

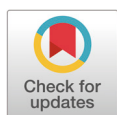
Evaluation of crude protein levels in White Pekin duck diet for 21 days after hatching

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Abstract

In poultry diets, a requirement of crude protein is one of the most important factors in poultry productivity. Besides, the Pekin duck requirement of crude protein is still not clear. This experiment was conducted to determine the crude protein requirement of Pekin duck on diet formulation by investigation of growth performance, carcass trait, and analysis of blood parameter for a hatch to 21-day (d) of age. A total of 432 male White Pekin ducks were randomly allocated to six levels of crude protein (i.e., 15%, 17%, 19%, 21%, 23%, and 25%) to give six replicate pens per treatment with 12 ducklings per each pen. Body weight and feed intake were measured weekly by calculating feed conversion ratio and protein intake. Two ducklings each pen was euthanized via cervical dislocation for analysis of carcass trait and plasma blood on 21-d of age. Data were applied on both prediction linear-plateau and quadratic-plateau models by estimation of the crude protein requirements. Data were applied on both prediction linear-plateau and quadratic-plateau models by estimation of the crude protein requirements. The level of crude protein requirements of Pekin ducks for 21 days after the hatch was estimated to be 20.63% and 23.25% diet for maximum daily gain, and minimum feed conversion ratio, respectively.

Keywords: Growth performance, Linear-plateau model, Pekin duck, Quadratic-plateau model

INTRODUCTION

Dietary crude protein is known to play a major role in maximizing growth performance. However, it is one of the main factors affecting the cost of Pekin duck diets [1,2]. High protein poultry diets are not only expensive but also cause environmental problems owing to ammonia emissions and nitrogen excretion [3]. However, adverse environmental impacts can be alleviated simply by decreasing the level of protein in poultry diets [4-8].

Despite the importance of dietary crude protein level on the growth performance of birds, only a couple of studies have demonstrated that feeding birds with a higher dietary crude protein improved their average daily gain and feed efficiency [9-11]. However, it is often reported that excessive intake of

Competing interests

The authors declare no conflict of interest related to this work.

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Availability of data and material

Upon reasonable request, the datasets of this study can be available from the corresponding author.

Authors' contributions

Conceptualization: Heo JM.
Data curation: Cho HM, Wickramasuriya SS, Macelline SP, Hong JS.
Formal analysis: Cho HM.
Methodology: Cho HM.
Software: Cho HM.
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Investigation: Cho HM.
Writing - original draft: Cho HM.
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Ethics approval and consent to participate

The experimental protocol used in this study was approved by the Animal Ethics Committee of the Chungnam National University (CNU-01175).

dietary crude protein (i.e., over 22.5%) deteriorates growth performance in birds [12,13]. Conversely, birds fed insufficient dietary crude protein (i.e., under 20.0%), were shown to have compromised growth performance due to amino acid imbalance [14].

The National Research Council (NRC) recommended a 16% crude protein level in Pekin duck diets for 14–49 days [15]. However, this study was conducted many years ago and is, therefore, no longer applicable to the formulation of modern diets. It has been reported that feeding 19% crude protein level provided for 15–35 days is suitable for maximizing growth performance and carcass traits without wasting crude protein [16]. However, limited data are available for recommendations for optimal crude protein levels in White Pekin duck diets 21 days after hatching. Therefore, the objective of this study was to examine crude protein level requirements in White Pekin duck diets 21 days after hatching by evaluating growth performance and carcass traits.

MATERIALS AND METHODS

Experimental design

An experiment was conducted using 432 males White Pekin ducklings in a completely randomized design with six levels of crude protein ($n = 6$ replicate pens per treatment and 12 ducklings per pen). Ducklings were fed their respective experimental diets from the d one to twenty-one.

Birds, housing, and diets

One-d-old male White Pekin ducklings were obtained from a local hatchery (Jang Sung duck farm, Jangseong, Jeonnam, Korea). On the same d, ducklings were weighed individually and randomly allocated to one of six dietary treatments with varying levels of crude protein content (15%, 17%, 19%, 21%, 23%, and 25% respectively). Twelve birds were housed in each pen ($1.7 \times 1.3 \text{ m}^2$), with a mean BW of $55.8 \pm 0.31 \text{ g}$ (mean \pm standard error of mean [SEM]). These ducklings were reared on floor pens littered with rice husk and each pen was equipped with 3 nipple drinkers and a feeder. Birds were offered the experimental diet on an *ad libitum* basis for the period of the study; the freshwater was available at all times, and lighting was continuous for 24 hours. The ambient temperature was maintained at 32°C from days one to three, and then gradually decreased to 25°C until the ducklings were 21 days of age. Six dietary treatments were formulated to contain a level of crude protein content from 15% to 25% in a 2.0% scale (Table 1). Diets were iso-caloric and formulated to meet or exceed NRC [15] specifications for ducklings 21 days of age. Crystalline amino acids (lysine, methionine, isoleucine, valine, arginine, leucine) also were added to the diet to meet or exceed dietary amino acid requirements in diets. All experimental diets were prepared as mash form.

Growth performance evaluation

Body weight (BW) was recorded at the beginning and on d 7, 14, and 21 of the experimental periods. Pen-based feed consumption was recorded together with BW to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR).

Post-mortem procedure and sample collection

On d 21, two ducklings (closest to the median BW) were selected from each pen. Individual live BW of the selected birds was recorded and euthanized via cervical dislocation for sample collection after bleeding. Blood samples were collected from the brachial vein into vacutainer tubes coated with lithium heparin (BD Vacutainer, BD SSTTM, Franklin Lakes, NJ, USA). Collected blood samples were quickly transferred to a laboratory for plasma separation. After evisceration, empty

Table 1. Composition of experimental diets (% as fed)

Item	Dietary protein content (%)					
	15	17	19	21	23	25
Corn	68.98	64.14	59.30	54.45	49.61	44.77
Wheat	10.00	10.00	10.00	10.00	10.00	10.00
Soybean meal (44%)	13.00	17.40	21.80	26.20	30.60	35.00
Fish meal	3.00	3.60	4.20	4.80	5.40	6.00
Vegetable oil	1.00	1.03	1.06	1.09	1.12	1.15
Limestone	1.00	1.00	1.00	1.00	1.00	1.00
Monocalcium phosphate	1.50	1.50	1.50	1.50	1.50	1.50
Vitamin-mineral premix ¹⁾	0.10	0.08	0.06	0.04	0.02	-
Salt	0.30	0.30	0.30	0.30	0.30	0.30
Lysine HCl	0.30	0.24	0.18	0.12	0.06	-
L-Arginine	0.25	0.20	0.15	0.10	0.05	-
DL-Methionine	0.15	0.16	0.17	0.18	0.19	0.20
L-Threonine	0.09	0.07	0.05	0.04	0.02	-
L-Tryptophan	0.10	0.10	0.09	0.09	0.08	0.08
L-Isoleucine	0.13	0.10	0.08	0.05	0.03	0.00
L-Valine	0.10	0.08	0.06	0.04	0.02	0.00
Calculated composition ²⁾						
Metabolizable energy (kcal/kg)	3,196	3,197	3,198	3,199	3,200	3,201
Crude protein (%)	15.43	17.29	19.15	21.01	22.87	24.73
Calcium (%)	0.80	0.83	0.85	0.87	0.89	0.92
Phosphorus (%)	0.39	0.40	0.40	0.41	0.42	0.42
Lysine HCl (%)	0.98	1.08	1.18	1.28	1.38	1.47
Methionine (%)	0.43	0.47	0.51	0.55	0.60	0.64
Methionine + Cysteine (%)	0.70	0.77	0.83	0.90	0.97	1.03
Isoleucine (%)	0.71	0.77	0.84	0.90	0.97	1.04
Valine (%)	0.79	0.86	0.93	1.01	1.08	1.15
Arginine (%)	1.08	1.18	1.28	1.38	1.48	1.58
Leucine (%)	0.68	0.78	0.87	0.96	1.06	1.15
Analyzed composition						
Crude protein (%)	15.67	17.51	19.37	21.25	23.06	24.88

¹⁾Supplied per kilogram of total diets: Fe (FeSO₄ · H₂O), 80 mg; Zn (ZnSO₄ · H₂O), 80 mg; Mn (MnSO₄ · H₂O) 80 mg; Co (CoSO₄ · H₂O) 0.5 mg; Cu (CuSO₄ · H₂O) 10 mg; Se (Na₂SeO₃) 0.2 mg; I, (Ca(IO₃) · 2H₂O) 0.9 mg; vitamin A, 24,000 IU; vitamin D₃, 6,000 IU; vitamin E, 30 IU; vitamin K, 4 mg; thiamin, 4 mg; riboflavin, 12 mg; pyridoxine, 4 mg; folacine, 2 mg; biotin, 0.03 mg; vitamin B₈, 0.06 mg; niacin, 90 mg; pantothenic acid, 30 mg.

²⁾The values are calculated according to the values of feedstuffs in NRC [14].

bodies were weighed. Drumsticks (skinless) and breast meat were removed from carcasses and weighed. The empty BW, drumstick, and breast meat weight were expressed as proportions relative to slaughter live BW.

Sample preparation and laboratory analysis

Collected blood samples were centrifuged (1248R, GYROZEN, Gimpo, Korea) at 3,000×g for 10 min at 4°C. Plasma samples were separated and stored at -80°C before analysis that the concentrations of total protein (TP), blood urea nitrogen (BUN) were determined using commercial kits (Asan Pharmaceutical, Seoul, Korea) for an automatic biochemical blood analyzer (Model HITACHI

7180 chemistry analyzer, HITACHI, Tokyo, Japan).

Nitrogen Excretion calculation

Average nitrogen excretion was determined using pre-derived equations as shown below according to Belloir et al. (2017).

$$\text{Nitrogen intake (g)} = \frac{\text{Feed intake (g)} \times \text{Crude protein in the diet (\%)}}{6.25}$$

$$\text{Nitrogen retention} = 29 \text{ g/kg} \times \frac{\text{Body weight gain (g)}}{1,000}$$

$$\text{Nitrogen excretion (g)} = \text{Nitrogen intake (g)} - \text{Nitrogen retention}$$

Statistical analysis

Data were analyzed as a completely randomized design, using the general linear model procedure of ANOVA of SPSS software version 24 (IBM Corp, 2016), with a pen used as the experimental unit for growth performance data. Data from selected individual birds for carcass traits and blood metabolites were pooled to get an average value per pen before statistical analysis. When dietary treatment was significant ($p < 0.05$), means were separated using Tukey's multiple range test procedures of SPSS software version 24. To determine the optimum crude protein level, linear-plateau and quadratic-plateau regression analysis were conducted using a Nutritional Response Model (Version 1.1; [17]) as described previously by [18].

RESULTS

Growth performance

Health and growth performance were good for all birds over the 21 days of the experiment. BW, ADG, and FCR were higher in White Pekin ducks fed the higher crude protein diet ($p < 0.05$) on days 7 and 14 than those in White Pekin ducks fed the lower crude protein diet. However, compared to the White Pekin ducks fed higher crude protein diets from days 1 to 21, the ducks fed the diet containing under 21% crude protein exhibited improved BW, ADG, and FCR on d 21 ($p < 0.05$, Table 2). On days 7 and 14, compared to the ducks fed the lower crude protein diets, White Pekin ducks fed diets with less than 19% crude protein exhibited an increase in ADFI.

The level of crude protein required to improve growth performance from hatch to 21 d was determined using both linear-plateau and quadratic-plateau models (Figs. 1 and 2). The requirements for attaining maximum ADG were crude protein levels of 19.71% (linear-plateau model) and 21.55% (quadratic-plateau model). Synthetically, there suggested crude protein levels of 20.62% on ADG from hatch to d 21. The maximum proportion of FCR was attained in birds fed diets containing crude protein levels of 21.30% (linear-plateau model) and 25.19% (quadratic-plateau model). Based on both the linear-plateau model and quadratic-plateau model, the suggested crude protein requirement for improving the proportion of FCR was 23.25% fed from hatch to d 21.

Protein intake

The effect of dietary protein level on the voluntary protein intake of White Pekin ducks is shown in Table 3. Increased protein intake for 21 days after hatching was observed ($p < 0.05$) with increasing dietary protein levels in White Pekin ducks exposed to a higher protein feed.

Table 2. Effect of different content on body weight, average daily gain, average daily feed intake, feed conversion ratio of male Pekin ducks for 21 days after hatching¹⁾

Item	Dietary protein content (%)						SEM	p-value
	15	17	19	21	23	25		
Body weight (g/bird/day)								
Day 7	268.1 ^e	280.6 ^d	290.1 ^c	297.4 ^{bc}	305.5 ^{ab}	308.0 ^a	2.30	0.001
Day 14	736.0 ^d	761.2 ^c	793.9 ^b	802.5 ^b	810.9 ^b	837.0 ^a	5.50	0.001
Day 21	1,329.2 ^c	1,383.4 ^b	1,430.7 ^a	1,465.1 ^a	1,454.9 ^a	1,429.6 ^a	8.90	0.001
Average daily gain (g/bird/day)								
Day 7	30.32 ^e	32.11 ^d	33.44 ^c	34.50 ^{bc}	35.65 ^{ab}	36.02 ^a	0.331	0.001
Day 14	66.84 ^c	68.66 ^c	71.97 ^b	72.17 ^b	72.20 ^b	75.57 ^a	0.531	0.001
Day 21	84.73 ^c	88.89 ^{bc}	90.98 ^{ab}	94.66 ^a	92.00 ^{ab}	84.66 ^c	0.853	0.001
Day 1–21	60.63 ^c	63.22 ^b	65.46 ^a	67.11 ^a	66.62 ^a	65.42 ^a	0.424	0.001
Average daily feed intake (g/bird/day)								
Day 7	36.99 ^c	38.16 ^{bc}	40.48 ^a	38.53 ^{abc}	39.31 ^{ab}	37.46 ^{bc}	0.308	0.008
Day 14	97.55 ^{bc}	96.11 ^{bc}	101.49 ^a	96.18 ^{bc}	94.91 ^c	99.32 ^{ab}	0.553	0.003
Day 21	148.63	151.17	152.17	147.43	148.89	143.07	1.074	0.188
Day 1–21	94.39	95.15	98.04	94.04	94.37	93.28	0.484	0.070
Feed conversion ratio (g/g)								
Day 7	1.22 ^a	1.19 ^b	1.21 ^b	1.12 ^c	1.10 ^c	1.04 ^c	0.013	0.001
Day 14	1.46 ^a	1.40 ^{ab}	1.41 ^{ab}	1.33 ^c	1.32 ^{bc}	1.31 ^{ab}	0.009	0.001
Day 21	1.76 ^a	1.70 ^{ab}	1.67 ^{ab}	1.56 ^c	1.62 ^{bc}	1.70 ^{ab}	0.016	0.002
Day 1–21	1.48 ^a	1.43 ^b	1.43 ^b	1.34 ^c	1.35 ^c	1.35 ^c	0.010	0.001

¹⁾Each value is the mean of 6 replicates (12 birds per cage).^{a–e}Means in the same row with different superscripts differ in the level of crude protein content ($p < 0.05$).

Carcass trait

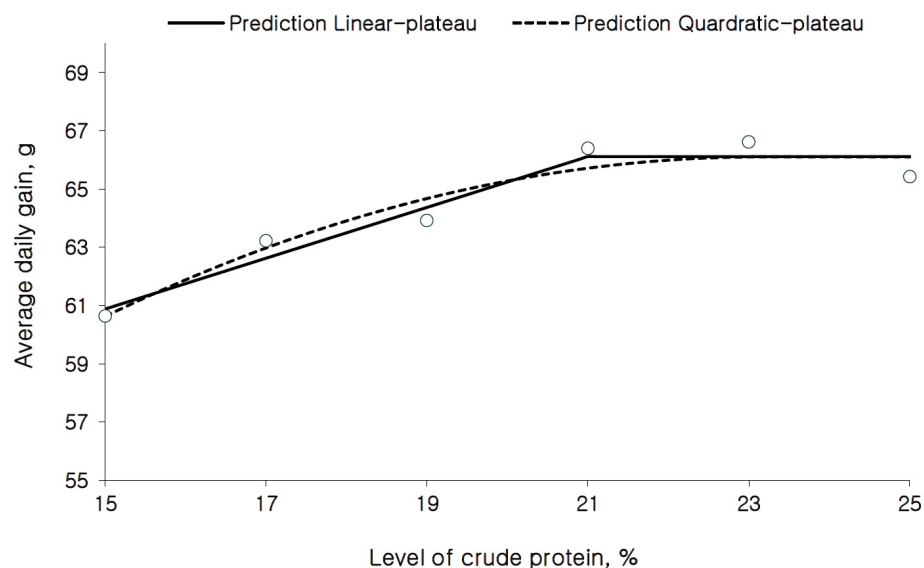
No significant differences ($p > 0.05$) were observed in empty body weight, drumstick, and breast meat of ducks exposed on diverse levels of crude protein, on d 21 after hatching (Table 4).

Urea nitrogen and total protein in the blood

White Pekin ducks exposed to a higher protein diet, showed ($p < 0.05$) increased blood urea nitrogen and total protein levels on d 21 after hatching (Table 5).

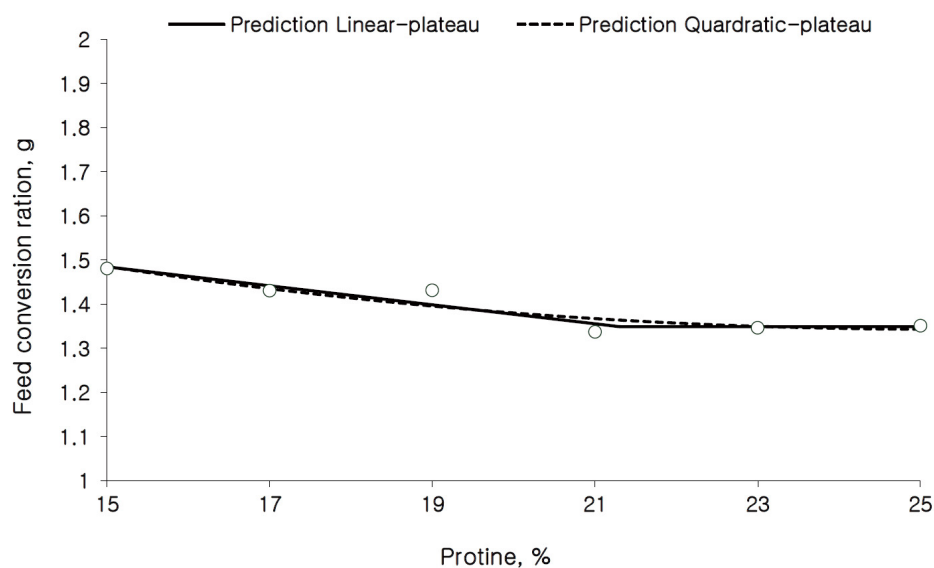
DISCUSSION

In this study, the crude protein requirements for improved growth performance of White Pekin ducks, from hatch to d 21 were evaluated. Although a previous study on the crude protein requirements of White Pekin duck diet exists, it may not be completely applicable to the formulation of new diets due to the evolved genotype of White Pekin ducks. Baéza et al. [19] reported the crude protein requirements for improved FCR in White Pekin duck diet from d 1 to 21% to be 23.2%, which may not apply to diets with varying energy levels (i.e., up to 3,000 kcal/kg metabolizable energy) in feed formulation. Moreover, Jiang et al. [20] recommended crude protein requirements based on only two crude protein levels (17% and 21%). In addition, due to differences in protein digestibility between White Pekin ducks and broiler chickens, the crude protein requirement for broiler chickens cannot be adequately applied to White Pekin ducks [21], as White Pekin ducks have a higher basal endogenous amino acid loss than do broiler chickens. Therefore, it is important to evaluate the crude protein requirements for White Pekin ducks under various conditions.



	Requirement	SEM	R ²	p-value	Recommendation
Linear plateau	19.71	1.536	94.76	0.001	20.63
Quadratic plateau	21.55	1.693	94.23	0.001	

Fig. 1. Crude protein requirements of Pekin ducks for hatch to 21 d of age for average daily weight gain determined by a quadratic-plateau model was 19.71 [$Y = 66.38 - 1.2088(21.55 - x)^2$, $R^2 = 94.76$] (open line), and by a linear-plateau was 21.55 [$Y = 66.34 - 0.1365(21.55 - x)^2$, $R^2 = 94.23$] (closed line). Data points (°) represent the least-square means of dietary treatment (n = 6). SEM, standard error of mean.



	Requirement	SEM	R ²	p-value	Recommendation
Linear plateau	21.30	0.002	91.19	0.001	23.25
Quadratic plateau	25.19	0.002	87.06	0.011	

Fig. 2. Crude protein requirements of Pekin ducks for hatch to 21 d of age for feed conversion ratio determined by a quadratic-plateau model was 21.30 [$Y = 1.35 - 0.0215(21.30 - x)^2$, $R^2 = 91.19$] (open line), and by a linear-plateau was 25.19 [$Y = 1.34 - 0.0014(1.34 - x)^2$, $R^2 = 87.06$] (closed line). Data points (°) represent least-square means of dietary treatment (n = 6). SEM, standard error of mean.

Table 3. Effect of different content on protein intake and nitrogen utilization of male Pekin ducks for 21 days after hatching¹⁾

Item	Dietary protein content (%)						SEM	p-value
	15	17	19	21	23	25		
Protein intake (g)								
Day 7	5.55 ^e	6.49 ^d	7.69 ^c	8.09 ^b	9.04 ^a	9.36 ^a	0.202	0.001
Day 14	14.63 ^f	16.34 ^e	19.28 ^d	20.20 ^c	21.83 ^b	24.83 ^a	0.498	0.001
Day 21	22.29 ^f	25.70 ^e	28.91 ^d	30.96 ^c	34.24 ^b	35.77 ^a	0.703	0.001
Day 1–21	14.16 ^f	16.18 ^e	18.63 ^d	19.75 ^c	21.71 ^b	23.32 ^a	0.46	0.001
Nitrogen retention (g)								
Day 1–21	36.92 ^c	38.50 ^b	39.87 ^a	40.87 ^a	40.57 ^a	39.84 ^a	0.258	0.001
Nitrogen excretion (g)								
Day 1–21	10.65 ^f	15.85 ^e	22.72 ^d	25.49 ^c	32.36 ^b	38.52 ^a	1.383	0.001

¹⁾Each value is the mean of 6 replicates (12 birds per cage).^{a–f}Means in the same row with different superscripts differ in the level of crude protein content ($p < 0.05$).**Table 4.** Effect of different content on carcass trait of male Pekin ducks on 21 day of age¹⁾

Item	Dietary protein content (%)						SEM	p-value
	15	17	19	21	23	25		
Empty body weight (%)	70.51	71.14	71.24	70.85	71.00	71.32	0.171	0.791
Drumstick (%)	7.31	7.45	7.74	7.51	7.74	7.67	0.060	0.204
Breast meat (%)	6.33	6.50	6.86	7.34	6.36	7.22	0.154	0.235

¹⁾Each value is the mean of 6 replicates (1 bird per cage).**Table 5.** Effect of different content on the blood of male Pekin ducks on 21 day of age¹⁾

Item	Dietary protein content (%)						SEM	p-value
	15	17	19	21	23	25		
Blood urea nitrogen (mg/dL)	0.49 ^c	0.51 ^c	0.59 ^{bc}	0.59 ^{bc}	0.73 ^{ab}	0.81 ^a	0.026	0.001
Total protein (g/dL)	3.44 ^c	3.45 ^c	3.47 ^c	3.51 ^{bc}	3.71 ^{ab}	3.86 ^a	0.038	0.001

¹⁾Each value is the mean of 6 replicates (1 bird per cage).^{a–c}Means in the same row with different superscripts differ in the level of crude protein content ($p < 0.05$).

Protein sources are the most important components of poultry diets after energy sources. Protein is essential in poultry diets because, it supplies amino acids for the growth of muscle and synthesis of egg protein. The synthesis of muscle requires, physiologically, 20 amino acids in poultry diets. Ten of these twenty amino acids cannot be synthesized, while the other ten can be synthesized slowly in the interior of a poultry body. Therefore, sufficient amino acids must be supplied in poultry diets [22]. However, there are limitations in terms of the expense associated with the proportion of the protein source and the environmental contamination resulting from excessive protein diets.

Studies evaluating crude protein requirements have been conducted using a variety of methods (e.g., a statistical model for analysis) to ensure the accuracy of the estimated nutrient requirements [23,24]. Although the statistical model for analysis shows appropriate fit, its output cannot be regarded as the optimal level of nutrient requirement because it does not consider the physiological differences among the individuals in a population [25]. In addition, a broken-line analysis is generally applied by underestimating nutrient requirements [18]. To supplement the analysis of the statistical model method, both the linear-plateau model and the quadratic-plateau model methods have been used in recent studies on the estimation of nutrient requirements [26–28]. Consequently, the aforementioned methods (i.e., an average of the linear-plateau and quadratic-plateau models)

were used to estimate the crude protein requirements of the White Pekin duck diet for 21 days after hatching, by evaluating the growth performance and carcass traits of the ducks.

In this study, when the level of protein was increased, BW, ADG, and FCR improved over the first 14 days, whereas data acquired on days 14–21 showed different results. The diet containing 21% crude protein elicited a greater improvement in BW, ADG, and FCR of the ducks from d 14–21 than a diet containing 25% crude protein. This result is probably due to higher concentrations of the ratio of amino acids as compared to the ratio before the diet experiments. Based on an average of both the linear-plateau and quadratic-plateau model analysis, the optimum requirements of crude protein for were 20.63% and 23.25%, for maximal ADG and FCR, respectively, for 21 days after hatching. Similarly, Baéza et al. [19] reported that a level of 21% crude protein in the diet could improve feed efficiency; however, crude protein levels higher than 21% may result in an increase in the nitrogen content of White Pekin duck feces. Jiang et al. [29] reported improved ADG in White Pekin ducks in response to the crude protein level of 20.89% in the diet, from d 1 to 21. Moreover, Xie et al. [30] observed that a diet containing 19.58% crude protein improved the BW, ADG, and feed efficiency of White Pekin ducks from d 1 to 19.

According to recent reports, significant differences were observed in the carcass traits exhibited by White pecking ducks in response to different crude protein levels. According to Baéza et al. [19], compared to that with a lower crude protein diet, a higher crude protein diet was observed to increase the breast weight of Pekin ducks, as measured on d 21. However, our results for the carcass trait were not affected by the crude protein requirements probably due to sex, metabolizable energy, and amino acid balance.

CONCLUSION

Crude protein levels of 20.63%, and 23.25% base on both the linear-plateau model and the quadratic-plateau model were respectively recommended to improved ADG, FCR for White Pekin ducks from hatch to 21 days.

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