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Research Article
Productive Performance and Histological Evaluation of Delayed Post Hatch Feed Access Broilers Fed Threonine Supplemented Diet

[1]Hanaa Mohamed Ghanem, [2]Samah El Sayed Lashen, [3]Rania El Sayed Mahmoud and [4]Mahmoud Azzam

[1]Department of Animal Husbandry and Wealth Development, Faculty of Veterinary Medicine, Mansoura University, 35516 Mansoura, Egypt
[2]Department of Cytology and Histology, Faculty of Veterinary Medicine, Mansoura University, Egypt
[3]Department of Nutrition and Nutritional Deficiency Diseases, Faculty of Veterinary Medicine, Mansoura University, Egypt
[4]Department of Poultry Production, Faculty of Agriculture, Mansoura University, 35516 Mansoura, Egypt

Abstract

Background and Objective: Chicks may expose to delayed post hatch feed access due to several causes which may have consequences for poultry production. The present study investigated the impacts of different delayed time's access of feed on the production performance, histological examination and carcass traits in broiler chicks and how to substitute the detrimental effect of this delay by supplementation of threonine. **Methodology:** A total of 300 one-day old broiler chicks "Cubb 500" were used. They were alienated into 5 experimental groups, each group was further alienated into 6 replicates of 10 birds. Several productive traits as body weight gain, feed intake, feed efficiency, mortality and carcass traits were measured. In addition, villus height, villus width, crypt depth and villus surface area were also measured. **Results:** Results showed that group IV which fastened 24 h after hatch and fed threonine supplemented diet showed the highest performance traits. Control group showed the least yolk residual and the highest feed intake. Group of bird fastened either 24 or 48 h showed the least weight gain compared to other groups. Regarding to carcass traits, group of bird fastened for 48 h showed the least giblets weight and dressing percentage, while group IV showed the highest values of carcass traits. **Conclusion:** The productive performance of chicks was improved following early access to diet after hatch through skip all obstacles that prevent early chicks feed access.

Key words: Feed access, threonine, intestinal villi, productive performance, chicks feed

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Corresponding Author: Hanaa Mohamed Ghanem, Department of Animal Husbandry and Wealth Development, Faculty of Veterinary Medicine, Mansoura University, 35516 Mansoura, Egypt Tel: 00201063592965 Fax: 0020502379952

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

It has been shown that in most commercial broiler farm the newly hatched birds remain several period of time approximately may reach to 72 h without feed and water after removal from the incubator[1], till the majority of chicks have hatched. Besides, the hatchery handling and processing operating procedures such as sex determination, vaccination, packaging and transportation to production facilities are also responsible for delay in access to feed[2]. All these factors make gap between hatching time and chick's access to diet consequences in a lowering of the physiology of the gastrointestinal tract[3], consequently undesirable bird performance such as decreasing final body weight, decreasing immune response, retarding growth and increasing mortality[4]. During the last few years, protein and energy cost have fluctuated dramatically for the poultry feed industry[5]. As a consequence, formulations of feed are directed toward economic analysis rather than optimal bird performance. In addition, use of alternate ingredients and amino acid formulation on a digestible basis[6]. Poultry cannot synthesize threonine making it a nutritionally essential amino acid. It is a great concern in poultry nutrition to add essential amino acids such as threonine, which consider the third limiting amino acid in rations for broilers since over 60 years ago after methionine and lysine[7]. Thus, this study was planned to determine the effects of delayed broiler chick access to feed on the productive traits, histological examination and carcass traits and how to substitute the detrimental effect of this delay through addition of threonine to the diet.

MATERIALS AND METHODS

This study was reviewed and approved by the Animal Care and Welfare Committee of Faculty of Veterinary Medicine, Mansoura University, Egypt through the period from August, 2016 till March, 2017. It was conducted at the Experimental Unit related to Department of Animal Husbandry. This unit is supported by the latest equipment that maintains the well-being of birds.

Experimental birds and management: A total of 300 one-day old broiler chicks "Cubb 500" with mean of initial body weight was 35.56±0.50 g, were alienated into five investigational treatments, each treatment was further alienated into 6 replicates of 10 chicks each. The birds were raised on deep litter system, prepared by air suction and fans for ventilation and supplied by all equipments meets all the requirements necessary for the welfare of chicks. The environmental house temperature and humidity were initially maintained at 35-37°C and 60-70%, respectively and after 3 days gradually decreased (temperature 0.5°C every day and humidity decreased 50-60%). Through the whole experiment, the lighting program was constant (23 Light: 1 Dark). Water was presents freely to the chicks through the whole experimental period. The control and experimental diets (Table 2 and 3) is formulated according to Cobb-vantress.com[8]. The diets of the experiment were weighed, mixed and prepared biweekly to avoid any change in its composition.

Experimental design: This research was randomized planned into 5 treatments (Table 1) includes, first one had free access to feed immediately after hatch (control) and fed normal basal control starter, grower and finisher diets containing the recommended levels of required elements specified for Cobb 500 broiler performance and nutrition supplement, the second one was exposed to delayed feed access till 24 h post hatch and fed control diet as group one, the third group was exposed to delayed feed access till 48 h post hatch and fed basal diet as group one, the forth one was starved for 24 h post hatch then fed controlled diet supplemented with threonine at 120% of the control diet, while the last one was starved for 48 h post hatch then fed diet supplemented with threonine at 120% of the control diet.

Data measurements

Productive performance: Indices for evaluating growth performance are live body weight was recorded at the 1st day of the experiment then biweekly till the finish of the study. The average body weight gain was calculated per chick in each subgroup every corresponding week. Feed consumption and feed efficiency were determined.

Histological examination: At 21 days of the experiment, 5 birds with body weights near to the mean were selected from each group. Subsequent bird slaughtering, specimens measure about (1×1 cm) from the first part of small intestine were collected and fixed in 10% neutral-buffered formalin for 24 h. Fixed samples were rinsed several times in 70% ethanol to get rid of the fixative before the subsequent steps of tissue processing then washed beneath a running water for 5 min.

Table 1: Arrangements of chicks into different groups

Groups	N	Treatments
I	60	Chicks had free access to feed immediately after hatch (control)
II	60	Chicks were exposed to delay feed access till 24 h post hatch
III	60	Chicks were exposed to delay feed access till 48 h post hatch
IV	60	Chicks were exposed to delay feed access till 24 h post hatch+threonine supplementation at 120% of the recommended level of the control diet
V	60	Chicks were exposed to delay feed access till 48 h post hatch+threonine supplementation at 120% of the recommended level of the control diet

Table 2: Ingredient percentages and nutritive value of control diet (starter, grower and finisher diets)

Ingredients (%)	Starter (1-10 days)	Grower (11-22 days)	Finisher (23-42 days)
Yellow corn	59.41	63.47	69.17
Corn gluten	4.53	3.00	6.30
Soybean meal	30.00	26.80	18.92
Oil	2.60	3.40	2.60
Limestone	1.90	1.83	1.74
Dicalcium phosphate	0.41	0.33	0.20
Common salt	0.30	0.30	0.30
Mineral and vitamin premix	0.25	0.25	0.25
Lysine	0.39	0.39	0.38
Methionine	0.13	0.15	0.08
Threonine	0.08	0.08	0.06
Calculated value			
Crude protein (%)	21.50	19.50	18.50
ME (kcal kg^{-1})	3034.50	3106.80	3179.00
Calcium (%)	0.90	0.84	0.76
Available phosphate (%)	0.46	0.43	0.38
Methionine (%)	0.50	0.48	0.43
Lysine (%)	1.30	1.22	1.05
Threonine (%)	0.86	0.78	0.70

For each kg of the diets, vitamin A: 9,000,000 IU, vitamin D3, 2,000,000 IU, vitamin B1: 1,800 mg, vitamin B2: 6,600 mg, vitamin B3: 10,000 mg, vitamin B6: 3,000 mg, vitamin B12: 15 mg, vitamin E: 18,000 mg, vitamin K3: 2,000 mg, vitamin B9: 1,000 mg, vitamin B5: 30,000 mg, folic acid: 21 mg, nicotinic acid: 65 mg, biotin: 14 mg, choline chloride: 500,000 mg, Mn: 100,000 mg, Zn: 85,000 mg, Fe: 50,000 mg, Cu: 10,000 mg, I: 1,000 mg, Se: 200 mg

Table 3: Ingredient percentages and nutritive value of diet supplemented by threonine at 120% of the recommended level of the control diet in (starter, grower and finisher diets)

Ingredients (%)	Starter (1-10 days)	Grower (11-22 days)	Finisher (23-42 days)
Yellow corn	61.40	66.56	68.80
Corn gluten	4.30	3.79	4.30
Soybean meal	28.13	23.55	20.34
Oil	2.39	2.65	3.30
Limestone	1.90	1.84	1.75
Dicalcium phosphate	0.42	0.33	0.20
Common salt	0.30	0.30	0.30
Mineral and vitamin premix	0.25	0.25	0.25
Lysine	0.46	0.44	0.39
Methionine	0.15	0.15	0.12
Threonine	0.30	0.28	0.25
Calculated value			
Crude protein (%)	20.78	18.90	17.90
ME (kcal kg^{-1})	3034.30	3107.00	3181.20
Calcium (%)	0.89	0.84	0.77
Available phosphate (%)	0.45	0.42	0.38
Methionine (%)	0.51	0.48	0.44
Lysine (%)	1.32	1.19	1.07
Threonine (%)	1.40	0.95	0.80

For each kg of the diets, vitamin A: 9,000,000 IU, vitamin D3: 2,000,000 IU, vitamin B1: 1,800 mg vitamin B2: 6,600 mg, vitamin B3: 10,000 mg, vitamin B6: 3,000 mg, vitamin B12: 15 mg, vitamin E: 18,000 mg, vitamin K3: 2,000 mg, vitamin B9: 1,000 mg, vitamin B5: 30,000 mg, folic acid: 21 mg, nicotinic acid: 65 mg, biotin: 14 mg, choline chloride: 500,000 mg, Mn: 100,000 mg, Zn: 85,000 mg, Fe: 50,000 mg, Cu: 10,000 mg, I: 1,000 mg, Se: 200 mg

The specimens were dehydrated in the series of ascending grade of ethyl alcohol (70, 80 and 90% and absolute alcohol) then cleared in xylene and impregnated in liquid paraffin and sectioned to 7 μm thickness using rotatory microtome.

Embedded sections were mounted onto glycerol-albumin-coated glass slides then kept at 60°C in incubator. Sections were stained with hematoxylin-eosin (H and E) that used to evaluate the tissue general morphology. Histological examinations were conducted under Olympus CX41 microscope.

Histomorphometry: Histomorphometric investigation was done on H and E stained sections. The measured parameters were villus height (VH), (measured from the base to the tip of the villus), villus width (VW) (at half height), villus surface area (AVSA), was determined as a product of villus height and width and crypt depth (CD) (measured from the crypt-villus junction to the base of the crypt)[9]. These parameters were measured from only complete, steeply oriented 6 villi. This experiment affected villus height, crypt depth and villus surface area in duodenum of broiler chicks.

Carcass traits: Five chicks were randomly taken from every subgroup at the end of study, weighed, slaughtered to complete bleeding and weighed again. The chicks from each treatment were dressed, eviscerated and the following parameters were measured: Weight of carcass, dressing percentage and the weight of giblets (heart, liver and gizzard).

Statistical analysis: Data were analyzed using general linear model (GLM) procedure of the statistical analysis system package. Preliminary test was applied to the percentage data before comparison and analysis and found that data was homogeneous and did not need a transformation to the corresponding arcsine angle in-addition a non-significant effect of replicate. All data are expressed as the least square mean (LSM)±SE. Also, the resultants p-values were also expressed $p<0.05$ was considered to be statistically significant.

RESULTS

The data concerning average body weight, daily gain, feed intake, feed efficiency and yolk residual are presented in Table 4. Results showed that group IV which fastened 24 h after hatch and fed threonine supplemented diet showed the highest performance traits compared to other groups. Control group showed the least yolk residual (0.61) compared to other treatments and this may be related to early consumption of feed post hatch which facilitate the action of intestine to make absorption to the yolk. Group of bird fastened either 24 or 48 h showed the least weight and gain compared to other groups. Also control group showed the highest feed intake compared to other fastened group. Regarding to carcass traits, results in Table 5 showed that group of bird fastened for 48 h showed the least giblets weight and dressing percentage while group IV which fastened 24 h post hatch and fed threonine supplemented diet showed the highest carcass traits compared to other groups.

Histomorphometric findings: The average data for villus height, villus width, villus surface area and crypt depth are presented in Table 6. Results showed that all measured parameters from starved group were lower than that of

Table 4: Effect of delayed feed access on the productive performance

	Groups					
Traits	I	II	III	IV	V	p-value
Yolk residual	0.61±0.05^{d}	0.85±0.08^{c}	1.05±0.04^{a}	0.80±0.08^{c}	0.95±0.09^{b}	<0.05
Weight	1.902±0.35^{c}	1.870±0.48^{d}	1.791±0.18^{e}	2.032±0.45^{a}	1.990±0.66^{b}	<0.01
Gain	1.595±0.56^{c}	1.559±0.57^{d}	1.492±0.65^{e}	1.826±0.81^{a}	1.700±0.11^{b}	<0.05
FI	3.40±0.42^{a}	3.35±0.31^{ab}	3.30±0.41^{b}	3.08±0.05^{d}	3.23±0.21^{c}	<0.05
FE	0.46±0.42^{c}	0.46±0.40^{c}	0.45±0.41^{d}	0.59±0.35^{a}	0.52±0.05^{b}	<0.05

FI: Feed intake, FE: Feed efficiency. Means of different levels within the same column having different superscripts are significantly different

Table 5: Effect of delayed feed access on the carcass traits

	Groups					
Traits	I	II	III	IV	V	p-value
Giblets wt.	83.00±0.57^{c}	71.66±0.80^{d}	67.00±0.15^{e}	90.00±0.55^{a}	84.55±0.35^{b}	<0.01
Dressing (%)	84.71±0.03^{c}	80.26±0.03^{d}	78.35±0.05^{e}	87.45±0.01^{a}	85.65±0.01^{b}	<0.01

Means of different levels within the same column having different superscripts are significantly different

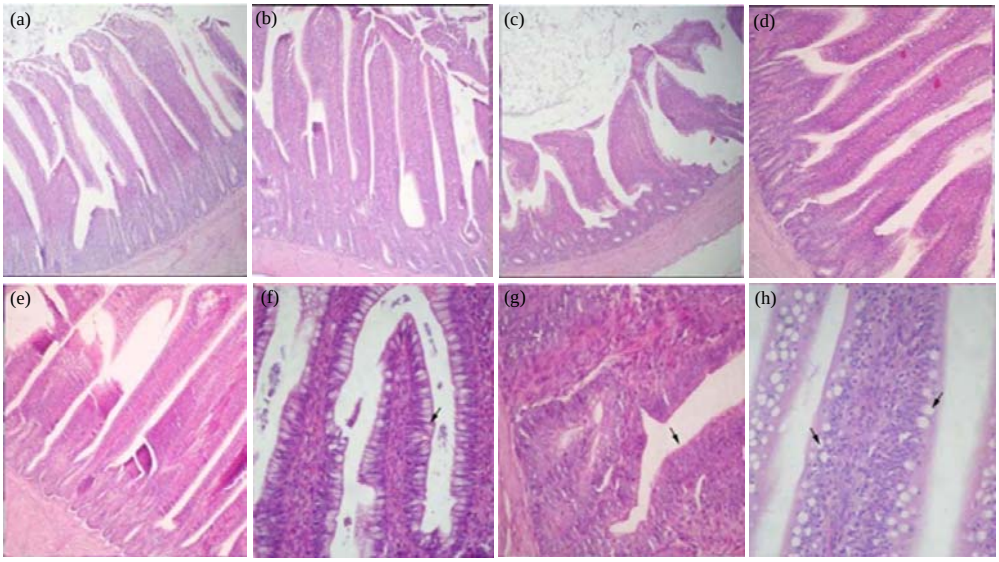

Fig. 1(a-h): Histomorphological characteristics of duodenal tissue in (a) Control group, (b) The effect of delayed feed access of post-hatch (24 h), (c) The effect of delayed feed access of post-hatch 48 h, (d, e) On duodenum villus height, villus weight and crypt depth of broiler chickens measured and effect of threonine supplement at 21 days of age (f) Normal columnar epithelium and goblet cell in control group, (g) Degenerative changes in fasted group and (h) With restore the architecture of goblet cell in threonine group. Arrows refers to columnar epithelium with goblet cell

Table 6: Effect of delayed feed access of post-hatch on histological examination

	Groups					
Traits	I	II	III	IV	V	p-value
Villus height	304.33±11.22	257.17±27.64	180.00±15.86	255.83±33.27	178.67±16.03	<0.001*
Villus weight	57.83±12.14	68.17±26.52	72.50±10.89	37.83±5.340	27.17±3.970	<0.001*
Villus surface area	17666.00±4247.30	17517.83±7390.39	13073.83±2409.33	9598.33±1301.86	4831.00±639.98	<0.001*
Crypt depth	89.50±13.26	62.33±26.55	51.50±2.17	45.50±3.27	40.67±7.50	<0.001*

*Significant

control one. Group IV which fastened 24 h after hatch and fed threonine supplemented diet showed the highest performance traits compared to other groups. Control group showed significantly higher villus parameter (VH, VW, AVSA and CD) of duodenum in the birds that started to fed after hatch compared to birds that fed 24 and 48 h after hatch showed a tendency of restored duodenal villi development. With increased fasted time showed significantly decreased VH, VW, AVSA and CD. Birds started to feed at 24 h post-hatch showed highest VH, VW, AVSA and CD and lowest VH, VW and AVSA observed in broiler chicks that started to feed with 48 h delayed. Supplementation of threonine is essential for adequate gut functions which had significantly effect ($p<0.001$).

Histological findings: The effect of fasting and threonine supplementation on histological structure in combination with villus height, villus width, villus surface area and crypt depth of duodenum are shown in Fig. 1 a-e. Samples collected from duodenal villi of control group displayed the normal architecture of the lining epithelium that composed of number of columnar cell with normal goblet cell (Fig. 1f). The morphological alterations of columnar epithelial cells of villi were investigated in the starved bird for 24-48 h, the lining epithelium showed degenerative changes and weekly stained goblet cells (Fig. 1g). Dietary treatments groups with threonine showed a tendency for an increase in goblet cells density (Fig. 1h).

DISCUSSION

There was significant ($p>0.05$) dissimilarity of the residual yolk weight between the first threonine investigational groups, there was decline in residual yolk weight of birds in all groups, but the reduction rate was different in the first threonine group than other groups. This

result is in agreements with Noy and Sklan[10], who stated that feeding early after hatch, compared to delay feeding seems to stimulate yolk consumption, Also, Noy and Sklan[11] and Speake *et al.*[12], stated that 18 h after hatch deprivation of feeding may results in decreasing in utilization of residual yolk and more yolk weights than was that chicks have free access to feed and water immediately post hatch, Moran[13] and Pinchasov and Noy[14]. An observable reduction in yolk weight from 48-41% per day for fed and feed-deprived broiler chicks, respectively[15]. It was found that during inspection of the rate of residual yolk consumption, chicks subjected to delayed access to diet appeared retardation in the use of yolk comparing to 'Early-fed access' birds, this may be related to increased intestinal mechanical (anti peristaltic) activity, Noy *et al.*[16].

It was observed significant increase in body weight and daily gain loss with delayed feed access and this could be attributed to lower feed intake and poor development of digestive tract. Results showed significant body weight increase in control group in comparison to the 2nd and 3rd group, this decrease in body weight and daily gain in the other groups was in agreements with Saki[17], who found that deprivation of feed after hatch results in reduction in broiler performance. During the first 2 weeks, feed conversion ratio and feed intake may improved through early access to feed of broilers, while during the starter phase a short time of feed deprivation (12 h) may possibly impact the productive performance and feed consumption of the chicks[18]. When chicks reached feed early post hatch, the value provided by diet are corresponding to the yolk nutrients and this will activate fast growth performance inchicks[19,20]. The in sufficient advance of the gastrointestinal tract post hatch, especially with deprivation of feed may have detrimental effect on the productive performance of chicks[21]. After-hatch deprivation of feed from broilers for 36 h resulted primarily in decreased developments of all parts and segments of the gastrointestinal tract than make that of chick's subjected to early access of feed post hatch[22].

For groups fed threonine supplemented diet, chicks fasted for 24 h and take threonine supplemented diet (group IV) achieved the best performance traits compared to the other groups. These results were in accordance with Abbasi *et al.*[23], who revealed that, the best FCR and BWG values were obtained in broilers fed diets supplemented with 120% of threonine requirements and assumed that threonine supplementation enhanced the growth performance by improving intestinal health through increase villus height to crypt depth ratio and that indicated slower tissue turnover and lower demands to compensate for villus atrophy. In addition, the response of broiler performance to threonine levels was time-dependent lead to decreased BWG only during starter period as the threonine supplementation at least up to 110% of recommended levels caused a significant improvement ($p<0.05$) in body weight gain immediately after the starter phase[24]. The best performance at levels of threonine supplementation by improving nitrogen retention and high threonine intake these results were supported by Estalkhzir *et al.*[25]. Weight gains and feed conversion ratio significantly improved in broilers fed threonine higher diets compared to control- feeding diets[26]. The highest feed intake was achieved in the diets containing threonine at 110 and 115% higher than the required level and addition of threonine increased growth and breast muscle weight gain and that might lead to consumption of more feed by broiler chickens in order to achieve rapid growth[27]. Data of feed conversion ratio showed that diet supplemented with threonine resulted in increased feed conversion ratio as a result of increased feed intake and decreased weight gain from 1-6 weeks of age and these results were supported by Namroud *et al.*[28].

On the other hand, it was found that increasing the levels of L-threonine in the diet did not affect body weight gain[29-32]. Threonine supplementation did not affect feed intake, also, increasing L-Threonine level in broiler diets did not affect feed intake up to 42 days of age and this was recorded by Khan *et al.*[6] .Threonine supplementation did not affect feed conversion ratio[33].

Concerning carcass traits, it was noticed that control group had the highest giblets weight and dressing percentage compared to that exposed to delayed access to feed time. Moreover, chicks fastened 24 h after hatch and fed threonine supplemented diet showed the highest carcass traits compared to other groups. These results were in accordance with Djouvinov *et al.*[34] and Ciftci and Ceylan[35], who reported that carcass weights and relative weights of breast and leg significantly increased in broilers fed threonine (110, 120 and 130%) supplemented. On the contrary, Deschepper and De Groote[36] was not found any differences in carcass yield using threonine supplemented diet but resulted in a higher carcass fat content.

Bregendahl *et al.*[37] concluded that threonine supplementation has no effect on improving carcass weight percentage. Also, Dozier *et al.*[38] found that the total carcass yield was not affected by dietary threonine supplementation. In harmony with this finding, Noy and Sklan[39] cleared that feeding chicks immediately after hatch encourage the morphological maturity of jejunum and duodenum. At the same consequences Yamauchi *et al.*[40] reported that the deprivation of feed for 24-48 h after hatch depressed the villi

length. By 36 h fasting post-hatch, intestinal villi volume of small intestine was decreased[21]. Ferket and Uni[41] stated that a few days before hatch, most parts of the gastrointestinal tract starts to grow to be completely developed after chicks take feed as the absence of diet immediately after hatch cause depression in the surface area of the intestinal villi[42].

CONCLUSION

Beginning of feed utilization immediately post hatch is an important task which is necessary for the growth of digestive system of chicks, which in turn affects the chick's performance. So delay feed access to chicks especially after hatch due to any cause have several consequences on the chick's performance and the whole economy and to decrease these consequences, it is recommended to add threonine to the diet in order to substitute the deficiency in body performance.

SIGNIFICANCE STATEMENT

This study discovers the possible elucidated effect of delayed feed access post hatch and the maximum time for that delay and in order not to affects poultry performance which obstacle the world of poultry production. This study will help the researcher to discover that the early neonatal chick access to feed through decrease the time of transportation from the hatchery to the poultry farm or purchase broiler chicks from near hatchery is most vital practical key to overcome this problem.

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