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# The Effects of Threonine on Performance Parameters, Carcass Traits, Visceral Organ Indices and Serum Biochemical Parameters of Linwu Ducks, Aged 4 to 8 Weeks

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#### ■Keywords

Linwu duck; threonine requirement; growth performance; carcass traits; biochemical parameters.



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# **ABSTRACT**

This study was conducted to establish dietary threonine (Thr) levels for Linwu ducks, aged 4 to 8 wk. Experimental diets formulated to contain 0.56, 0.61, 0.66, 0.71, 0.76, and 0.81% Thr fed to Linwu ducks. A total of 360 healthy Linwu female ducks with similar body weight (1183.89±3.83 g) were randomly divided into six groups, with five replicates in each group, and 12 ducks in each replicate. Samples were collected at 8 wk for the determination of growth performance, carcass traits, visceral organ indices, and serum biochemical parameters. As a result of this study, Thr level had no significant influence on the final weight, the daily gain, feed/gain ratio, and average daily intake (p>0.05). Similarly, there were no significant effects of dietary Thr on carcass traits and visceral organ indices (p>0.05). The pancreatic index was highest among all the treatments when the dietary Thr level was 0.66%. The different dietary Thr levels had no significant effect (p>0.05) on the concentration of total protein (TP), triglyceride (TG), total cholesterol (TC), glucose (GLU), superoxide dismutase (SOD), malondialdehyde (MDA), glutathione peroxidase (GSH-Px), and glutathione (GSH). However, the serum MDA concentration in the 0.66% treatment was lower (P=0.068) than in the other treatments. In conclusion, Thr at 0.66% concentration may have an antioxidant activity and exert positive effect on Linwu ducks.

# **INTRODUCTION**

Threonine (Thr) is an essential amino acid in poultry diets. It is the third most limiting amino acid and plays an important role in animal growth and development (Ayasan et al., 2009; Canogullari et al., 2009; Xie et al., 2014). Thr is a typically deficient amino acid in corn, sorghum and soybean meal based rations and it becomes one of the major factors in limiting the birds' performance (Kidd, 2000). Supplementation of Thr in the diets of broiler chicks and white Pekin ducklings was reported to improve their growth performance (Horn et al., 2010; Ayasan & Okan, 2011). Khan et al. (2006) conducted an experiment on commercial broiler chicks 20~35d of age with different levels of digestible Thr and observed significant improvement in body weight gain. Moreover, Carzo et al. (2009) reported that the whole carcass yield and breast meat were increased with increased Thr in combination with glycine in Ross 308 male broilers. However, Thr did not affect the relative percentages of liver, thigh, and gizzard in Ross 308 broiler chicks (Estalkhzir et al., 2013). Baeza & Leclercq (1998) designed an experiment to assess the effects of Thr supplementation in finishing diets used for raising Muscovy ducks and found that Thr requirement was greater than 3.5 g/kg and less than 4.5 g/kg.

Linwu duck is a special product in the Linwu County, Hunan province, China. It is one of the most famous ducks in China with characteristics of



fast growth, large body, strong adaptability, high feed reward, and delicate meat. Currently, the dietary Thr requirement of Linwu ducks has not been determined, and no reference values have been established for animal production. Thus, the objective of the current study was to determine the Thr requirement of Linwu ducks, aged 4 to 8 wk, based on their growth performance, carcass traits, visceral organ indices, and serum biochemical parameters.

# **MATERIALS AND METHODS**

All the protocols used in the study were approved by the Hunan Institute of Animal Science and Veterinary Medicine Animal Care and Use Committee. (Permit Number: 2017-0306)

# **Animals and housing**

Three hundred and sixty Linwu ducks (a local breed in southern China) were randomly divided into six groups (60 ducks/group) and housed in pens with a plastic floor for 4 wk. Each group had five replicates (12 ducks/pen). The ducks had free access to water and feed. The climatic conditions and lighting program were computer-operated and followed the commercial recommendations. Environmental temperature throughout the period of experiment was 25°C. During the experimental period, ducks were exposed to natural light—dark cycle.

# **Experimental diets**

All the feeds were manufactured at the Human Institute of Animal Science and Veterinary Medicine. Basal diet, which was corn and soybean meal based, was formulated to meet the nutrient needs of the Linwu ducks based on the nutrient recommendations of NRC (1994) (Table 1). The Thr content in the basal diet was 0.56%. The other diets consisted of basal diet supplemented with 0.05, 0.10, 0.15, 0.20, and 0.25% L-Thr, respectively, so as to have total Thr content of 0.61, 0.66, 0.71, 0.76, and 0.81%, respectively, in the five experimental diets. The contents of other nutritional ingredients in the diets were the same as in the basal diet.

# **Growth performance**

The body weight (BW) of ducks in each pen was recorded on a weekly basis from the first to the final day of the experiment. Feed intake in each pen during the period between two consecutive weight determinations was estimated by measuring the remaining feed on the same days on which the ducks

**Table 1** – Composition and nutrient levels of the basal diet (air-dry basis)

Ingredients	Content %	Nutrient levels <sup>2</sup>	Content	
Corn	46.00	ME / mcal/kg	2.75	
Soybean meal (44.0%CP)	5.40	СР	16.96	
Corn gluten meal(60.0% CP)	6.20	Ca	0.88	
Cottonseed meal(46.1% CP)	5.50	Total P	0.71	
Wheat bran	9.30	NPP	0.34	
Wheat middling	10.00	Lys	0.91	
Rice bran	8.00	Met	0.42	
Corn barn	4.00	Met+Cys	0.70	
Limestone	1.45	Thr	0.56	
CaHPO <sub>4</sub>	1.20	Trp	0.16	
Lys	0.40	Linoleic acid	2.03	
DL-Met	0.15			
NaCl	0.30			
Soybean oil	1.10			
Premix <sup>1</sup>	1.00			
Total	100.00			

 $^1$  The premix provides following for per kg diet: VA 5 000 IU, VD 800 IU, VE 20 IU, VK 0.5 IU, Niacin 60 mg, Pantothenic acid 60 mg, Pyridoxine 2.5 mg, Riboflavin 4.0 mg, Biotin 0.2 mg, Folic acid 0.6 mg, Thiamine 2 mg, Cu 8 mg, Fe 80 mg, Mn 50 mg, Zn 60 mg, Se 0.20 mg, and I 0.40 mg.  $^2$ Calculated values.

were weighed. The values of average daily gain (ADG), average daily feed intake (ADFI), and feed:gain ratio (F/G) were calculated as feed per gain based on the weight of feed consumed divided by BW gain per pen.

# **Sample collection**

At the end of feeding, four ducks were randomly selected from each pen and fasted for 12 h before sampling. The ducks were euthanized by severing the jugular vein. Each carcass was dissected into deboned skinless thighs and breasts immediately after the slaughter. The weight of each carcass (with feet and head) and its breast fat was recorded. Blood samples were centrifuged at  $4000 \times g$  for 5 min, and serum was separated and packed in tubes. Heart, liver, spleen, pancreas, gizzard, glandular stomach, and bursa of Fabricius were removed, cleaned, and weighed.

# Samples analysis

The concentrations of serum total protein (TP), glucose (GLU), triglyceride (TG), and total cholesterol (TC) were analyzed using an automatic biochemistry analyzer (URIT-8000, USA). Total superoxide dismutase (T-SOD), glutathione peroxidase (GSH-Px), glutathione (GSH), and malondialdehyde (MDA) in the serum were determined using colorimetric methods with a UV-visible spectrophotometer UV-2401PC (Shimadzu Co., Kyoto, Japan). All the serum biochemical parameters were determined by methods of commercial kits that were bought form a commercial company (Jiancheng Bioengineering Institute, Nanjing, China).



# **Calculations and statistical analysis**

The different carcass traits and organ index were calculated as follows according to Tong *et al* (2014): Percentage of eviscerated yield (%) = (eviscerated yield/live-weight)×100; Percentage of breast (%) = (weight of breast/eviscerated yield) ×100; Percentage of crureus (%) = (weight of crureus/eviscerated yield) ×100; Percentage of abdominal fat (%) = (weight of abdominal fat/eviscerated yield+weight of abdominal fat)×100; Organ index (%) = (weight of organ/live-weight) ×100.

Data were analyzed using the GLM procedure of SPSS 21.0 software (Lu *et al.* 2000) for one-way ANOVA to assess the main effects of dietary Thr level. Significant differences between the treatments were

determined at p<0.05 using Duncan's new multiple range tests. The values given in the tables are mean values and pooled SEM.

# **RESULTS**

# **Growth performance**

The effects of dietary Thr level on the growth performance of Linwu ducks are presented in Table 2. There were no significant (p>0.05) effects of treatment on the final weight, average daily gain, average daily feed intake, and F/G ratio of animals. However, F/G was gradually reduced with the increase in dietary Thr levels; the value of F/G was minimum when the dietary Thr level was 0.76%.

Table 2 – Effect of dietary Thr level on the growth performance of Linwu ducks, aged 4 to 8 weeks

Items		Dietary Thr level (%)						
items	0.56	0.61	0.66	0.71	0.76	0.81	SEM	p value
IW (g)	1180.33	1181.33	1187.00	1180.00	1189.00	1185.67	8.36	1.000
FW (g)	1513.57	1582.43	1564.49	1565.52	1591.68	1527.07	10.58	0.212
ADG (g)	11.90	14.32	13.48	13.77	14.38	12.75	0.32	0.178
ADFI (g)	129.51	123.85	116.62	114.45	111.28	121.16	2.67	0.389
F/G	11.29	8.90	8.74	8.44	7.83	9.51	0.38	0.110

Note: IW=initial weight, FW=final weight, ADG=average daily gain, ADFI=average daily feed intake, and F/G=feed/gain ratio

#### **Carcass traits**

As shown in Table 3, the different carcass traits of Linwu ducks showed no significant difference (p>0.05) at different dietary Thr levels. However, the dressing

percentage and percentage of breast muscle in Linwu ducks was higher (p>0.05) in the treatments in which the dietary Thr level was 0.66%. The percentages of half-eviscerated yield and eviscerated yield were highest when the dietary Thr level was 0.71%.

Table 3 – Effect of dietary Thr level on carcass traits of Linwu ducks, aged 4 to 8 weeks

Items%		Dietary Thr level (%)						
Items 70	0.56	0.61	0.66	0.71	0.76	0.81	- SEM	<i>p</i> value
Dressing percentage	88.07	88.62	89.32	88.07	85.95	86.78	0.45	0.289
Percentage of half-eviscerated yield	81.09	81.41	81.11	82.22	80.02	79.55	0.42	0.504
Percentage of eviscerated yield	72.93	73.19	72.75	74.05	71.64	71.78	0.40	0.543
Percentage of breast muscle	12.97	14.12	14.52	14.49	12.93	13.91	0.25	0.262
Percentage of leg muscle	9.67	10.20	11.17	9.94	10.50	11.19	0.23	0.275
Percentage of abdominal fat	1.71	1.57	1.49	1.76	2.00	1.21	0.11	0.459

# **Visceral organ indices**

The dietary Thr level had no significant effect (p>0.05) on the visceral organ indices of Linwu ducks

(Table 4). However, the pancreatic index was highest among all the treatments when the dietary Thr level was 0.66%.

Table 4 – Effect of dietary Thr level on visceral organ indices of Linwu ducks, aged 4 to 8 weeks

Items %		Dietary Thr level (%)						
	0.56	0.61	0.66	0.71	0.76	0.81	SEM	P value
Spleen index	0.60	0.65	0.61	0.63	0.59	0.58	0.03	0.847
Pancreatic index	2.28	2.09	2.86	2.48	2.51	2.37	0.08	0.120
Proventriculus index	2.90	3.00	3.32	2.83	2.72	3.11	0.08	0.374
Heart index	6.85	7.45	7.45	6.73	6.86	7.49	0.13	0.278
Liver index	17.97	16.53	18.01	17.62	16.75	17.61	0.37	0.820
Gizzard index	25.10	25.32	26.51	25.15	26.54	25.08	0.56	0.925



# **Serum biochemical indices**

The dietary Thr level had no significant effect (p>0.05) on the concentration of TP, TG, GLU, SOD, MDA, GSH-Px, and GSH (Table 5). However, it had significant effect on the concentration of TC, which was highest in the 0.66% treatment

and was significantly higher (p<0.05) than the corresponding value in the 0.81% treatment. Although there was no significant effect on the concentration of MDA, it was lower (p=0.068) in the 0.66% treatment compared to that in the other treatments.

Table 5 – Effect of dietary Thr level on serum biochemical indices of Linwu ducks, aged 4 to 8 weeks

Items -		Dietary Thr level (%)						
	0.56	0.61	0.66	0.71	0.76	0.81	SEM	<i>p</i> value
TP(g/L)	40.80	44.20	41.00	43.20	42.60	40.80	0.91	0.863
TC(mmol/L)	5.30 <sup>ab</sup>	5.96ª	6.09ª	5.53 <sup>ab</sup>	5.85ª	4.79 <sup>b</sup>	0.13	0.016
TG(mmol/L)	0.92	0.62	0.76	0.81	0.73	0.87	0.06	0.718
GLU(mmol/L)	9.97	11.49	11.24	9.91	10.28	9.42	0.31	0.351
SOD(U/ml)	133.93	113.93	120.91	131.33	122.96	127.11	3.24	0.511
MDA(nmol/ml)	5.92	6.36	5.03	5.24	7.08	6.08	0.24	0.068
GSH-PX(U/ml)	494.63	459.51	438.05	481.95	479.02	459.51	9.45	0.605
GSH(µmol/L)	39.05	42.12	36.76	25.59	31.65	31.28	2.14	0.416

Note: in the same row, values with different capital letter superscripts mean significant difference ( $\rho$ <0.05), while with the same or no letter superscripts mean no significant difference ( $\rho$ >0.05).

# **DISCUSSION**

Thr is the third most limiting amino acid in poultry diets behind the total sulfur-containing amino acids and lysine (Kidd, 2000; Baylan et al., 2006). A suitable amount of Thr in diet can maintain animal health and improve its production. Dietary Thr levels had no significant effect on the feed intake and feed conversion ratio. The results of the present study are in accordance with the study performed on Korean native ducks during 22-56 d with different levels of Thr and observed no significant improvement in body weight gain (Choo et al. 2014). Zhang et al. (2016) carried out an experiment by determining the dietary Thr requirement of Pekin ducks from hatch to 14 d of age and observed that body weight, body weight gain and feed intake were not significantly affected by increasing dietary Thr. However, Xie et al. (2014) reported that the weight and feed intake of Pekin ducks increased and F/Gratio of ducks decreased linearly or quadratically as the dietary Thr level increased from 5.0 to 8.2 g Thr/kg. Similar results were observed by Horn er al. (2009), Jiang et al. (2017) who reported that the growth performance was enhanced when dietary supplementation with Thr in ducks. In addition, Zhang et al. (2014) detected the Thr requirement of Pekin ducks from 15 to 35 d of age, and reported that the average daily weight gain increased significantly with the dietary Thr concentration. Bi et al. (2012) conducted an experiment on Pekin ducks from hatch to 21 d with graded concentration of Thr in diets

and reported significant improvement with increased dietary Thr levels.

In our study, dietary Thr had no significant effect on the production performance of ducks. This inconsistency in the animal production performance among different studies could be ascribed to the difference in the types of experimental animals, dose of the supplements, and housing environments, and other factors.

Carcass traits are important economic indicators that reflect the meat quality of commercial meat ducks. Several factors have been shown to influence the poultry carcass traits; these include genotype (Kokoszynski et al., 2015), age (Murawska, 2012), dietary energy (Fan et al., 2008), and dietary amino acid (Wang et al., 2004; Tang et al., 2011). Ciftci & Ceylan (2004) found that breast yield of broiler chickens was increased with the increasing dietary Thr, but abdominal fat was not affected. Similarly, Maqbool (2014) used six Thr levels (0.48, 0.54, 0.60, 0.61, 0.66, 0.72, and 0.78%) to investigate the effects of dietary Thr on carcass traits of Pekin ducks from 15 to 35 days of age, and observed that the breast yield was significantly increased at higher Thr levels, but abdominal fat and leg meat yield were not affected. These results are in agreement with the findings of Dozier et al. (2000) who indicated that the total carcass yield and proportions of abdominal fat were not affected by the dietary Thr concentration (in the range from 0.50 to 0.80%), but breast fillet weight and its relative yield were maximum at 0.62% dietary Thr level. The study was performed



to evaluate the effect of choice feeding based on Thr and observed significant effects on carcass parameters (Ayasan & Okan, 2014). Our results are in agreement with those of previous studies. Moreover, we observed that the dressing percentage and the percentage of breast muscle were highest at 0.66% dietary Thr level. Similarly, Bons (2000) reported that the Thr requirement for Pekin ducks from 21 to 49 days was 0.66%, based on the index of percentage of breast muscle.

The feed consumed by animals is digested, absorbed, and utilized for the work performed by the various organs. The development of internal organs is closely linked to growth, development, and health of animals, and to some of their other attributes. Generally, the organ index can reflect the development of organs that is often used in animal nutrition (Steczny *et al.*, 2017). As in the previous study, Azzam & El-Gogary (2015) found that dietary Thr levels had no significant differences in their effects on the weights of liver, gizzard, and pancreas (*p*>0.05). In the present study, the pancreatic, proventriculus, and liver indices were highest at 0.66% dietary Thr level.

Liver is not only extremely active in oxidizing triglycerides to produce energy, but it can also synthesize large quantities of TC. In the present study, blood TC was highest at 0.66% dietary Thr level. The reason for this could be that the index of liver was highest at 0.66% dietary Thr level and it could catabolize large amounts of amino acids. Generally, MDA is used as a biomarker for free radical-induced damage and endogenous lipid peroxidation (Xie et al., 2017). Li et al. (2016) reported that the 0.67% dietary Thr group had the lowest serum MDA concentration in Xinyang green-shell laying hens. Chen et al. (2017) reported that a higher level of Thr reduced the MDA content in the serum of male broilers (P = 0.012). The results from our study indicate that 0.66% Thr concentration may have an antioxidant activity and exert a positive effect on animal health.

# CONCLUSION

In conclusion, under the conditions of the present study, the optimum level of Thr in the diet of Linwu ducks, aged 4 to 8 wk, could improve their performance and played an important role in maintaining their health. However, further research is warranted to confirm these results and to better assess the actual requirement of Thr by Linwu ducks at different stages of life, the information for which is not available from the current literature.

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