



## Effects of Dietary Methionine Levels on Choline Requirements of Starter White Pekin Ducks

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**ABSTRACT:** A 2×5 factorial experiment, using 2 dietary methionine levels (0.28% and 0.48%) and 5 dietary choline levels (0, 394, 823, 1,239, and 1,743 mg/kg), was conducted to study the effects of dietary methionine status on choline requirements of starter white Pekin ducks from 7 to 28 days of age. Four hundred eighty 7-d-old male White Pekin ducks were randomly allotted to ten dietary treatments, each containing 6 replicate pens with 8 birds per pen. At 28 d of age, weight gain, feed intake, and feed/gain were measured and the legs of all ducks from each pen were examined for incidence of perosis. Perosis and growth depression were observed in choline-deficient ducks and supplementation of choline reduced perosis and significantly increased weight gain and feed intake regardless of dietary methionine levels ( $p < 0.05$ ). In addition, significant positive effects of dietary methionine supplementation on weight gain, feed intake, and feed/gain were observed at any choline level ( $p < 0.05$ ). Supplementation of 1,743 mg/kg choline in diets alleviated the depression of weight gain and feed intake caused by methionine deficiency at 0.28% methionine level. The interaction between choline and methionine influenced weight gain and feed intake of ducks ( $p < 0.05$ ). At 0.28% methionine level, 1,743 mg/kg choline group caused 4.92% and 3.23% amount of improvement in weight gain and feed intake compared with 1,239 mg/kg choline group, respectively. According to the broken-line regression, the choline requirements of starter Pekin ducks for weight gain and feed intake were 1,472 and 1,424 mg/kg at 0.28% methionine level and 946 and 907 mg/kg at 0.48% methionine level, respectively. It suggested the choline recommendations of starter Pekin ducks on a semi-purified diet were 1448 mg/kg at 0.28% methionine level and 927 mg/kg at 0.48% methionine level, respectively. Compared with the adequate methionine level, methionine deficiency markedly increased the choline requirements of ducks. (**Key Words:** Duck, Choline, Methionine, Growth Performance)

### INTRODUCTION

Choline, a water-soluble vitamin, plays important roles in the synthesis of the membrane phospholipids (Hollenbeck, 2010), neurotransmitter synthesis (Blusztajn and Wurtman, 1983; Wessler et al., 2001) and methyl-group metabolism (Zeisel and Blusztajn, 1994). Poor growth, hepatic fatty infiltration, and perosis were all observed in choline-deficient Pekin ducks (Bernard and Demers, 1949; Wen et al., 2014). NRC (1994) provided choline recommendations for chickens, turkeys, geese, and quails, but the recommendation of this vitamin was missing for

ducks. At present, although the effects of different choline levels on growth performance of Tsaiya ducks was reported by Lien and Jan (1999), the data about the requirement of this vitamin was not given in their study and new literature on choline requirements of ducks was lacking until now.

On the other hand, choline is the methyl donor that provides the one-carbon unit in the conversion of homocysteine to methionine (Zeisel and Blusztajn, 1994). The interrelationship between choline and methionine remains a subject of ongoing debate. Ketola and Nesheim (1974) suggested that the choline requirement of chicks is elevated by high dietary methionine levels. However, some researchers reported more dietary choline was required when methionine is deficient in poultry (Miles et al., 1983; Harms and Miles, 1984). At present, studies on the interrelationship between methionine and choline in Pekin ducks are scarce and the effect of dietary methionine levels

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on choline requirements of ducks is not clear. Therefore, the objective was to estimate the choline requirements of white Pekin ducks with deficient and adequate methionine levels and to evaluate the effects of methionine status on the requirements of choline for ducks.

## MATERIALS AND METHODS

This study was approved by the animal care and use committee of the Institute of Animal Sciences of Chinese Academy of Agricultural Sciences, and all efforts were made to minimize the suffering of animals.

### Birds and housing

A 2×5 factorial experiment, using 2 dietary methionine levels (0.28% and 0.48%) and 5 dietary choline levels (0, 394, 823, 1,239, and 1,743 mg/kg), was conducted to study the effects of methionine status on choline requirements in white Pekin ducks from 7 to 28 d of age. A total of 480 seven-d-old male White Pekin ducklings with average body weight of 132±3 g were randomly divided into ten dietary treatments, each containing 6 replicate pens with 8 birds per pen. These birds were kept in plastic-wire-floor pens (200 by 100 by 40 cm) in an environmentally controlled experimental building. The temperature was kept at 28°C (at floor level) from 7 to 10 d of age, and then it was reduced gradually to room temperature until 28 d of age. All birds had free access to water and feed at any time and lighting was continuous. Water was provided by drip-nipple water supply lines and feed was fed in pellet form.

### Experimental diets

The basal diet was formulated using corn starch and isolated soybean protein that were low in methionine and choline content (Table 1). To produce experimental diets, the basal diets were supplemented with different levels of *DL*-methionine (0 and 0.2%) and choline (0, 400, 800, 1,200 and 1,600 mg/kg). Choline was added in the form of choline chloride (silica carrier, purity≥50%, Hebei Be-Long Feed Additive Co., Ltd, Heibe, China). The choline concentration was analyzed by ion exchange chromatography according to Zhai et al. (2013). In brief, powdered feed samples (2 g) were extracted with 25 mL of ultrapure water in a 50-mL polypropylene centrifuge tube and homogenized for 1 min. Then feed samples were extracted by using ultrasound bath for 30 min at 70°C. The supernatant was transferred into a 50-mL volumetric flask after extracts were centrifuged. After repeating the procedure, the volume of the supernatant was increased to 50 mL with ultrapure water. The supernatant was loaded into a plastic 2.5-mL syringe and filtered by a 0.45-µm filter membrane. This solution was then used for choline quantification by an ion chromatograph. Choline standards

**Table 1.** Ingredient composition and nutrient content of the basal diet (% as-fed basis)

Item	Content
Ingredient (%)	
Corn starch	63.0
Isolated soybean protein	25.7
Rice hull powder	5.0
Soybean oil	2.0
Dicalcium phosphate	2.2
Limestone meal	0.8
Premix <sup>1</sup>	1.0
Sodium chloride	0.3
Calculated values	
Metabolizable energy <sup>2</sup> (kcal/kg)	3,480
Ca (%)	0.90
Nonphytate P (%)	0.42
Analyzed values <sup>3</sup>	
Crude protein (%)	22.12
Total lysine (%)	1.29
Total threonine (%)	0.78
Total tryptophan (%)	0.20
Total methionine (%)	0.28
Choline <sup>4</sup> (mg/kg)	0

<sup>1</sup> Premix provided the following per kg of diets: Cu, 10 mg; Fe, 60 mg; Zn, 60 mg; Mn, 80 mg; Mg, 500 mg; K, 2,000 mg; Se, 0.3 mg; I, 0.2 mg; retinyl acetate, 10,000 IU; cholecalciferol, 3,000 IU; DL- $\alpha$ -tocopheryl acetate, 20 IU; menadione sodium bisulphate, 2 mg; thiamin, 2 mg; pyridoxine hydrochloride, 4 mg; cobalamin, 0.02 mg; calcium-D-pantothenate, 20 mg; nicotinic acid, 50 mg; folic acid, 1 mg; riboflavin 8 mg; biotin, 0.2 mg.

<sup>2</sup> Values were calculated according to the MEn of feedstuffs for poultry provided by NRC (1994).

<sup>3</sup> Analyzed in duplicate.

<sup>4</sup> Below the level of detection.

were choline chloride (purity ≥98%, Sinopharm Chemical Reagent Co., Ltd., Shanghai, China). The analyzed levels of dietary choline were 0, 394, 823, 1,239, and 1,743 mg/kg diet, respectively. Contents of methionine in basal diet were analyzed by ion-exchange chromatography with an amino acid analyzer (L-8900, Hitachi, Tokyo, Japan) after acid hydrolysis according to the method recommended by the Standardization Administration of China (2000). The analyzed methionine concentration in the basal diet was 0.28%. Crude protein in the basal diet was determined by the Kjeldahl method (method 954.01; AOAC, 2000). All diets were firstly prepared in mash form and then all pelleted (2.5 mm in diameter).

### Measurements

At 28 d of age, weight gain, feed intake and feed/gain of ducks from each pen were measured after 12 h of fasting. Feed intake and feed/gain were all corrected for mortality. In addition, the legs of all ducks from each pen were examined for incidence of perosis according to symptom

descriptions of Evans et al. (1942). The symptoms of perosis were divided into four types: Enlarged hock joint; twisting, turning, or bowing leg; slipped tendon; crippled. When single or more symptoms were observed in one or both legs of ducks, the bird was considered to have perosis. The perosis of all ducks were examined by the same person every time.

### Statistical analysis

Data were analyzed by two-way analysis of variance procedure of SAS (SAS Institute, 2003) with the pen being used as the experimental unit. Linear and quadratic polynomial contrasts were performed to determine the effects of dietary choline. The variability in the data was expressed as the standard error of the means and a probability level of  $p < 0.05$  was considered to be statistically significant. In study, broken-line regression model analysis (Robbins et al., 2006) was used to estimate dietary choline requirement at each supplemental methionine level using the NLIN procedure of SAS. The model was as follows:  $y = l + u(r - x)$ , where  $y$  = weight gain,  $x$  = dietary choline level,  $r$  = choline requirement,  $l$  = the response at  $x = r$  and  $u$  = the slope of model. In this model,  $y = l$  when  $x > r$ . In addition, according to the statistical method suggested by Sterling et al. (2003), a t-test was used to determine if a difference existed for the choline requirements established at the two methionine levels.

## RESULTS AND DISCUSSION

In previous studies, a diet containing a large percentage of corn starch and isolated soybean protein had been successfully used to determine the choline requirement and the availability of choline in feedstuffs for broilers (Molitoris and Baker, 1976; Pesti et al., 1981; Emmert and Baker, 1997). Therefore, in order to reduce choline content in the basal diet, purified diets containing corn starch and isolated soybean protein were used in this study as they contained little choline. The choline concentration in basal diet was analyzed by ion exchange chromatography, but it was not detected. The reason may be associated with the detection limit, precision, and recovery. So the choline concentration in basal diet was given as zero. Previous studies have used measurements of perosis and growth depression as the characteristic symptom of choline-deficiency in ducks (Wen et al., 2014). In the present study, a lack of supplementation of choline caused perosis and depressed weight gain and feed intake, which indicated that the basal diet was choline deficient and was hence appropriate for estimation of choline requirements of Pekin ducks. Perosis caused by choline deficiency was reduced by increasing dietary choline level. The reason for perosis caused by choline deficiency may be that the content of

phosphatidylcholine in the bone cell was decreased, because choline is a component of the membrane phospholipids (Hollenbeck, 2010). Additionally, choline deficiency could cause a decrease in choline kinase activity (Li et al., 2005); previous study has shown that choline kinase was required for normal endochondral bone formation (Li et al., 2014).

As shown in Table 2, supplementation of methionine significantly improved weight gain and feed intake and decreased feed/gain ( $p < 0.05$ ). In poultry, this positive effect of methionine on growth performance has been shown by many researchers (Bornstein and Lipstein, 1964; Blair et al., 1986; Ohta and Ishibashi, 1995). In addition, supplementation of choline caused significant improvement in weight gain and feed intake (Table 2). This result is in accordance with a previous study in chickens by Ketola and Nesheim (1974), who reported that the addition of choline could improve the body weight gain of chicks either in the presence or absence of methionine supplementation. In this study, the data also indicated that methionine had a greater growth-promoting effect than choline because increasing dietary methionine significantly decreased feed/gain ( $p < 0.05$ ), but it was not true for increasing choline. In chicks, excess choline was found to efficiently compensate for dietary methionine deficiency (Quillin et al., 1961; Pesti et al., 1980; Baker et al., 1983). This study found similar results. As shown in Table 2, at low methionine level, 1,743 mg/kg choline could improve weight gain and feed intake further. However, at normal methionine level, weight gain and feed intake showed no increase at the 1,743 mg/kg choline level. It indicated that supplementation of choline in diets could alleviate the depression of weight gain and feed intake caused by methionine deficiency. Methionine plays an important role in poultry growth, and it is the first-limiting amino acid in common poultry diets. The early growth of young birds is mainly due to the deposition of body protein, and feed intake is an important factor affecting body protein synthesis (Kita et al., 1996). Dietary methionine deficiency caused an imbalance in the diet's amino acid profile, which altered feed intake. This pattern in ducks has also been observed by Chen et al. (1991). This interaction between choline and methionine was also found in fish studies. In the absence of methionine, choline can provide a portion of the methionine requirement (Kasper et al., 2000; Wu and Davis, 2005). Because choline is a methyl donor and can donate a methyl to homocysteine to form methionine (Zeisel and Blusztajn, 1994), which was clearly shown by the interaction between choline and methionine in this study ( $p < 0.05$ ).

A linear increase in weight gain and feed intake was obtained as dietary choline was increased from 0 to 1,734 mg/kg at 0.28% methionine level (Table 2). However, at 0.48% methionine level, weight gain and feed intake increased significantly as dietary choline increased from 0

**Table 2.** Effect of dietary choline on growth performance and perosis of Pekin ducks from 7 to 28 d of age at two dietary methionine level<sup>1</sup>

Dietary treatment			Weight gain	Feed intake	Feed/gain	Incidence of perosis <sup>3</sup> (%)
Dietary Met <sup>2</sup>	Analyzed choline <sup>2</sup>	Calculated choline	(g/d/bird)	(g/d/bird)	(g:g)	
0.28	0	2	12.13 <sup>e</sup>	29.49 <sup>e</sup>	2.48	100
	394	402	13.30 <sup>e</sup>	32.53 <sup>de</sup>	2.46	45
	823	802	16.80 <sup>cd</sup>	39.89 <sup>bc</sup>	2.38	22
	1,239	1,202	21.13 <sup>ab</sup>	49.23 <sup>a</sup>	2.35	5
	1,743	1,602	22.17 <sup>a</sup>	50.82 <sup>a</sup>	2.32	0
0.48	0	2	15.94 <sup>d</sup>	35.96 <sup>cd</sup>	2.26	100
	394	402	19.15 <sup>bc</sup>	42.01 <sup>b</sup>	2.20	15
	823	802	22.05 <sup>a</sup>	50.58 <sup>a</sup>	2.31	4
	1,239	1,202	23.11 <sup>a</sup>	52.17 <sup>a</sup>	2.30	0
	1,743	1,602	22.99 <sup>a</sup>	51.38 <sup>a</sup>	2.26	0
Pooled SEM			0.57	1.19	0.04	
Main effect						
Dietary Met level		0.28	17.10 <sup>b</sup>	40.39 <sup>b</sup>	2.40 <sup>b</sup>	
		0.48	20.65 <sup>a</sup>	46.42 <sup>a</sup>	2.26 <sup>a</sup>	
Pooled SEM			1.29	2.71	0.03	
Dietary choline level		0	14.04 <sup>d</sup>	32.73 <sup>d</sup>	2.38	
		394	16.22 <sup>c</sup>	37.27 <sup>c</sup>	2.33	
		823	19.43 <sup>b</sup>	45.24 <sup>b</sup>	2.35	
		1,239	22.12 <sup>a</sup>	50.70 <sup>a</sup>	2.32	
		1,743	22.58 <sup>a</sup>	51.09 <sup>a</sup>	2.29	
Pooled SEM			1.65	3.66	0.01	
Probability						
Met			<0.001	<0.001	0.048	
Choline			<0.001	<0.001	0.896	
Met×choline			0.046	0.045	0.908	
Choline linear			<0.001	<0.001	0.584	
Choline quadratic			0.028	0.104	0.974	

SEM, standard error of the means.

<sup>1</sup> Values are the means of 6 replicates of 8 ducks. <sup>2</sup> Analyzed value.<sup>3</sup> Incidence of perosis = the number of ducks with perosis per treatment/the total ducks number per treatment.<sup>a-c</sup> Mean values in the same column with no common superscript differ significantly (p<0.05).

to 1,239 mg/kg and then reached a plateau at the ranges of dietary choline from 1,239 to 1,743 mg/kg. Pekin ducks showed different growth response with increasing choline level at two methionine levels. This data indicates that more choline was needed by ducks at the low methionine level. Broken-line regression analysis has been used to estimate the choline requirements in broilers (Pesti et al., 1980), ducks (Wen et al., 2014), and fish (Mai et al., 2009; Jiang et

al., 2012). Therefore, the choline requirements of ducks at two methionine levels were estimated by broken-line regression (Table 3). According to this regression, the choline requirements of White Pekin ducks for weight gain and feed intake were 1,472 and 1,424 mg/kg at 0.28% methionine level and 946 and 907 mg/kg at 0.48% methionine level, respectively (Table 3). The t-test showed that the choline requirement at 0.28% methionine level was

**Table 3.** Choline requirements for weight gain and feed intake at different supplemental methionine levels according to broken-line regression

Methionine level (%)	Response criterion	Broken-line model	p-value	R <sup>2</sup>	Choline requirement (mg/kg) <sup>1</sup>
0.28	Weight gain	Y = 22.17 - 0.00738 × (1,472 - x)	0.029	0.947	1,472 ± 212 <sup>a</sup>
	Feed intake	Y = 50.82 - 0.0161 × (1,424 - x)	0.024	0.959	1,424 ± 187 <sup>a</sup>
0.48	Weight gain	Y = 23.05 - 0.00741 × (946 - x)	0.002	0.982	946 ± 31 <sup>b</sup>
	Feed intake	Y = 51.775 - 0.0178 × (907 - x)	0.005	0.981	907 ± 48 <sup>b</sup>

<sup>1</sup> It was expressed as requirement ± standard error.<sup>a,b</sup> Values in the same column with no common superscript differ significantly (p<0.05).

much greater than the requirements of choline at 0.48% methionine level ( $p < 0.05$ ). Therefore, it was concluded that methionine deficiency markedly increased the choline requirements for ducks, and this reason for this may be that in the presence of low methionine there is greater reliance on any surplus choline above the requirement of the bird, as this can be oxidized to betaine which can provide methyl for conversion of homocysteine to methionine (Setoue et al., 2008).

### CONCLUSION

In conclusion, choline deficiency could cause growth depression and perosis of starter Pekin ducks. In this study it was found that the choline requirements of male Pekin ducks from 7 to 28 d of age for weight gain and feed intake were 1,472 and 1,424 mg/kg at 0.28% methionine level and 946 and 907 mg/kg at 0.48% methionine level, respectively. It suggested the choline recommendations of starter Pekin ducks in semi-purified diet were 1,448 mg/kg at 0.28% methionine level and 927 mg/kg at 0.48% methionine level, respectively. Compared with the adequate methionine level, methionine deficiency markedly increased the choline requirements of ducks.

### 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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