.55 <sup>a</sup>	145	3.49 <sup>b</sup>	143	3.53 <sup>ab</sup>	143	<0.001

<sup>a-d</sup> Means within a day with different superscripts are significantly different ( $P < 0.05$ ).

<sup>1</sup> LME-LCP: low metabolizable energy and low crude protein diet.

<sup>2</sup> STD: standard diet.

<sup>3</sup> N: number of birds.

concentrations of CK than those in the other groups throughout the experiment but lower concentrations of T-CH and BUN from days 56–84. Similar levels of BUN were found between Groups C and D throughout the experiment. Birds in small cages that received STD (Group D) had lower blood serum concentrations of CK and GSH than those in the other groups throughout the experiment. There were no significant differences ( $P > 0.05$ ) in CORT among the four groups throughout the experiment. The concentrations of TG and T-CH were higher in Groups B and C than in Groups A and D ( $P < 0.05$ ) throughout the experiment.

#### Antibody Titer

As shown in Table 5, there were no significant differences in immune responses to H5N1 between any groups at 56, 70, and 84 days of age. The antibody titer of H9N2 in Group A was similar to that in the other groups at 56 days of age but was higher at 70, 84, and 98 days. No significant differences in the antibody titer of NDV were observed between Groups A and D throughout the experiment. The antibody titer of NDV in Group B was higher than that in the other groups at 70, 84, and 98 days of age.

#### Discussion

The differences in various indicators among groups showed

the adaptability of birds to different-sized cages. Our previous study (Li *et al.*, 2019) revealed that larger cages would provide more space for birds and promote the growth performance of Jinghong chickens. However, further studies are required to investigate the superiorities of large cage in different breeds. In the present study, different dietary energy and protein contents were used to evaluate the effect of different-sized cages on Gushi chicks.

Theoretically, birds raised in larger cages should have larger space allowances and altered dynamics of space use, which could contribute to growth, than those in small cages at an equal stocking density (birds/m<sup>2</sup>), which have less activity space (Appleby, 2004; Widowski *et al.*, 2017). Body weight in Group D was greater than that in the other groups from days 14–56, which suggested that energy and protein supply were more important for growth than cage size when birds were young and small. The larger space allowances did not yield apparent benefits, because of the small size of the chicks at this stage; the space available in the small cages was enough for their activities. However, the body weights of chicks were similar between Groups A and D from days 70–98, which indicated that the large cages promoted weight gain during this period despite being fed LME-LCP diet. Moreover, shank length and shank girth were greater in

**Table 4. The consumption and cost of feed per bird among the four groups of Gushi chickens during different growth periods**

Age (days)	Item	LME-LCP <sup>1</sup>			STD <sup>2</sup>		P-value
		Group A	Group B	Group C	Group D		
1-14	Mean feed intake	10.01	9.22	9.52	9.97	0.720	
15-28	(g/bird/day)	17.81	18.39	16.41	17.21	0.556	
	Total feed intake (g/bird)	389.49	386.61	362.99	380.49		
	Feed prices (rmb/kg)		2.7		3.10		
	Cost (rmb/bird)	1.05	1.04	0.98	1.18		
29-42	Mean feed intake	29.35 <sup>a</sup>	24.51 <sup>bc</sup>	23.55 <sup>b</sup>	26.82 <sup>ac</sup>	<0.001	
43-56	(g/bird/day)	46.58 <sup>a</sup>	42.25 <sup>b</sup>	33.69 <sup>c</sup>	32.92 <sup>c</sup>	<0.001	
	Total feed intake (g/bird)	1063.12	934.52	801.38	836.41		
	Feed prices (rmb/kg)		2.7		3.07		
	Cost (rmb/bird)	2.87	2.52	2.16	2.57		
57-70	Mean feed intake	53.15	50.75	51.93	53.61	0.153	
71-84	(g/bird/day)	56.30	56.31	53.55	54.78	0.200	
85-98	Total feed intake (g/bird)	56.48	57.19	53.63	57.42	0.264	
	Total feed intake (g/bird)	2323.15	2299.48	2227.67	2321.22		
	Feed prices (rmb/kg)		2.57		2.95		
	Cost (rmb/bird)	5.97	5.91	5.73	6.85		
1-98	Total feed intake (g/bird)	3775.76	3620.61	3392.04	3538.12		
	Total Cost (rmb/bird)	9.89	9.47	8.87	10.60		

<sup>a-d</sup> Means in the same time period with different superscripts are significantly different ( $P < 0.05$ ).

<sup>1</sup> LME-LCP: low metabolizable energy and low crude protein diet.

<sup>2</sup> STD: Standard diet.

Group A than in the other groups during the late stage of the experiment (from days 84-98), which also reflected the positive effects of large cages on shank growth as the birds aged. These effects were probably due to the larger free space and increased exercise/activity of the birds in the large cages, which are conducive for growth and feed intake (Widowski *et al.*, 2016; Li *et al.*, 2019). Similarly, Widowski *et al.* (2003) found that increasing space allowance during rearing improved the body weight of pullets. Birds in Group C, which were grown in small cages and fed an LME-LCP diet, had lower body weight, shank length, and shank girth than those in the other groups (from 56-98 days), as expected.

Providing space allowance does have consequences for feed intake in caged laying chickens (Widowski *et al.*, 2017). The feed intake in Groups A and B was higher than that in Group D; this could be due to the relatively larger space in the cage. This is consistent with the finding of Sohail *et al.* (2004) who reported that increasing cage space per hen increased 14-week average feed intake. Similarly, Jalal *et al.* (2006) reported that feed intake increased by 6.30 g/hen per day as cage space increased from 342-690 cm<sup>2</sup>/hen. In addition, birds from Group A had a higher feed intake, which resulted in a lower total cost of feed consumption than that observed for Group D (6.70% reduction per bird). This finding is similar to that in a previous study (Morris, 1968), in which a low-calorie diet could reduce the cost of feeding.

The release of CK is thought to be proportional to the intensity and duration of exercise (Apple, 1981), while exercise can briefly lower serum TG (Oscari *et al.*, 1972) and cholesterol (Johnson *et al.*, 1959) levels. Hens are social animals and sometimes gather under natural conditions (Xiang *et al.*, 2016); larger spaces appear in larger cages, which motivates the activities of birds. In this study, we observed a higher CK level and lower TG and T-CH levels in birds from Group A than in those from Group B and C, which indicated that large-sized cage is conducive for facilitating movements of birds and lowering their blood lipids.

GSH, an important intracellular antioxidant, plays a vital role in antioxidant defense mechanisms (Schulz *et al.*, 2010). Previous studies have stated that small space allowance reduced serum GSH-Px level but did not change the serum GSH level in Ross-308 broiler chickens (Simsek *et al.*, 2009), and plasma GSH-Px levels in Jinghong layer breeder males from small cages were higher than that in those from large and middle cages (Li *et al.*, 2019). In this study, serum GSH levels were lower in the birds from Group D than in those from the other groups throughout the experiment, which did not show the superiority of large-sized cage or low-calorie diets in oxidation resistance. These differences among studies could be attributed to the differences in dietary nutrient levels and chicken breeds.

Low space allowance in cages could induce chronic stress in birds, which affects CORT, an indicator of physiological

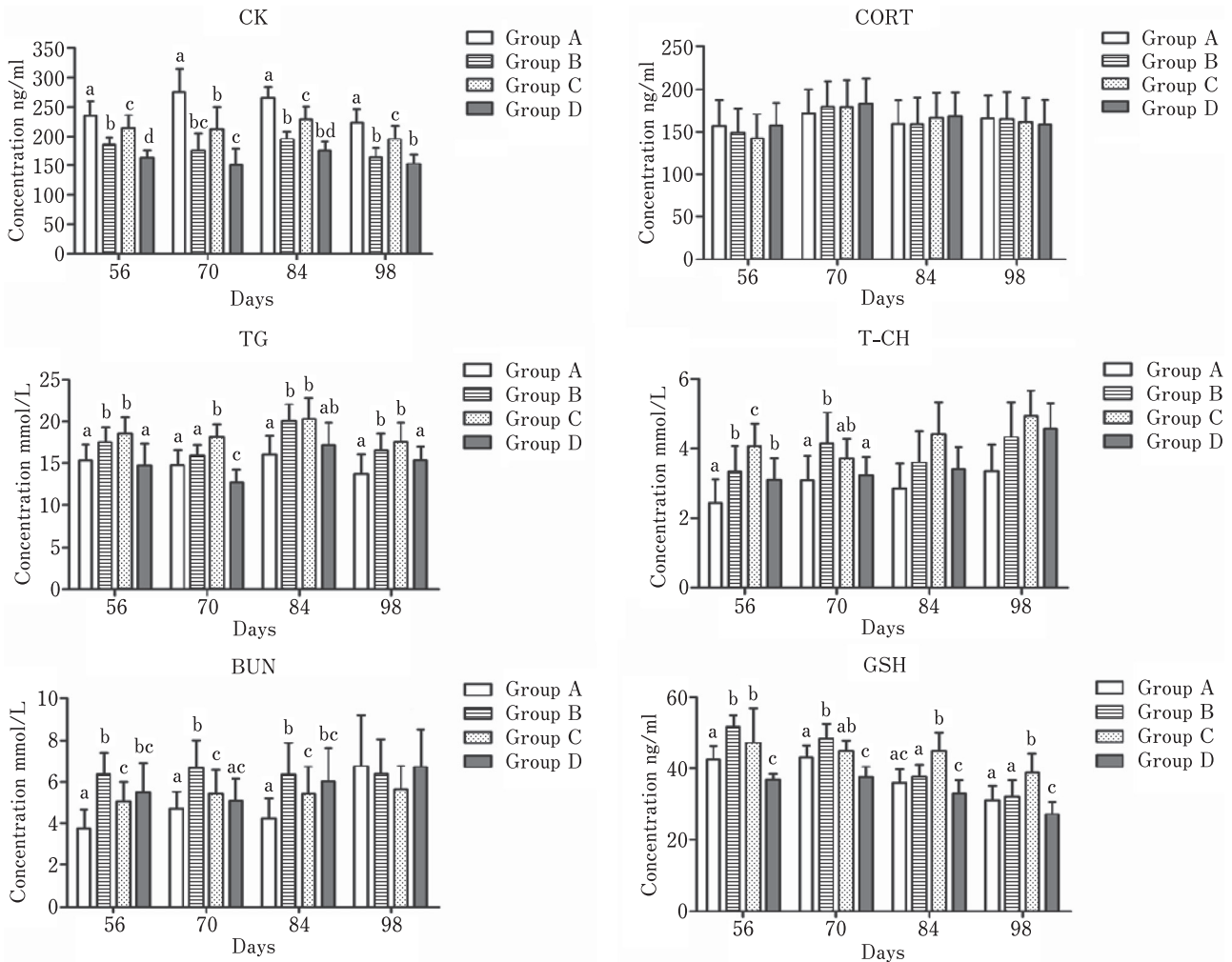


Fig. 1. Blood serum concentrations of CK, CORT, TG, T-CH, BUN, and GSH in Gushi chickens from the four groups during different growth periods. Group A, B, and C were large, medium, and small cages, respectively, and fed LME-LCP diet; Group D was small cages and fed STD diet; <sup>a-d</sup> Means within a day with different superscripts are significantly different ( $P < 0.05$ ).

stress (Villagra *et al.*, 2009). Kang *et al.* (2013) reported that plasma CORT levels were significantly ( $P < 0.05$ ) lower in hens housed in floor pens with larger space allowance than that in those housed in conventional cages with lower space allowance. Cheng and Muir (2004) reported that laying hens showed a significantly lower plasma CORT level in single bird cages (525 cm<sup>2</sup>/bird) than that in 10-bird cages (419 cm<sup>2</sup>/bird). However, Houshmand *et al.* (2012) noted a negligible effect of stocking density on CORT in caged broilers, and Thaxton *et al.* (2006) summarized that stocking density did not cause physiological adaptive changes that were indicative of stress. Similarly, no significant difference in serum CORT levels was found among the four groups in the present study.

Antibody titer of birds was influenced by housing system, stocking density, and cage sizes (Onbaşlılar and Aksoy, 2005;

Arbona *et al.*, 2011). Birds in large cages showed higher antibody titers against H5N1 and H9N2 avian influenza viruses than those in middle and small cages in our previous study (Li *et al.*, 2019). However, there were no significant differences in antibody titers among the four groups during most of the experimental period in the present study, except the higher level of antibody titers against H9N2 in Group A and B compared to Group D at 98 days of age and the higher antibody titers against NDV in Group B and C as compared with Group D at 84 days of age, which indicated that neither dietary energy or protein nor cage size had great effects on immunity levels. This may be due to the differences in management procedures and environmental conditions in their housing.

In summary, large cages with LME-LCP diets increased shank growth as chicks grew and optimized CK, TG, and T-

**Table 5. The antibody titer of the four groups of Gushi chickens during different growth periods**

Antibody titer <sup>1</sup>	Group	Age (days)			
		56 <sup>th</sup>	70 <sup>th</sup>	84 <sup>th</sup>	98 <sup>th</sup>
H5N1	A	9.13	10.00	9.42	6.53
	B	9.24	9.60	9.54	7.28
	C	9.18	9.60	8.91	6.66
	D	9.26	10.10	8.55	7.03
	<i>P</i> -value	0.995	0.297	0.649	0.174
H9N2	A	10.52	12.00	12.19	11.40 <sup>a</sup>
	B	10.52	11.83	12.00	11.83 <sup>a</sup>
	C	10.46	11.33	11.94	11.24 <sup>ab</sup>
	D	10.20	11.45	11.52	10.63 <sup>b</sup>
	<i>P</i> -value	0.905	0.153	0.161	0.012
NDV	A	6.30	9.20	9.94 <sup>ab</sup>	9.90
	B	6.67	10.00	10.75 <sup>a</sup>	10.62
	C	6.86	9.57	10.62 <sup>a</sup>	10.00
	D	6.40	9.59	9.83 <sup>b</sup>	10.20
	<i>P</i> -value	0.445	0.068	0.047	0.115

<sup>a-b</sup> Means within a day with different superscripts are significantly different ( $P < 0.05$ ).

<sup>1</sup> H5N1: avian influenza viruses H5N1 (Re-5 strain); H9N2: avian influenza viruses H9N2 (Re-2 strain); NDV: Newcastle disease virus; Titers are reported as logarithms (base 2)

CH levels in blood serum. The reduction in feed cost by 6.70% per bird compared with the small-sized cages with STD diets indicated the cost-effectiveness of the former conditions. Overall, the current findings provide support for the advantages of large-sized cages with LME-LCP diets during rearing, at least in growing Gushi chickens. Further studies are needed to evaluate the effects of large cage on production performances and welfare traits in larger populations with different chicken breeds.

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### Conflicts of Interest

The authors declare no conflict of interest.

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