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Research article

The interactive effects of threonine and crude protein level on apparent nutrients digestibility and nitrogen balance in local Muscovy ducks

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Abstract

This study aimed to determine the effects of threonine (Thr) and crude protein (CP) on the apparent nutrients, amino acid digestibility, and nitrogen balance of local Muscovy ducks at 8 and 10 weeks of age. The study was a 3x2 factorial design for two phases with three levels of CP (17, 18, and 19% CP) and two levels of Thr (0.7 and 0.8% Thr) for the first phase while three levels of CP (15, 16, and 17% CP) and two levels of Thr (0.5 and 0.6% Thr) for the second phase. The birds were fed and given water ad libitum for the entire experiment. The results showed that CP and Thr increased apparent nutrient digestibility, especially for DM digestibility ($P < 0.05$). Besides, Thr addition also increased OM, and NDF digestibility ($P < 0.05$). In addition, nitrogen balance was better in high-dose Thr and CP diets ($p<0.05$). For amino acids, when increased Thr and CP levels, it increased the digestibility of Isoleucine, Threonine, and Valine ($P < 0.05$) while Thr levels increased Methionine, Phenylalanine, Serine, Tyrosine, and CP levels increased Isoleucine, Leucine, Lysine, Threonine, Valine, Alanine, Glutamic, Proline digestibility ($P < 0.05$). However, this study could not record any interactions between Thr and CP in the diet throughout the experiment ($P > 0.05$). It can be concluded that supplementation with 0.8% Thr and 19% CP for 5-8 weeks of age and 0.6% Thr and 17% CP for 8-10 weeks of age in the local Muscovy duck diet increased the digestibility of apparent nutrients and amino acids and balanced the nitrogen substance in the duck's body.

Keywords: Crude protein, Digestibility, Muscovy, Nitrogen retention, Threonine

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INTRODUCTION

The poultry industry in Vietnam has been developing in recent years. The increase in both the quality and quantity of poultry breeds was focused on meeting the demand of humans. Local Muscovy ducks, called Cairina moschata, are a common breed for both egg and meat production (Downs et al., 2017). In addition, ducks were also a source of income for communities in the Mekong Delta Vietnam.

To this point, experts have carried out a multitude of experiments to enhance the digestibility of poultry. The reduction of feedstuff costs while maintaining the efficiency of utilizing low- or high-protein diets supplemented with crystalline amino acids to achieve or surpass minimum amino acid standards is one of the most significant roles that nutritionists play (NRC, 1994). The first step toward optimizing the growth and productive performance of poultry and animals, as well as reducing the negative effects on the environment, is to ensure that they are receiving the appropriate nutrition. Additionally, the incorporation of L-threonine as the third feed grade amino acid was facilitated by developments in optimum protein formulation methods as well as the expression of amino acids in dietary formulation on a digestible basis (Kidd et al., 2021). Threonine, the third limiting amino acid after methionine and lysine, is the two amino acids that are limited in broilers and is an essential amino acid for broilers (Berres et al., 2007). Thr is important in the development and normal functioning of the gut. In addition, Thr is necessary for the creation of uric acid and pancreatic enzymes, the synthesis of protein, collagen, and elastin in the body, and antibodies (Debnath et al., 2019). Moreover, the majority of the nitrogen that is expelled by birds is a result of undigested feedstuffs and amino acids that are not in proper proportion to the immediate requirements of the body for the synthesis of tissue (Alagawany et al., 2016). An increase in digestibility may improve the use of protein in the diet and reduce the amount of nitrogen that is produced. The measurement of apparent amino acid digestibility based on the analysis of ileal digestion is favored over the analysis of excreta. This is because ileal digestion contains more amino acids that are truly digested (Foltyn et al., 2015). The supplementation of Thr in the diets improved the growth performance and gut health of quail (Rasheed et al., 2018). Thr had a positive effect on growth performance. It also regulated the expression levels of genes related to amino acid transportation and protein deposition (Jiang et al., 2019). Better growth performance, amino acids, gut health, and ileal digestibility of protein were shown by the effects of CP and Thr interaction (Ahmed et al., 2020).

Previous studies have shown the effects of CP and Thr on growth performance. However, few studies have focused on the effects of these substances on duck production. Thus, the study aims to determine the suitable level of threonine and protein interactions in the diets apparent nutrient digestibility and nitrogen balance for local Muscovy ducks.

MATERIALS AND METHODS

Location and ethics statement

The study was conducted at Experimental Farm in Tra Vinh University, Tra Vinh city. The experiment followed the Ethical Management Process in Experimental Animals of Can Tho University No. 3965/QĐ-ĐHCT, published on October 15, 2021. In addition, to provide animal welfare, all procedures also followed the standard of Vietnam regulation (in Vietnamese).

Animals and trial design

To carry out this study, local Muscovy ducks were purchased from Tra Vinh province and afterward raised there. One-day-old ducklings were selected, brooded, and fed a meal that was delivered *ad libitum* from 1 to 28 days old. Two separate phases of the experiment were carried out when the chicks were 5-8 and 8-10 weeks of age. To determine the effects of Thr and CP on digestibility, the ducks were hand-picked and weighed individually, with an average initial live weight of 750 g at the 5^{th} week and 2050 g at the 7^{th} week of the experiment as one of the fastest growing phases of Muscovy ducks in Vietnam. These ages correspond to 8 and 10 weeks, respectively. All birds received vaccinations against the avian influenza virus and the duck plague virus at two and three weeks old, respectively. To experiment, all of the ingredients - corn, broken rice (BR), soybean meal (SBM), rice bran (RB), fish meal (FM), and dicalcium phosphate (DCP) – were purchased at a nearby animal feed shop in the province of Tra Vinh. All the materials for the experimental diets (Table 1), including feed, were analyzed to ascertain their chemical composition, and the mashes for the diets were prepared and provided.

Table 1 Amino acid composition of 335 feed ingredients in the diet

Criteria (%)	Corn	Rice bran (RB)	Broken rice (BR)	Fish meal (FM)	Soybean meal (SBM)
Essential amino acid					
Arginine	0.29	0.89	0.55	2.89	2.22
Isoleucine	0.26	0.53	0.36	2.14	1.74
Leucine	0.69	0.89	0.69	3.70	2.59
Lysine	0.25	0.53	0.22	3.89	2.15
Methionine	0.17	0.24	0.18	1.33	0.68
Histidine	0.06	0.19	0.08	0.47	0.28
Phenylalanine	0.29	0.50	0.43	1.96	1.78
Threonine	0.21	0.42	0.26	2.09	1.41
Valine	0.29	0.61	0.43	2.49	1.53
Nonessential amino acid					
Alanine	0.43	0.72	0.41	3.01	1.48
Aspartic	0.66	1.31	0.85	4.78	3.48
Glutamic	1.22	1.93	1.45	7.79	6.02
Glycine	0.24	0.51	0.27	2.66	1.33
Proline	0.69	0.87	0.37	1.94	1.84
Tyrosine	0.25	0.43	0.29	1.68	1.35

The first phase, 5-8 weeks old

At 5 weeks of age, local Muscovy ducks with 72 heads were assigned to a two-factorial design experiment, the first factor was CP concentrations of 17, 18, and 19%, and the second one was Thr concentrations of 0.7% and 0.8%. There were six treatments, 3 replicates per treatment, 4 birds per replicate, and sex parity. Dietary feed materials and chemical composition for local Muscovy ducks aged 5-8 weeks are shown in Table 2.

Table 2 Feed composition for the first phase (from 5-8 weeks of age)

Ingredients (%)	Thr 0.7%			Thr 0.8%		
	CP17	CP18	CP19	CP17	CP18	CP19
Corn	23.77	19.3	18.0	24.0	19.09	18.0
Rice bran	41.2	39.01	38.03	40.68	39.1	38.0
Broken rice	18.0	22.0	22.0	18.0	22.0	22.0
Fish meal	7.70	8.50	9.70	7.70	8.50	9.70
Soybean	8.00	9.80	11.0	8.00	9.80	11.0
Vit-Premix mineral	0.30	0.30	0.30	0.30	0.30	0.30
Lysine	0.15	0.07	-	0.15	0.07	-
Methionine	0.04	0.02	-	0.04	0.04	-
Threonine	0.14	0.10	0.07	0.23	0.20	0.16
DCP	0.50	0.50	0.50	0.50	0.50	0.50
Cr_2O_3	0.40	0.40	0.40	0.40	0.40	0.40
Total	100	100	100	100	100	100
DM	88.6	88.7	88.8	88.6	88.8	88.8
CP	17.0	18.0	19.0	17.0	18.0	19.0
ME (MJ/kg, DM)	12.96	12.97	12.97	12.95	12.96	12.96

DM: dry matter, ME: metabolizable energy, Vit: vitamin

The second phase, 8-10 weeks old

A total of 72 local Muscovy ducks aged 10 weeks were chosen based on their average live weight. Using a factorial design with two variables, three levels of CP (15%, 16%, and 17%) and two levels of Thr (0.5% and 0.6%), they were then randomly allotted to one of six treatments. There were 3 duplicates per treatment, with 04 birds in each replicate, balanced for sex. Table 3 displays the dietary feed ingredients and chemical composition of local Muscovy ducks aged 8 to 10 weeks.

Table 3 Feed composition for the second phase (from 8-10 weeks of age)

Ingredients (%)	Thr 0.5%			Thr 0.6%		
	CP15	CP16	CP17	CP15	CP16	CP17
Corn	50.7	49.3	46.7	50.6	49.3	46.8
Rice bran	27.8	27.1	27.2	27.8	27.0	27.1
Broken rice	8.00	8.00	8.00	8.00	8.00	8.00
Fish meal	6.20	7.50	7.50	6.20	7.50	7.50
Soybean	6.00	7.00	9.50	6.00	7.00	9.50
Vit-Premix mineral	0.30	0.30	0.30	0.30	0.30	0.30
Lysine	0.16	0.08	0.02	0.16	0.08	0.02
Methionine	0.06	0.04	0.02	0.06	0.04	0.02
Threonine	0.03	-	-	0.13	0.10	0.06
DCP	0.50	0.50	0.50	0.50	0.50	0.50
Cr_2O_3	0.40	0.40	0.40	0.40	0.40	0.40
Total	100	100	100	100	100	100
DM	88.6	88.6	88.7	88.7	88.6	88.7
CP	15.0	16.0	16.9	15.0	16.0	16.9
ME (MJ/kg, DM)	13.81	13.79	13.79	13.80	13.80	13.79

Diet and feeding

Diets typically consist of ingredients such as corn, BR, RB, SBM, and FM. All diets were supplemented with a vitamin and trace element mixture at a rate of 0.3%. Before each feeding occasion, chromic oxide at a concentration of 0.4% was carefully combined with the other components of the feed diet (Kim et al., 2014). Each experimental period lasted 19 days divided into three stages. Before the research began, birds were gradually introduced to the experimental meals over seven days period. After this, there was an adaptation period that lasted seven days, and then there was a collecting phase that lasted five days. The birds were fed in groups of three times each day to prevent any spillage (7:30, 13:00, and 17:00 a.m.). During the adaptation period of seven days, the ducks were allowed to eat *ad libitum* to measure their true feed consumption. Nevertheless, to reduce the number of feed refusals that occurred during the trial period, the feeding level was maintained at a level that was somewhat lower than the feed intake (90%). To calculate feed intake, the daily feeds and leftovers were weighed and collected first in the morning. Drinkers were filled with fresh water around the clock and offered to the birds.

Housing and management

During the experiment, a total of 72 ducks were randomly distributed among 18 wire cages that were 0.6 x 0.8 x 0.6 meters. Feeders and drinking nipples were positioned beneath the cages, and a plastic tray was positioned beneath each cage to collect waste. Feeders and drinking nipples were also positioned beneath each cage. A plastic cover was placed beneath the feeder of the cages in the morning so that any stray feed could be collected before it was distributed. The cages were placed within a well-ventilated building that had natural light during the day and artificial illumination at night so that the birds could eat whenever they pleased throughout the whole day. Cages, feed, and the plastic trays holding it were cleaned thoroughly every morning.

Sampling procedure for excreting

To determine the amount of feed intake and nutrients consumed as well as to conduct chemical tests, feed samples and total excreta were collected throughout the final five days of the trials. At 7:00, 13:00, and 19:00 each day, enormous amounts of excreta were collected and frozen at -20 degrees Celsius (Kim et al., 2014). Feathers, scales, and other debris were not collected to the greatest extent possible. After being frozen, the feces were thawed, placed in pens, and dried at temperatures between 55 and 60°C for 24 hours before being tested. After the dried excreta were homogenized, weighed, and powdered to the point where they could pass through a 0.5 mm sieve, representative samples were obtained and stored in airtight plastic containers at 4°C for subsequent testing. The samples were kept until later testing (Ravindran et al., 1999).

Chemical analysis

In this study, the method developed by AOAC was also used to determine the levels of Cr_2O_3 in both the diets and the excreta (1990). In addition to analyzing amino acids found in excreta, we analyzed amino acids found in representative samples of BR, RB, FM, and SBM (AOAC, 2000).

Every bird was weighed separately. To calculate daily feed intake, the total amount of feed used by each pen's four ducks was used. Excreta were quantitatively collected.

(Nutrient intake - nutrient in excreta)/nutrient intake) x 100 = apparent nutrient digestibility (%)

Using Cr_2O_3 as an indigestible marker, apparent excreta amino acid digestibility was estimated. (Ravindran et al., 1999; Bryden et al., 2009), as shown below:

$$\text{Apparent amino acid digestibility} = \frac{(AA/Cr_2O_3)_d - (AA/Cr_2O_3)_e}{(AA/Cr_2O_3)_d}$$

where:

$(AA/Cr_2O_3)_d$ = amino acid ratio to the dietary indicator of indigestibility

$(AA/Cr_2O_3)_e$ = ratio of amino acids to an indigestible marker in excreta.

Intake of nitrogen from feed minus nitrogen excreta equals nitrogen retention (Carvalho et al., 2012)

Nitrogen retention / $BW^{0.75}$= (The ratio of nitrogen expelled in excreta to nitrogen absorbed from the feed) / $BW^{0.75}$ (g of N per kg of $BW^{0.75}$)

Statistical analysis

Minitab Reference Manual Release 16.1.0 was used to analyze the data using ANOVA with the general linear model technique. The analysis followed a 3x2 factorial design. Paired comparisons were carried out using Tukey's technique, where the F test indicated a significant difference at $p<0.05$.

RESULTS

Apparent nutrient digestibility at 8 weeks age (%) of Muscovy ducks

At 8 weeks of age, significant effects of CP and Thr on local Muscovy ducks were recorded through apparent nutrient digestibility (Table 4). DM digestibility was significantly noted in this study ($p < 0.05$), with the highest DM digestibility in the study of 19% CP. Thr supplementation also showed a significant effect in the treatment of 0.8% Thr on DM, OM, and NDF digestibility ($p < 0.05$). The study did not record positive effects on EE, CF, and ADF by CP and Thr levels in the diet ($p > 0.05$) as well as no interaction between CP and Thr on apparent nutrient digestibility ($p > 0.05$).

Table 4 The effects of CP and Thr on apparent nutrient digestibility at week 8 (%)

Criteria	Thr levels		CP levels			SEM/p		
	Thr 0.8%	Thr 0.7%	CP 19%	CP 18%	CP 17%	Thr level	CP level	CP*Thr
DM	78.9	74.9	78.7^{a}	77.4^{ab}	74.6^{b}	0.76/0.003	0.94/0.028	1.33/0.067
OM	81.1	77.3	80.8	79.7	77.1	0.82/0.007	1.01/0.057	1.43/0.153
EE	79.1	77.5	79.9	77.9	77.1	1.01/0.293	1.24/0.291	1.75/0.465
CF	38.5	33.1	40.5	36.8	30.1	5.23/0.483	6.40/0.525	9.05/0.127
NDF	49.2	40.4	50.0	45.6	38.8	2.79/0.047	3.42/0.105	4.85/0.088
ADF	41.9	34.7	43.4	39.6	32.1	4.19/0.244	5.13/0.318	7.26/0.075

a, b, c – means within a row with different superscripts are significantly different at $p < 0.05$

Nutrient digestible at 8 weeks age (g/bird) of Muscovy ducks

The positive effects of CP and Thr were shown in DM, OM, EE, NDF, and ADF digestibility (Table 5) ($p < 0.05$). The highest DM and OM digestibility was recorded in the CP 19% and Thr 0.8% treatments. In addition, the CP factor had a significant effect on EE, with the highest digestibility in the CP 19% treatment, while an effect on NDF showed in the supplementation of 0.8% Thr ($p < 0.05$). This study did not record any significant changes in CF and ADF.

Table 5 The effects of CP and Thr on nutrient digestible at week 8 (g/bird)

Criteria	Thr levels		CP levels			SEM/p		
	Thr 0.8%	Thr 0.7%	CP 19%	CP 18%	CP 17%	Thr level	CP level	CP*Thr
DM	70.8	64.4	72.0^{a}	68.0^{ab}	62.8^{b}	1.81/0.027	2.22/0.038	3.14/0.244
OM	67.5	61.7	78.6^{a}	65.0^{ab}	60.2^{b}	1.74/0.038	2.13/0.049	3.01/0.303
EE	5.28	4.97	5.56^{a}	5.08^{ab}	4.74^{b}	0.15/0.157	0.18/0.024	0.25/0.420
CF	1.59	1.30	1.71	1.47	1.15	0.21/0.348	0.25/0.331	0.36/0.084
NDF	7.43	5.84	7.55	6.65	5.70	0.48/0.037	0.59/0.126	0.83/0.082
ADF	2.44	1.89	2.55	2.21	1.75	0.23/0.126	0.28/0.178	0.40/0.045

a, b, c – means within a row with different superscripts are significantly different at $p < 0.05$

The effects of CP and Thr on nitrogen balance at 8 weeks of age in Muscovy ducks

For the CP factor, nitrogen intake, nitrogen retention, the ratio of nitrogen retention to nitrogen intake, N intake/$W^{0.75}$ and N retention /$W^{0.75}$ were highest in the 19% CP treatment and lowest in the 17% CP treatment ($p < 0.05$), while nitrogen in the exrecta showed no significant differences between treatments ($p > 0.05$). In contrast, the diets with 0.8% Thr showed significant effects on N excreta ($p < 0.05$) and no differences in N intake and N intake/$W^{0,75}$ ($p > 0.05$). There was no interaction between treatments (Table 6).

Table 6 The effects of CP and Thr on nitrogen balance at week 8

Criteria	Thr levels		CP levels			SEM/p		
	Thr 0.8%	Thr 0.7%	CP 19%	CP 18%	CP 17%	Thr level	CP level	CP*Thr
N-int, g	2.58	2.48	2.78^{a}	2.53^{b}	2.28^{c}	0.05/0.162	0.06/0.001	0.09/0.570
N-exc, g	0.64	0.77	0.69	0.69	0.74	0.02/0.001	0.02/0.284	1.70/0.234
N-ret, g	1.94	1.70	2.09^{a}	1.84^{b}	1.54^{c}	0.05/0.008	0.06/0.001	0.09/0.269
N-ret/N-int, %	74.7	68.5	75.1^{a}	72.6^{a}	67.2^{b}	1.04/0.001	1.27/0.003	1.81/0.370
N-int/$W^{0.75}$, *	1.63	1.55	1.75^{a}	1.58^{ab}	1.45^{b}	0.04/0.220	0.05/0.003	0.07/0.656
N-ret/$W^{0.75}$, *	1.22	1.07	1.32^{a}	1.15^{ab}	0.97^{b}	0.04/0.017	0.05/0.001	0.07/0.354

*: g/kgW0.75
a, b, c – means within a row with different superscripts are significantly different at $p < 0.05$
N: nitrogen; Int.: intake; Exc: excreta; Ret: retention; W0.75: metabolizable body weight.

Apparent amino acid digestibility at 8 weeks age (%) of Muscovy ducks

The effects of CP and Thr on apparent amino acid digestibility were shown in Table 7. Supplementation with CP affected the digestibility of 4 of 9 essential amino acids, including arginine, leucine, threonine, and valine ($p<0.05$). For nonessential amino acids, only 3 of 7 were significantly different ($p<0.05$). The highest digestibility for all amino acids was in the 19% CP treatment, and the lowest one was at the 17% CP treatment. For Thr, supplementation showed no effects on 2 of 9 essential amino acids (isoleucine and histidine) and 1 of 7 nonessential amino acids (aspartic) ($p>0.05$), and the rest of the amino acids in this study recorded significant changes ($p<0.05$).

Table 7 The effects of CP and Thr on apparent amino acid digestibility at week 8

Criteria	Thr levels		CP levels			SEM/p		
	Thr 0.8%	Thr 0.7%	CP 19%	CP 18%	CP 17%	Thr level	CP level	CP*Thr
Essential amino acids								
Arginine	89.7	85.4	89.9[a]	87.3[ab]	85.4[b]	0.80/0.003	0.99/0.024	1.39/0.218
Isoleucine	81.9	78.8	82.9	80.5	77.8	1.16/0.080	1.42/0.072	2.01/0.945
Leucine	86.1	82.9	87.2[a]	84.8[ab]	81.7[b]	2.01/0.031	1.13/0.016	1.59/0.595
Lysine	87.8	80.9	86.7	84.2	82.2	1.23/0.002	1.51/0.151	2.14/0.117
Methionine	84.3	78.9	84.4	81.1	79.2	1.496/0.022	1.78/0.154	2.52/0.799
Histidine	79.2	76.0	81.8	77.7	73.2	2.89/0.459	3.53/0.263	4.50/0.772
Phenylalanine	89.4	83.2	88.2	86.6	84.1	0.97/0.001	1.19/0.089	1.69/0.124
Threonine	83.7	72.4	81.2[a]	77.8[ab]	75.2[b]	1.19/0.001	1.45/0.042	2.06/0.065
Valine	86.1	79.6	86.8[a]	82.4[ab]	79.4[b]	1.01/0.001	1.24/0.004	1.76/0.932
Non-Essential amino acids								
Alanine	85.8	80.9	84.3	83.8	81.9	1.31/0.022	1.60/0.555	2.27/0.054
Aspartic	85.5	83.8	86.6	84.5	82.8	1.13/0.287	1.38/0.193	1.96/0.082
Glutamic	86.8	83.6	87.4[b]	84.8[ab]	83.3[b]	0.70/0.008	0.86/0.018	1.21/0.062
Glycine	80.0	72.3	79.7	75.5	73.3	1.49/0.003	1.82/0.078	2.58/0.077
Proline	86.3	80.0	86.8[a]	82.8[ab]	79.8[b]	1.29/0.005	1.59/0.025	2.24/0.185
Serine	84.1	78.7	83.7	81.8	78.7	1.43/0.020	1.76/0.171	2.48/0.368
Tyrosine	84.4	75.6	85.5[a]	79.4[ab]	75.2[b]	1.78/0.004	2.17/0.018	3.08/0.417

a, b, c – means within a row with different superscripts are significantly different at $p < 0.05$

Apparent nutrient digestibility at 10 weeks age (%) of Muscovy ducks

At 10 weeks of age, the significant effects of CP and Thr on apparent nutrient digestibility are shown in Table 8 ($p<0.05$). The digestibility increased when the levels of CP in the diets increased. However, only treatment with 17% CP in the diet showed the highest DM digestibility. Similarly, a total of 0.6% Thr also showed the highest DM digestibility ($p<0.05$). In addition, OM and NDF were also recorded in this study, with a significant difference between the treatments of 0.6% Thr and 0.5% Thr ($p<0.05$). Other criteria were not different ($p>0.05$), and there were no interactions between treatments.

Table 8 The effects of CP and Thr on apparent nutrient digestibility at week 10 (%)

Criteria	Thr levels		CP levels			SEM/p		
	Thr 0.6%	Thr 0.5%	CP 17%	CP 16%	CP 15%	Thr level	CP level	CP*Thr
DM	81.3	76.9	81.7[a]	78.9[ab]	76.7[b]	0.93/0.006	1.14/0.027	1.61/0.193
OM	84.8	81.5	85.0	83.1	81.3	0.78/0.012	0.95/0.051	1.35/0.284
EE	88.5	83.3	89.2	85.3	83.2	2.09/0.101	2.56/0.279	3.62/0.174
CF	42.8	36.5	43.5	39.5	35.9	3.82/0.268	4.67/0.538	6.16/0.078
NDF	59.1	51.5	60.7	55.4	49.8	2.27/0.036	2.78/0.052	3.94/0.161
ADF	45.9	36.7	45.2	42.5	36.2	4.41/0.168	5.39/0.505	7.63/0.378

a, b, c – means within a row with different superscripts are significantly different at $p < 0.05$

Nutrient digestible at 10 weeks age (g/bird) of Muscovy ducks

Nutrients digested by local Muscovy ducks from week 10 are recorded in Table 9. For both CP and Thr, the effects were shown in DM, OM, EE and NDF (p<0.05). The highest digestibility was observed in the treatment with 17% CP and 0.6% Thr. However, this study did not note any statistically significant differences in CF and NDF for both CP and Thr supplementation (p>0.05). The study did not find any interactions between CP and Thr (p>0.05).

Table 9 The effects of CP and Thr on nutrient digestible at week 10 (g/bird)

Criteria	Thr levels		CP levels			SEM/p		
	Thr 0.6%	Thr 0.5%	CP 17%	CP 16%	CP 15%	Thr level	CP level	CP*Thr
DM	76.1	67.2	78.6[a]	70.9[ab]	65.5[b]	2.03/0.009	2.48/0.009	3.51/0.551
OM	74.7	67.0	77.0[a]	70.3[ab]	65.3[b]	1.87/0.013	2.29/0.012	3.24/0.644
EE	5.57	4.91	6.02[a]	5.09[b]	4.61[b]	0.15/0.010	0.18/0.001	0.26/0.190
CF	1.73	1.36	1.84	1.52	1.27	0.16/0.125	0.19/0.158	0.27/0.124
NDF	9.96	8.10	10.4[a]	8.93[ab]	7.72[b]	0.47/0.016	0.57/0.018	0.81/0.265
ADF	2.56	1.88	2.61	2.25	1.80	0.25/0.075	0.30/0.204	0.43/0.392

a, b, c – means within a row with different superscripts are significantly different at p < 0.05

The effects of CP and Thr on nitrogen balance at 10 weeks of age in Muscovy ducks

As the CP level in the diet increased, N intake, N retention, N retention/N intake ratio, and N retention/$W^{0.75}$ increased gradually and reached a higher value (p<0.05) in the treatment of 17% CP, corresponding to DM intake and high CP level in the diet (Table 10). Similarly, when threonine levels were increased, all of the above parameters were higher (P<0.05) in the treatment of 0.6% Thr as compared with the treatment of 0.5% Thr, except for N intake/$W^{0.75}$, there was not significantly different (p>0.05).

Table 10 The effects of CP and Thr on nutrient balance at week 10

Criteria	CP levels			Thr levels		SEM/p		
	CP 15%	CP 16%	CP17%	Thr 0,5%	Thr 0,6%	Thr level	CP level	CP*Thr
N-int, g	2.04[c]	2.29[b]	2.58[a]	2.22	2.39	0.05/0.031	0.06/0.001	0.08/0.778
N-exc, g	0.51	0.48	0.45	0.52	0.44	0.02/0.014	0.03/0.304	0.04/0.356
N-ret, g	1.53[c]	1.81[b]	2.13[a]	1.69	1.95	0.05/0.004	0.06/0.001	0.09/0.726
N-ret/N-int, %	74.6[b]	78.7[ab]	82.6[a]	76.1	81.4	0.98/0.002	1.20/0.003	1.69/0.226
N-int/$W^{0.75}$, *	1.19[b]	1.35[ab]	1.49[a]	1.30	1.39	0.03/0.074	0.04/0.001	0.06/0.345
N-ret/$W^{0.75}$, *	0.89[c]	1.06[b]	1.24[a]	0.99	1.14	0.04/0.014	0.04/0.001	0.06/0.624

*: g/kgW0.75
a, b, c – means within a row with different superscripts are significantly different at p < 0.05
N: nitrogen; Int.: intake; Exc: excreta; Ret: retention; W0.75: metabolizable body weight.

Apparent amino acid digestibility at 10 weeks age (%) of Muscovy ducks

When increasing the level of CP in the diet, the digestibility of 9 essential amino acids tended to increase gradually from the 15% CP treatment to the 17% CP treatment (Table 11). However, only 5 essential amino acids (isoleucine, leucine, lysine, threonine and valine) were found to be significantly higher (P<0.05) in the treatment of 17% CP. Similar results were found when increasing dietary levels of threonine, with 5 essential amino acids (isoleucine, methionine, phenylalanine, threonine, and valine) being higher (P<0.05) in the treatment of 0.6% Thr. For nonessential amino acids, the increase in CP up to 17% increased the apparent nonessential amino acid digestibility, including alanine, aspartic, glutamic and proline, while only serine showed a significant effect when the Thr level was increased (p<0.05).

Table 11 The effects of CP and Thr on nutrient balance at week 10

Criteria	Thr levels		CP levels			SEM/p		
	Thr 0.6%	Thr 0.5%	CP 17%	CP 16%	CP 15%	Thr level	CP level	CP*Thr
Essential amino acids								
Arginine	91.5	90.4	92.6	90.9	89.3	0.76/0.356	0.92/0.080	1.31/0.644
Isoleucine	84.3	80.7	84.9[a]	82.9[ab]	79.8[b]	0.75/0.005	0.91/0.007	1.29/0.537
Leucine	86.7	86.5	88.6[a]	87.7[ab]	83.3[b]	0.91/0.869	1.11/0.011	1.57/0.154
Lysine	88.5	86.2	89.9[a]	86.9[ab]	85.3[b]	0.88/0.086	1.07/0.028	1.52/0.178
Methionine	84.5	74.3	81.6	79.3	77.2	2.12/0.006	2.60/0.501	3.68/0.345
Histidine	84.8	84.4	87.5	84.2	82.2	2.12/0.900	2.60/0.378	3.68/0.727
Phenylalanine	90.3	86.8	89.1	88.1	88.4	1.08/0.041	1.32/0.873	1.88/0.787
Threonine	84.8	77.8	88.1[a]	81.3[ab]	74.5[b]	1.72/0.014	2.11/0.002	2.98/0.098
Valine	86.0	83.1	86.8[a]	84.4[ab]	82.5[b]	0.77/0.018	0.94/0.022	1.33/0.281
Non-Essential amino acids								
Alanine	85.1	83.1	87.2[a]	84.9[ab]	80.3[b]	1.10/0.216	1.35/0.010	1.91/0.418
Aspartic	88.2	85.7	89.2	87.1	84.6	0.97/0.098	1.19/0.056	1.68/0.511
Glutamic	87.6	86.9	89.1[a]	88.3[ab]	84.3[b]	0.69/0.475	0.85/0.004	1.20/0.727
Glycine	82.9	79.5	83.8	81.5	78.4	1.65/0.164	2.02/0.204	2.86/0.166
Proline	88.4	87.5	90.0[a]	87.6[ab]	86.2[b]	0.63/0.336	0.77/0.012	1.09/0.148
Serine	85.2	78.9	84.5	82.4	79.4	1.88/0.036	2.30/0.325	3.26/0.798
Tyrosine	87.7	84.7	87.3	87.3	83.9	1.11/0.090	1.37/0.184	1.94/0.475

a, b, c – means within a row with different superscripts are significantly different at p < 0.05

DISCUSSION

Regarding chicken farming, Thr and CP are the two nutrients in the diet that are necessary for optimal economic and production efficiency. Poultry fed diets with high crude protein have an accumulation of amino acids and an increase in their nitrogen waste output. If low-protein poultry diets are supplemented with crystalline amino acids in a pattern that fulfils the requirements for maintenance and tissue accretion, there is a possibility that nitrogen retention efficiency will be boosted (Manegar et al., 2019). The current breed of broiler poultry has undergone significant changes in a variety of aspects, including genetic selection, management procedures, and feed composition, which have resulted in a quicker and more successful growth performance than in past years. These broiler poultry are exposed to a variety of stresses, including the threat of disease, the presence of unsanitary conditions, and the effects of a harsh environment. Because of this, diets must be formulated effectively.

As mentioned in the study of Ahmed et al. (2020), growth performance, gut morphology, carcass traits, and ileal digestibility of protein and AA were improved by the effects of Thr and CP in the diet. This result was similar to that of this study, which showed an increase in apparent nutrient digestibility when CP and Thr were increased. This demonstrates that the gut uses a significant amount of Thr given through the intestinal lumen rather than arterial blood since dietary Thr deficit causes a drop in the number of intestinal goblet cells and mucin content, which cannot be restored by intravenous Thr (Law et al., 2007). The brush membrane of the intestinal epithelium, which is home to certain transporters and digestive enzymes (Na+/K+- atp enzyme, alkaline phosphatase, aminopeptidase N, and sucrase-isomaltase), makes it possible for nutrients to be absorbed (Ma and Ma, 2019). The intestinal mucosa takes in an adequate amount of Thr, which is then utilized by it, and this adds to the mucosa's ability to preserve its integrity. As a result, there is a possibility that Thr could contribute to an improvement in the capacity of the intestinal tract to digest and absorb nutrients. In addition, the generation of uric acid and protein in the body requires Thr to be successful (Debnath et al., 2019). Because of this, ducks can absorb nutrients more effectively when given CP and Thr supplements.

Dietary amino acids undergo a process called oxidative catabolism, which results in the production of ammonia in the body. This happens because of the constant turnover of proteins in the body and the oxidation of the excess amino acids that are left over after protein synthesis (Wu, 2009). Ammonia can build up if an excessive amount of amino acids that contain nitrogen go through the process of catabolism, which most likely results from the breakdown of amino acids that are out of balance (Stern and Mozdziak, 2019). The ammonia that is created is then transformed into urea, which is carried by the blood to the kidneys so that it can be eliminated through urine. Nitrogen that the body is unable to use in its whole ought to be transformed and then eliminated from the body. In addition, the nitrogen in poultry faeces originates from metabolizable nitrogen, which the body of poultry is unable to absorb (Linh et al., 2022). The results of N retention obtained are similar to the findings of Kim et al. (2014) and Chalova et al. (2016) that increasing dietary amino acid supplementation might limit nitrogen discharge, leading to an increase in nitrogen retention.

At either 8 or 10 weeks of age, the addition of an amino acid such as Thr as a dietary supplement has the potential to improve the digestibility of other amino acids. The fact that Thr is removed in greater proportion by the small intestine in mammals suggests that it plays a role in the functionality and maintenance of the intestinal tract. Thr is one of the essential amino acids (van der Schoor et al., 2007). Through the small intestine, the body may utilize between 30 and 50% of the amino acid's arginine, proline, isoleucine, valine, leucine, methionine, lysine, phenylalanine, glycine, serine, and Thr (Najafi et al., 2017). This could be one of the reasons for the increased digestion of amino acids.

CONCLUSIONS

The inclusion of 0.8% threonine and 19% crude protein (5-8 weeks age) and 0.6% threonine and 17% crude protein (for 8-10 weeks age) in the diets improved the apparent nutrient digestibility, nitrogen retention, and apparent amino acid digestibility of local Muscovy ducks. The increase in crude protein and threonine levels in the diets leads to an increase in digestibility, especially DM, OM and EE. Essential and nonessential amino acid digestibility also increased when the supplementation of threonine and crude protein increased. As also recorded in this study, the nitrogen balance was greater for both the growing phases of 5-8 weeks and 8-10 weeks when the levels of threonine and crude protein increased. However, the addition of threonine and crude protein had no interaction effect on duck digestibility.

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AUTHOR CONTRIBUTIONS

NTL created and conducted the experimental design. NTKD analysed data for this study. NHQ wrote the manuscript. All authors checked and accepted the manuscript before submission.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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