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Effects of first feed intake time on growth performance, nutrient apparent metabolic rate and intestinal digestive enzyme activities in broilers

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Objective: This experiment studied the effects of first feed intake time post-hatch on growth performance, nutrient apparent metabolic rate and intestinal digestive enzyme activities in broilers.
Methods: Two thousand five hundred and twenty LingNan Yellow broilers were randomly allotted to seven treatments with six replicates of 60 each. The only experimental factor was the first feed intake time which was 18, 24, 30, 36, 42, 48, and 54 hours after hatching. The whole experiment lasted for 21 days.
Results: During the whole period, the 30 h treatment had the best body weight and average daily gain ($p<0.05$), followed by the 24 h group performance optimization. Also, the 30 h group was observed to have the best apparent metabolic rate for ether extract ($p<0.05$) and crude protein ($p<0.05$) and the highest activities of amylase, lipase and trypsin in small intestine. And the 24 h group was second only to the 30 h group in terms of the above two measures.
Conclusion: These results indicated that the appropriate first feeding time of LingNan Yellow broilers was 24 to 30 hours after hatching.

Keywords: First Feed Intake Time; Growth Performance; Intestinal Digestive Enzyme; Apparent Metabolic Rate; Broiler

INTRODUCTION

The importance of post-hatch early-life nutrition to lifelong health and performance for broilers is beginning to be recognized. Chicks out of the shell usually can't leave the hatchery until most of the eggs have hatched under practical conditions. The delayed time from hatching to onset of feeding is quite common in the poultry industry due to the variation in hatching time of different eggs. It is disadvantageous for chicks to hatch early because of the prolonged fasting period and potential dehydration [1]. It is generally more than 48 hours until chicks reach the farm after a series of procedures such as vaccination, sex identification and transportation. Under practical conditions, many birds were delayed access to feed for 36 to 48 h after hatching, and body weight (BW) decreased during this time [2]. Delaying access to feed and water had been documented to increase weight loss [3], leading to poorly starting flocks with reduced weight gains. For the growth of poultry, yolk sacs were usually considered as the primary source of nutrition. During the fasting period, the residual yolk which was not used during fetal life could supply nutrients for chicks instead of feed. However, the nutrients in the residual yolk were insufficient to satisfy the requirements for both maintenance and growth in broiler chicks [4]. Through the changes in the composition and content of the yolk at 12h and 130h after hatching, it was found that the egg yolk

nutrient was rapidly used and timely first feeding could promote the utilization of yolk [5]. Sklan and Noy indicated that the secretion of trypsin and amylase (AMS) into the intestine was triggered by feed intake [6]. The final BW of delayed feeding was lower than that of early feeding (EF), which meant providing EF supplements could improve BW [3]. Noy and Sklan speculated that in addition to enhanced gastrointestinal growth in birds with immediate access to feed, early feed consumption causes birds to begin to grow earlier and are thus "older" nutritionally [2].

Now broilers reach slaughter weight physiologically younger and younger, which means the first week post-hatch represents a larger proportion (20%) in the whole life of broilers, so EF plays an important part in their growth. As researchers proposed, BW increases two to threefold during the first week [7-9].

There were some researches about first feeding time affecting growth performance from the perspective of intestinal enzymes. However, few studies were established to explore the apparent metabolic rate of nutrients and its relationship with intestinal enzymes. This paper explored nutrient apparent metabolic rate and intestinal digestive enzyme activities in broilers, the effects of different first feeding times on growth performance, and the optimal time for first feeding in broilers. And for the first time, this research viewed the effects of first feeding time on growth performance from the angle of apparent metabolic rate of nutrients in broilers.

MATERIALS AND METHODS

All procedures of the present study were coincided with the Chinese guidelines for animal welfare and were approved by the Zhejiang University Institutional Animal Care and Use Committee (Hangzhou, China).

Bird management and experimental design

Total of 2,520 LingNan Yellow male chicks were hatched from eggs produced by the same flock at a local hatchery (GuangDa Breeder Farm, JiaXing, China). Normal and healthy chicks were removed from the hatchery and weighed immediately, and randomly allotted to pens so that each pen of chicks had a similar initial weight and weight distribution. In order to minimize the variation of body weights caused by extended holding time in the hatchery, only birds emerging within four hours were taken for this study. Chicks were randomly assigned to seven treatments with six replicate pens of 60 broilers each (Xinxin Culture-Farm, JiaXing, China). All chicks were reared at floor pens with five-centimeter deep fresh wood shavings. During the experiment period, birds received 24 h light.

All groups received water *ad libitum* after hatching, but were fed only 18 h, 24 h, 30 h, 36 h, 42 h, 48 h, and 54 h post-hatch respectively. Times of post-hatch delayed in access to feed in this study were calculated from hatching time to first access to feed. Diets were formulated to meet nutrient requirements of broilers according to NRC [10]. Feeds were analyzed for crude protein (CP) and ether extract (EE) according to the methods of GB/T6432-1994 and GB/T6433-2006. Ingredient composition and analyzed nutrients are presented in Table 1. Immunization and feeding management practices were similar to commercial broiler rearing.

Sampling and measurements

Mortality was checked and recorded daily by each replicate respectively, and then the date of death and BW of chicks were recorded. In addition, the following data were collected and calculated on day 1, 7, and 21 of the experimental period: BW, average daily gain (ADG), average daily feed intake (ADFI) and gain:feed. On day 4, 7 and 21, 4 chickens from each replicate respectively were randomly selected to be slaughtered for sampling after 12-h fasting (water offered *ad libitum*). The contents of duodenum, jejunum and ileum were collected, and frozen in liquid nitrogen immediately. All samples were stored at –80°C prior to analysis. Digesta were weighed, homogenized with cold 0.86% NaCl, and aliquots of the homogenate taken for AMS, trypsin and lipase activity. The activities of AMS, trypsin, lipase and the concentration of protein were measured using kits (Nanjing Jiancheng Bioengineering Institute,

Table 1. Composition and nutrient content of the basal diet

Items	1-21 d
Ingredients (%)	
Corn	54.70
Wheat	5.00
Soybean meal	29.00
CGM	6.00
Soybean oil	1.00
NaCl	0.30
CaHPO4	1.70
Limestone	1.30
Premix[1)]	1.00
Total	100.00
Nutrient levels[2)] (%)	
ME (kcal/kg)	2,909
CP	20.96
Lys	1.10
Met	0.50
Met+Cys	0.85
Ca	0.99
TP	0.66

CGM, corn gluten meal; ME, metabolizable energy; CP, crude protein; TP, total phosphorus.
[1)] Supplied per kg of diet: vitamin A, 9,600 IU; vitamin D_3, 2,700 IU; vitamin E, 36 mg; vitamin K_3, 3.0 mg; vitamin B_1, 3.0 mg; vitamin B_2, 10.5 mg; vitamin B_6, 4.2 mg; vitamin B_{12}, 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.
[2)] ME is calculated value, other nutrient levels are measured values.

Nanjing, China). The activities of AMS, trypsin and lipase were expressed as international units per gram of protein.

On 14 day, four chicks from each replicate were randomly selected to rear in the cages for metabolic experiment. After three days' adjustment period, a four-day formal experiment was conducted. For the determination of EE and CP metabolism, excreta from each replicate were collected at 09:00 am and 16:00 pm every day. After freeze-drying, extra samples were ground to pass through a 40-mesh screen and then analyzed for CP and EE according to GB/T6432-1994 and GB/T6433-2006. And then the apparent metabolic rate of CP and EE were calculated.

Statistical analysis

Data was analyzed by using One-way analysis of variance procedure of SPSS 20.0 computer software. All the data were presented as the means of each treatment. The variability of data was expressed as standard error of the mean and a probability level of $p<0.05$ was considered to be statistically significant.

RESULTS

Table 2 shows the effects of first feeding time on growth performance and feed utilization of broilers. With the extension of the first feeding time, BW and ADG of 7 day increased, reaching the maximum at the 30 h group ($p<0.05$), followed by the 24 h group, but no significant difference between these two groups.

The effects of first feeding time on nutrient apparent metabolic rate of broilers is detailed in Table 3. The highest apparent metabolic rates of CP and EE were observed at the 24 and 30 group ($p<0.05$). And there was no significant difference between these two groups.

The effects of first feed intake time on intestinal enzyme activities of broilers at 4 d of age are given in Table 4. The AMS, lipase and trypsin enzyme activities of the 30 h group in duodenum, jejunum and ileum were the highest ($p<0.05$), but

Table 3. Effects of first feeding time on apparent metabolic rate of feed in broilers

Items (%)	Time of first feeding post-hatch (h)							
	18	24	30	36	42	48	54	SEM
CP	34.55^{b}	38.18^{a}	38.99^{a}	31.75^{c}	31.58^{c}	31.25^{c}	29.69^{d}	0.789
EE	84.58^{a}	84.27^{a}	84.58^{a}	84.34^{a}	83.91^{a}	82.67^{b}	79.78^{c}	0.368

SEM, standard error of the mean; CP, crude protein; EE, ether extract.
$^{a-d}$ Means (n = 6) within a row lacking a common superscript differ ($p<0.05$).

Table 2. Effects of first feeding time on growth performance in broilers

Items	Age (d)	Time of first feeding post-hatch (h)							
		18	24	30	36	42	48	54	SEM
BW (g)	1	37.50	36.20	36.69	36.83	37.25	36.61	36.95	0.160
	7	85.91^{bc}	90.79^{ab}	96.05^{a}	85.77^{bc}	83.70^{bc}	83.11^{bc}	79.92^{c}	1.380
	21	517	521	533	503	510	490	492	5.251
ADG (g)	7	6.92^{bc}	7.80^{ab}	8.48^{a}	6.99^{bc}	6.64^{bc}	6.64^{bc}	6.14^{c}	0.211
	21	22.85	23.06	23.62	22.19	22.49	22.59	21.65	0.231
ADFI (g)	21	42.80	42.86	43.27	42.70	42.64	42.15	42.25	0.868
G:F (g:g)	21	1.87	1.86	1.83	1.92	1.90	1.95	1.95	0.027

SEM, standard error of the mean; BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; G:F, gain:feed.
$^{a-c}$ Means (n = 6) within a row lacking a common superscript differ ($p<0.05$).

Table 4. Effects of first feeding time on intestinal digestive enzyme activities of broilers on d 4

Items	Enzyme (U/g protein)	Time of first feeding post-hatch (h)							
		18	24	30	36	42	48	54	SEM
Duodenum	AMS	13.70^{c}	48.86^{a}	45.65^{b}	10.32^{d}	9.09^{de}	6.74^{e}	6.50^{e}	3.885
	Lipase	303^{c}	336^{b}	367^{a}	300^{cd}	274de	271^{e}	175^{f}	12.87
	Trypsin	94.25^{c}	99.79^{b}	133^{a}	81.55^{d}	59.99^{e}	42.69^{f}	34.99^{g}	7.185
Jejunum	AMS	31.79^{c}	43.84^{b}	52.66^{a}	22.75^{d}	11.68^{e}	11.42^{e}	6.90^{f}	3.643
	Lipase	447^{b}	473^{b}	605^{a}	447^{b}	402^{c}	325^{e}	370^{d}	18.72
	Trypsin	221^{c}	294^{b}	397^{a}	203^{d}	172^{e}	158^{e}	111^{f}	19.94
Ileum	AMS	54.45^{b}	80.69^{a}	57.11^{b}	45.65^{c}	42.08^{c}	29.37^{d}	25.12^{d}	3.903
	Lipase	626^{c}	651^{b}	742^{a}	467^{d}	418^{e}	341^{f}	359^{f}	32.68
	Trypsin	344^{c}	385^{b}	405^{a}	326^{d}	231^{f}	245^{e}	206^{g}	16.36

SEM, standard error of the mean; AMS, amylase.
$^{a-g}$ Means (n = 6) within a row lacking a common superscript differ ($p<0.05$).

AMS enzyme activities in duodenum and ileum was the best in the 24 h group ($p<0.05$).

The results presented in Table 5 show the effects of first feeding time on intestinal enzyme activities of broilers on 7 d of age. The AMS, lipase and trypsin activities in duodenum, jejunum and ileum were the highest in the 30 h group ($p<0.05$), while the activities of trypsin in duodenum and activities of lipase in jejunum were the highest in the 24 h group ($p<0.05$).

Table 6 shows the effects of first feeding time on intestinal digestive enzyme activities of broilers on 21 d of age. On the whole, the AMS, lipase and trypsin activities in duodenum, jejunum and ileum were the highest in the 30 h group ($p<0.05$), and then the 24 h group was second only to the 30 h group in terms of these three enzyme activities.

DISCUSSION

Initiation of BW growth in neonate chicks was directly linked to feed availability [4]. Several reports had demonstrated that delay in feed intake after hatching adversely affected post-hatch growth performance of chicks [4,11-13]. It was common for chicks to lose weight during holding period mainly because of dehydration and yolk consumption [3,14]. Post-hatch holding of chicks before access to feed caused weight loss during holding time and depressed growth rate after access to feed [15]. And Careghi et al [15] demonstrated a positive curvilinear relationship between percentage of chick weight loss and holding time. The results of this study showed that the BW and ADG of broilers fed at 30 h post-hatch were both significantly higher than those of other groups at 7 d of age, and the smallest BW and ADG were observed at the 54 h group. In addition, there was no significant difference between the 24 h and 30 h group in terms of BW and ADG. This is in agreement with those findings which reported that chicks in the EF group had a greater BW than that of the early fasting group [16,17]. Bhanja et al [18] conducted a more detailed grouping design of broiler feed times, and found that BW of each group feeding before 24 hours was greater than that of after 24 hours. Panda et al [19] observed that newborn chicks began to grow after 24 hours of ingestion, whereas fasting for too long led to a slowing of the gastrointestinal tract and immune system, and caused early weight loss [19]. Chicks just experiencing the shelling process have not yet resumed physical recovery. Under this circumstance, EF easily leads to excessive

Table 5. Effects of first feeding time on intestinal digestive enzyme activities of broilers on 7 d of age

Items	Enzyme (U/g protein)	Time of first feeding post-hatch (h)							
		18	24	30	36	42	48	54	SEM
Duodenum	AMS	27.16^{c}	31.09^{b}	40.66^{a}	22.80^{d}	20.35^{d}	21.32^{dc}	17.95^{e}	1.642
	Lipase	233.4^{b}	206.2^{c}	242.6^{a}	162.3^{d}	37.95^{e}	20.56^{f}	11.00^{g}	21.53
	Trypsin	221^{c}	776^{a}	256^{b}	178^{d}	91.03^{e}	61.45^{f}	37.97^{g}	52.54
Jejunum	AMS	26.37^{c}	34.84^{b}	56.53^{a}	24.24^{d}	21.74^{e}	18.4^{f}	17.84^{f}	2.818
	Lipase	629^{c}	1,091^{a}	817^{b}	547^{d}	97.01^{e}	128^{e}	68.90^{f}	82.55
	Trypsin	587^{c}	862^{b}	1,239^{a}	585^{c}	251^{d}	129^{e}	80.93^{e}	87.28
Ileum	AMS	29.53^{c}	50.34^{b}	59.37^{a}	29.34^{c}	25.91^{d}	24.91^{e}	24.78^{e}	2.904
	Lipase	485^{c}	1,040^{b}	1,143^{a}	486^{c}	132^{d}	123^{d}	33.73^{e}	92.66
	Trypsin	1,239^{b}	1,255^{b}	1,424^{a}	916^{d}	1,016^{c}	983^{c}	639^{e}	54.26

SEM, standard error of the mean; AMS, amylase.
[a-g] Means ($n = 6$) within a row lacking a common superscript differ ($p < 0.05$).

Table 6. Effects of first feeding time on intestinal digestive enzyme activities of broilers on 21 d of age

Items	Enzyme (U/g protein)	Time of first feeding post-hatch (h)							
		18	24	30	36	42	48	54	SEM
Duodenum	AMS	34.71^{a}	31.64^{ab}	52.44^{a}	23.02^{ab}	15.99^{ab}	11.82^{b}	10.90^{b}	4.670
	Lipase	299^{bc}	363^{b}	504^{a}	277^{bc}	248^{bc}	174^{cd}	149^{d}	26.47
	Trypsin	462^{b}	362^{c}	1,009^{a}	321^{c}	256^{d}	141^{e}	97.70^{e}	63.51
Jejunum	AMS	28.52^{ab}	27.52^{ab}	49.99^{a}	24.60^{ab}	10.49^{b}	13.85^{b}	6.00^{b}	4.000
	Lipase	284^{b}	366^{b}	809^{a}	167^{c}	143^{c}	92.57^{c}	115^{c}	53.09
	Trypsin	995^{b}	1,672^{a}	433^{c}	252^{d}	251^{d}	108^{d}	104^{d}	121.9
Ileum	AMS	30.57^{a}	34.84^{a}	36.62^{a}	25.12^{ab}	21.57^{ab}	20.78^{ab}	11.09^{b}	2.602
	Lipase	1,750^{ab}	1,543^{ab}	2,248^{a}	1,054^{bc}	1,031^{bc}	435^{c}	337^{c}	170.7
	Trypsin	685^{b}	1,274^{a}	1,268^{a}	589^{c}	444^{d}	384de	324^{e}	83.61

SEM, standard error of the mean; AMS, amylase.
[a-e] Means ($n = 6$) within a row lacking a common superscript differ ($p < 0.05$).

fatigue and thus affects the body growth and development, so chicks starting to feed should have a suitable transition period, but the transition period should not be too long, which may be the reason why the 24 h and 30 h group in this experiment weighted higher than the other groups ($p<0.05$).

Yang et al [20] indicated that energy, EE and CP contents of the yolk decreased exponentially, and these nutrients of fed goslings were decreased significantly faster than those of fasted goslings. It was reported that holding chicks without feed decreased both the height and the area of small intestinal villi versus chicks allowed access to feed immediately [21]. The present study showed that the apparent metabolic rate of nutrient in broilers increased with the time of EF and the apparent metabolic rate of EE and CP were better in both 24 h and 30 h groups ($p<0.05$). The apparent metabolic rate of EE and CP were significantly lower in the 54 h group than in the other groups. It can be seen, EF helps the digestive metabolism of exogenous nutrients in chickens, which may be due to exogenous nutrients promoting the absorption of chicken yolk to strengthen intestinal development and thus enhance the digestion and metabolism of exogenous nutrients; for another possibility, exogenous nutrients may directly stimulate the development of the gastrointestinal tract and thus enhance its digestion and absorption capacity [22].

Chicks ingesting feed showed increases in total intestinal trypsin, AMS and lipase activities that were correlated with intestinal weights and BW [6]. Exogenous feed enhanced yolk utilization, stimulated intestinal peristalsis and triggered secretion of pancreatic enzymes enabling efficient digestion of nutrients after 4 days' post-access to feed [20]. From day 4, secretion of pancreatic enzymes per gram of feed intake changed little with age [23]. Research had also shown that digestive enzymes might be limited by adequate nutrient utilization post-hatch in chicks and poults [24,25] and that the concentrations of these digestive enzymes increased post-hatch. Throughout the trail, there was a difference ($p<0.05$) in the effect of different feeding time on the digestive enzyme activity of broiler intestinal chyme. With the increase of feeding time, the concentration of intestinal AMS increased, reaching the maximum at 30 h with the exception of a maximum of duodenum and ileum at 24 h on day 4. And the 54 h group had the lowest concentration of AMS. The results of lipase and trypsin were similar to the result of AMS, almost all of them reached the maximum at 30 h group, followed by 24 h group. Besides, the results of 7 day and 21 day also suggested that 24 h group and 30 h group were the best two treatments. Our study indicated that there was an optimal feeding time of 24 to 30 hours which was different from that of Gonzales et al [13]. According to Gonzales et al [13], the maximum fasting period, which had no significant negative effect on final weight, was 24 h after the chicks were removed from the hatchers. This may be attributable to animal breeds, experimental conditions, setting criteria for opening time, animal physiology and other factors. In general, EF can improve the digestive enzyme activities of small intestine of broilers. Although the growth rate of lipase, AMS and trypsin were not the same, they were effective in promoting the improvement of digestive capacity in broilers. However, there was no systematic study on the mechanism of EF to promote digestive enzyme activity in broilers. Therefore, it was necessary to further explore the mechanism of EF to regulate the digestive enzyme activity of broilers from molecular or cytology.

It was possible, therefore, that the increase of digestive enzyme activity in the small intestine promoted the absorption and utilization of nutrients and increased the apparent metabolic rate of EE and CP, leading to higher ADG, ADFI, and gain:feed. This may be the reason why the appropriate start feeding time, which was from 24 to 30 hours in this study, improved broiler performance.

In conclusion, this experiment indicated that it was not beneficial for newly hatched chicks to be exposed to exogenous feed as soon as possible. On the contrary, feeding untimely and prematurely could cause negative effects. It was recommended that, in our experiment, the appropriate time access to feed was from 24 to 30 hours post-hatch.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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