Effects of dietary protein levels and protease supplementation on growth performance, carcass traits, meat quality, and standardized ileal digestibility of amino acid in Pekin ducks fed a complex diet

Q. D. Wang,* K. Y. Zhang,* Y. Zhang,† S. P. Bai,* X. M. Ding,* J. P. Wang,* H. W. Peng,* G. Tian,* Y. Xuan,* Z. W. Su,* and Q. F. Zeng*,[1]

**Institute of Animal Nutrition, Sichuan Agricultural University, Chengdu, Sichuan, China; Key Laboratory for Animal Disease-Resistance Nutrition of, Ministry of Education, Ministry of Agriculture and Rural Affaires, Sichuan Province 611130, China; and †Habio Bio-tech Co. Ltd., Mianyang, China, 610000*

ABSTRACT This study aimed to investigate to the effects of dietary CP levels and protease supplementation on growth performance, carcass traits, meat quality, nutrients utilization, and standardized ileal digestibility of amino acid in Pekin ducks fed a complex diet. A total of 960 14-day-old male ducks were weighed and randomly allotted to a 2 × 5 factorial arrangement of 10 treatments with 6 replicate pens per treatment and 16 ducks per pen fed to 49 D of age. Experimental factors included five dietary CP levels ranging from 13.5 to 17.5% and with or without protease (200 mg/kg) supplementation. Between day 28 to 34, the digestible and metabolizable trials were performed. Significant CP × protease interactions ($P < 0.05$) on breast meat yield, DM, energy and nitrogen utilization, as well as standardized ileal digestibility values of 7 amino acids were observed. Regardless of protease supplementation, ducks fed 13.5, 14.5, and 15.5% CP had a poorer ($P < 0.05$) growth performance and breast meat yield than ducks fed with 16.5 and 17.5% CP. Ducks fed 13.5% CP had a positive effect ($P < 0.05$) on meat quality, dietary DM, energy and nitrogen utilization as well as standardized ileal digestibility of amino acids. Protease supplementation increased ($P < 0.05$) DM and phosphorus retention and decreased ($P < 0.05$) shear force of breast meat, regardless of CP level; when CP = 14.5%, protease significantly increased ($P < 0.05$) breast muscle yield. The optimal CP requirement without or with protease supplementation for BWG and FI were 17.02 or 16.53% and 16.64 or 16.75%, respectively, based on linear broken-line regression.

Key words: duck, low-protein diet, meat quality, protease, standard ileal amino acid digestibility

2020 Poultry Science 99:3557–3566
https://doi.org/10.1016/j.psj.2020.03.047

INTRODUCTION

Dietary regimens capable of increasing duck production efficiency and reducing nitrogen (**N**) and phosphorus (**P**) excretion are of major interest to duck nutritionists. Over the past decades, numerous studies in broilers or pigs have been conducted on the use of synthetic amino acids (**AA**) in low-protein (**LP**) diets (Baeza and Leclercq, 1998; Bregendahl et al., 2002; Law et al., 2018). However, conflicting results from these studies prevent clear conclusions on the effects of these diets in practical broiler production. The only consistent result reported in the mentioned studies was the increased accumulation of abdominal fat in broilers fed LP diets (Namroud et al., 2008; Law et al., 2019). There are a few previous studies looking at dietary CP levels on meat ducks. A previous study from our laboratory by Zeng et al. (2015) demonstrated that ducks fed a corn–soybean meal diet from 15 to 35 D of ages with 15% CP had lower growth performance and breast meat yield than those fed diets with 17 or 19% CP, when dietary energy concentration and total AA to N ratio were kept constant.

Recently, the cost of feed ingredients, especially protein ingredients, has continued to increase. In addition, owing to high and unpredictable prices of soybean meal, alternative feedstuffs such as rapeseed meal, cottonseed meal, dried distillers grains with solubles could be valuable alternative proteins for feed formulations (Cowieson et al., 2009). Therefore, the LP diets based on many miscellaneous meals may be a beneficial choice

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Received November 5, 2019.
Accepted March 28, 2020.

[1] Corresponding author: zqf@sicau.edu.cn

in the duck industry. However, the effect of dietary CP levels is influenced by the digestibility of ingredients in feed, and there are no available data from studies of the LP diet based on diet with many miscellaneous meals in ducks.

Considering the magnitude of antinutritional factors such as protease inhibitors, nonstarch polysaccharides, phytate, and lectins present in the most of poultry feed, there may be an important opportunity to use commercial exogenous enzymes. Ghazi et al. (2002) found that supplemented protease had been applied to LP diets without deleterious effects on the performance of broiler chickens. Supplemental exogenous proteases have been found to improve protein digestibility, energy efficiency, and performance indices in broilers (Fru-Nji et al., 2011), even in a LP diet (Cowieson et al., 2017; Lei et al., 2017; Morales et al., 2017; Law et al., 2018; Mohammadigheisar and Kim, 2018), which indicated that reducing dietary CP levels with exogenous supplementation can be used for production. However, to our knowledge, no data are available concerning the supplementation of protease to reduced CP levels of diets in ducks. Therefore, the objective of the present study was to investigate the effects of dietary CP levels and protease supplementation on the growth performance, carcass traits, meat quality, nutrients utilization, and standardized ileal digestibility (**SID**) of AA (**SIDAA**) in Pekin ducks fed complex diets.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee of Sichuan Agricultural University approved all procedures used in the study (SAUPN-19-01).

Bird, Diets, and Management

Experiment (**Exp.**) 1 was a performance trial. A total of 960 one-day-old male Pekin ducklings were fed a standard starter diet containing 11.70 MJ/kg ME, 19.5% CP, 1.1% lysine (**Lys**), 0.45% methionine, 0.78% threonine (**Thr**), and 0.22% tryptophan from 1 to 14 D of age. On day 14, ducks were randomly assigned to 60 cage pens of 16 birds/cage so that ducklings had a similar initial BW in each pen. Ducks were reared in pens (2.2 × 1.2 × 0.9 m) in a temperature- and humidity-controlled room, and had free access to water and feed to 49 D of age.

A 5 × 2 factorial group arrangement was used with 5 dietary CP levels, without or with protease supplementation, totaling 10 treatments with 6 replicates of 16 ducks. Ten isocaloric diets were formulated containing 17.5, 16.5, 15.5, 14.5, and 13.5% CP of each diet in combination with or without protease supplementation (200 mg/kg as per the manufacturer's recommendation). The protease used in this study was produced by fermentation of *Bacillus licheniformis* (Mianyang Habio Bio-tech Co. Ltd., Mianyang, China). One unit of this protease enzyme activity is defined as the amount of enzyme that releases 1 μg of p-hydroxyphenylalanine from the Flynn-positive AA and peptide per minute at pH 8.5 and 37 °C (Ren et al., 2011). The enzyme activity unit in the present study is 1×10^5 U/g. Diets were fortified with synthetic feed-grade Lys, methionine, Thr, and tryptophan to provide the recommended levels of AA for Pekin ducks in accordance with the NRC (1994) (Table 1). The analyzed N content and AA compositions of the 10 experimental diets are presented in Table 2.

Data Collection and Measurements in Exp 1

On day 49, after 12 h of feed withdrawal, ducks were weighed, and feed consumption was obtained for each pen. Feed intake (**FI**), BW gain (**BWG**) and feed-to-gain (**F:G**) ratio were determined. Mortality was recorded, and the weights of dead birds were used to adjust the F:G ratio.

On day 49, one bird with weight that was closest to the average of the pen was selected and euthanized by exsanguination. After slaughtering, feathers were plucked, followed by evisceration to obtain dressed breast and leg meats. Carcass yield was determined as the carcass weight in relation to BW expressed as percentage of BW (%), whereas breast and leg meat yields were expressed as percentages of the carcass weight.

The pH of breast meat samples was measured from the same place on the right upper third of all samples using a pH meter (pH-2004; Selecta, Barcelona, Spain) as per previous study of Liao et al. (2018). Each meat sample was measured 3 times, and the average pH value of meat samples was calculated. The color profile (lightness [**L***], redness [**a***], and yellowness [**b***]) of breast meat was measured using a colorimeter (Minolta CR-400; Konica, Chiyoda-ku, Japan) at 3 points on the dorsal surface of each breast sample after 30 min of exposure to ambient air (Van Laack et al., 2000).

To measure shear forces, samples were cut into at least five pieces measuring 1 × 1 × 2 cm (rectangular section 1 × 1 and 2 cm along the fiber axis) and positioned with their muscle fibers perpendicular to the blades of a Warner–Bratzler TA. An AXT plus–Texture Analyzer (Stable Micro Systems, Haslemere, UK) was used for shredding (Zeferino, et al., 2016). The device descent speed was set to 10 mm/s. Drip loss was determined from 2 samples per plot, in accordance with the methodology previously described by Silva et al. (2019). Samples were cut into 2.5 cm^3 cubes, placed in hermetically sealed containers, and kept in a refrigerator at 4 °C for 24 h. Subsequently, samples were removed from the refrigerator and weighed to calculate percentage drip loss.

Digestibility and Metabolism Trial

Experiment 2 was a subsequent digestibility and metabolism trial of diets fed in Exp.1. On day 28, 2 birds per pen were randomly selected (12 ducks per treatment, 120 ducks in total) and transferred to metabolic cages (2 ducks per cage) and fed with the original diets mixed with titanium dioxide (**TiO_2**; 0.5%). An additional

Table 1. Composition and nutrient contents of the experimental diets (air-dry basis)%.

	Dietary crude protein levels %				
Items	13.5	14.5	15.5	16.5	17.5
Ingredients,%					
Corn	59.8	54.9	50	45.1	40.2
Cottonseed meal	4	5	6	7	8
Rapeseed meal	4.50	4.90	5.25	5.60	6.00
Wheat middlings	8	9	10	11	12
Rice bran	14.00	13.75	13.50	13.25	13.00
Feather meal	0.20	0.55	0.90	1.25	1.60
DDGS	4	6	8	10	12
Soybean oil	0.30	0.875	1.45	2.025	2.60
Calcium carbonate	1.22	1.24	1.26	1.28	1.29
Dicalcium phosphate	1.16	1.10	1.03	0.97	0.90
L-Lysine.HCl	0.82	0.78	0.74	0.70	0.66
DL-Methionine	0.320	0.305	0.290	0.275	0.260
Threonine	0.500	0.463	0.425	0.388	0.350
Tryptophan	0.10	0.09	0.08	0.07	0.06
Sodium chloride	0.35	0.35	0.35	0.35	0.35
Choline chloride (50%)	0.2	0.2	0.2	0.2	0.2
Vitamin premix [1]	0.03	0.03	0.03	0.03	0.03
Mineral premix [2]	0.5	0.5	0.5	0.5	0.5
Total	100.00	100.00	100.00	100.00	100.00
Calculated nutrients levels (%)					
ME (MJ/kg)	11.90	11.90	11.90	11.90	11.90
Crude protein	13.5	14.5	15.5	16.5	17.5
Calcium	0.79	0.79	0.79	0.79	0.79
Available phosphorus	0.35	0.35	0.35	0.35	0.35
Lysine	1.10	1.10	1.10	1.10	1.10
Methionine	0.53	0.53	0.53	0.53	0.53
Threonine	0.94	0.94	0.94	0.94	0.94
Tryptophan	0.22	0.22	0.22	0.22	0.22

Abbreviation: DDGS, dried distillers grains with solubles.

[1] Vitamin premix provides the following per kg of the final diet: vitamin A, 8,000 IU; vitamin D_3, 2,000 IU; vitamin E, 5 mg; vitamin K_2, 1 mg; vitamin B_1, 0.6 mg; vitamin B_2, 4.8 mg; vitamin B_6, 1.8 mg; vitamin B_{12}, 0.009 mg; niacin, 10.5 mg; DL-calcium, pantothenate, 7.5 mg; folic acid, 0.15 mg.

[2] Mineral premix provides the following per kg of the final diet: Fe ($FeSO_4 \cdot H_2O$), 80 mg; Cu ($CuSO_4 \cdot 5H_2O$), 8 mg; Mn ($MnSO_4 \cdot H_2O$), 70 mg; Zn ($ZnSO_4 \cdot H_2O$), 90 mg; I (KI), 0.4 mg; Se (Na_2SeO_3), 0.3 mg.

12 ducks from the 17.5% CP in the group without protease supplementation were randomly selected based on BW, assigned to 6 cages of 2 ducks, and fed with a N-free diet mixed with TiO_2 (0.5%) to determine basal endogenous AA losses as per the method of Han et al. (2017). After a 3-day adaptive period (day 28 to 30), the total excreta samples from each cage were collected for 72 h (at day 31 to 33). Excreta were collected and analyzed for DM, TiO_2, N, P, and energy to calculate AME in accordance with the method of Cowieson et al. (2009) and Zeng et al. (2015). On day 34, when the 72-hour excreta collection was completed, ducks were fed for 4 h and then were euthanized by cervical dislocation. The ileal digesta was gently rinsed with distilled water into plastic containers (Qin et al., 2017). The collected ileal samples from 2 birds within a cage were pooled and stored at −20 °C for subsequent analyses of DM, TiO2, and AA. These data were used to calculate SIDAA based on our previous studies of Han et al. (2017) and Qin et al. (2017).

Statistical Analysis

All data were analyzed by two-way ANOVA using the GLM procedure of SAS software (version 9.2; SAS Institute Inc., Cary, NC). The model included the main effects of dietary CP concentration, dietary protease supplementation, and their interaction. The pen was the experimental unit. The means showing significant treatment differences at $P \leq 0.05$ in ANOVA were then compared using Fisher's protected least significant difference procedure, and an alpha level of 0.05 was considered significant. All data were tested for normality using the UNIVARIATE procedure and common variance using the GLM procedure. In addition, the linear broken-line regression was computed by the NLIN procedure (Robbins et al., 2006), and CP requirement was estimated using linear broken-line regressions when a significant response occurred ($P < 0.05$) based on growth performance parameters. The R^2 was provided to compare these regressions (Pesti et al., 2009).

RESULTS

The analyzed dietary CP and AA concentrations for 10 diets on a formulated basis were determined and summarized in Table 2. The analyzed CP value verified that the diets were well mixed and the 10 experimental diets can be used with confidence for further study.

Table 2. Analyzed amino acid and nitrogen concentration of the experimental diets (as-fed basis).

	Without protease supplementation (-)					With protease supplementation (+)				
Item	13.5%	14.5%	15.5%	16.5%	17.5%	13.5%	14.5%	15.5%	16.5%	17.5%
CP, g/kg	13.24	14.29	15.26	16.48	17.27	13.60	14.50	15.52	16.54	17.27
Nonessential amino acids, g/kg										
Aspartic acid	0.88	1.05	1.10	1.15	1.11	0.93	1.01	1.08	1.15	1.15
Alanine	0.54	0.61	0.70	0.68	0.69	0.62	0.64	0.72	0.71	0.74
Cysteine	0.25	0.25	0.25	0.25	0.21	0.23	0.26	0.28	0.24	0.28
Glutamic acid	2.22	2.40	2.66	2.74	2.81	2.22	2.40	2.53	2.81	2.69
Glycine	0.51	0.54	0.58	0.60	0.64	0.52	0.59	0.60	0.65	0.65
Proline	0.85	0.86	1.00	0.98	0.97	0.83	0.89	0.87	0.96	0.89
Serine	0.50	0.64	0.65	0.70	0.68	0.55	0.58	0.63	0.69	0.65
Tyrosine	0.40	0.42	0.44	0.43	0.34	0.42	0.37	0.40	0.46	0.44
Essential amino acid, g/kg										
Arginine	0.81	0.90	1.04	1.07	1.07	0.87	0.94	0.97	1.17	1.07
Histidine	0.28	0.31	0.32	0.33	0.36	0.32	0.38	0.35	0.43	0.37
Isoleucine	0.40	0.45	0.45	0.48	0.40	0.45	0.45	0.48	0.52	0.49
Leucine	1.28	1.40	1.47	1.46	1.34	1.35	1.33	1.37	1.51	1.37
Lysine	0.94	1.08	1.07	1.03	0.99	1.00	1.14	1.11	1.14	1.07
Methionine	0.37	0.37	0.36	0.38	0.33	0.44	0.38	0.40	0.33	0.42
Phenylalanine	0.51	0.55	0.61	0.63	0.60	0.51	0.56	0.57	0.65	0.60
Threonine	0.73	0.87	0.83	0.88	0.75	0.83	0.85	0.87	0.90	0.82
Valine	0.55	0.56	0.63	0.63	0.61	0.54	0.59	0.63	0.63	0.66
Total NEAA[1]	6.16	6.76	7.37	7.53	7.44	6.33	6.75	7.12	7.67	7.49
Total EAA	5.87	6.49	6.77	6.91	6.44	6.27	6.61	6.77	7.29	6.87
EAA: NEAA	0.95	0.96	0.92	0.92	0.87	0.99	0.98	0.95	0.95	0.92
Total AA	12.02	13.25	14.15	14.44	13.88	12.60	13.35	13.88	14.96	14.36
Total AA:N[1]	5.77	5.89	5.89	5.56	5.11	5.88	5.85	5.68	5.74	5.28

[1] Abbreviations: EAA, essential amino acid; EAA : NEAA, the content of total EAA of diet/the content of total NEAA of diet; NEAA, nonessential amino acids; Total AA: N, the content of total AA of diet/Nitrogen content of diet.

Growth Performance

The effects of dietary CP levels and protease supplementation on BW, BWG, FI, and F:G ratio are displayed in Table 3. No significant ($P > 0.05$) CP × protease interactions were detected for growth performance of ducks during 15 to 49 D. CP levels had a significant effect ($P < 0.05$) on the growth performance of ducks. The BW(day 49), BWG, and FI of ducks fed 17.5% CP = 16.5% CP > 15.5% CP > 14.5% CP > 13.5% CP. The F:G of ducks fed 17.5, 16.5, 15.5, and 14.5% CP was lower ($P < 0.05$) than that of those fed 13.5% CP. Irrespective of dietary CP level,

Table 3. Effects of dietary protein levels and protease supplementation on growth performance of ducks from 15 to 49 D of age (Exp1).

CP%	Protease supplementation	49d BW/g	15–49d BWG/g	15–49d FI/g	15–49 D F:G
13.5	-	2,108[1]	1359	3,939	2.97
14.5	-	2,561	1,816	4,818	2.69
15.5	-	2,961	2,214	5,801	2.65
16.5	-	3,400	2,654	6,814	2.58
17.5	-	3,621	2,877	6,916	2.41
13.5	+	2,042	1,297	3,912	3.05
14.5	+	2,691	1,947	5,177	2.68
15.5	+	2,900	2,151	5,692	2.67
16.5	+	3,623	2,873	6,859	2.50
17.5	+	3,572	2,823	7,084	2.52
	SEM	120.8	128.0	218.7	0.11
Main effect					
CP%	13.5	2,075^{d}	1,328^{d}	3,926^{d}	3.01^{a}
	14.5	2,626^{c}	1,881^{c}	4,997^{c}	2.69^{b}
	15.5	2,930^{b}	2,182^{b}	5,747^{b}	2.66^{b}
	16.5	3,512^{a}	2,708^{a}	6,837^{a}	2.54^{b}
	17.5	3,596^{a}	2,850^{a}	7,000^{a}	2.47^{b}
	SEM	85.42	124.8	218.7	0.11
Protease supplementation	-	2,930	2,184	5,658	2.66
	+	2,965	2,218	5,754	2.66
	SEM	54.02	53.34	97.32	0.05
Source of variation		Probability			
P-value	CP	<0.05	<0.05	<0.05	<0.05
	protease	0.647	0.650	0.484	0.946
	CP*protease	0.658	0.654	0.844	0.680

$^{a–d}$Values within a column with no common superscripts differ significantly ($P < 0.05$).
Abbreviations: BWG, BW gain; F:G, feed-to-gain ratio.
[1] Values are the means of 6 replicates of 16 ducks each.

protease supplementation could numerically increase ($P > 0.05$) the BWG and FI of ducks aged from 15 to 49 D.

Carcass Traits

The CP level × protease interaction for breast meat yield was statistically significant ($P < 0.05$; Table 4). Protease supplementation had a much greater effect for breast meat yield in the 14.5% CP diet than any of the other CP levels diets. Dietary CP levels had significant effects ($P < 0.05$) on the yield of carcass and leg meat yields. Ducks fed diets with 16.5 and 17.5% CP had lower ($P < 0.05$) leg muscle yield than those on diets with 15.5, 14.5, and 13.5% CP. Interestingly, ducks fed diets with 13.5% CP had the highest carcass yield ($P < 0.05$) than those on diets with other 4 CP levels.

Breast Muscle Quality

There was no interaction ($P > 0.05$) between dietary CP levels and protease supplementation on the quality of breast muscle (Table 5). However, dietary CP levels had a significant effect ($P < 0.05$) on $\mathrm{pH}_{45\mathrm{min}}$ value, b*, shear force, and drop loss. With increasing dietary CP levels, the pH value and b* decreased ($P < 0.05$), and shear force and drop loss increased ($P < 0.05$). Regardless of dietary protein levels, protease supplementation significantly decreased ($P < 0.05$) the shear force of breast muscle.

Energy, N, and P Utilization

The CP level × protease interactions for DM, energy and N utilization, as well as dietary AME content were significant ($P < 0.05$; Table 6). Protease supplementation with a low-CP diet (13.5%) significantly improved ($P < 0.05$) energy availability and dietary AME content. Protease supplementation to a 17.5% CP diet significantly improved ($P < 0.05$) DM and N utilization compared with a diet without protease supplementation. The CP of 13.5, 16.5, and 17.5% diets had a higher ($P < 0.05$) retention of P than those of the 14.5 and 15.5% CP diets. Protease supplementation could significantly improve ($P < 0.05$) dietary P utilization regardless of CP levels.

Standardized Ileal Digestibility of AA

The CP level × protease interactions for SID of 7 AA (histidine [**His**], phenylalanine [**Phe**], Thr, cysteine [**Cys**], glycine [**Gly**], proline [**Pro**], and tyrosine [**Tyr**]) were statistically significant ($P < 0.05$; Table 7 and Table 8). Irrespective of protease supplementation, compared with 17.5% CP, 13.5% CP significantly increased ($P < 0.05$) the SID of 11 AA (His, isoleucine, Lys, leucine, Phe, Thr, Pro, Tyr), total essential AA (**EAA**), total nonessential AA (**NEAA**), and total AA; moreover, compared to 17.5% CP, the diet with 16.5% CP significantly increased ($P < 0.05$) the SID of 15 AA (His, isoleucine, Lys, leucine, Phe, Thr, Cys, Gly, Glu, Pro, Tyr, serine), total EAA, total NEAA, and total AA.

Table 4. Effects of dietary protein levels and protease supplementation on carcass traits of ducks at 49 D of age (Exp1).

		Carcass traits (%)				
CP%	Protease supplementation	Carcass	Eviscerated with giblet	Eviscerated	Breast muscle	Leg muscle
13.5	-	90.3[1]	82.8	75.1	6.79^{d}	11.5
14.5	-	88.7	83.3	76.0	6.83^{d}	11.4
15.5	-	88.3	83.4	76.7	$12.1^{b,c}$	10.4
16.5	-	89.1	85.1	78.2	$14.2^{a,b}$	9.41
17.5	-	88.2	83.5	76.6	15.3^{a}	9.04
13.5	+	89.8	83.3	75.4	7.31^{d}	10.9
14.5	+	88.4	83.3	76.5	11.6^{c}	10.3
15.5	+	88.6	83.2	75.9	11.5^{c}	10.0
16.5	+	88.1	83.9	76.5	15.5^{a}	8.96
17.5	+	88.0	83.3	76.4	15.6^{a}	8.92
	SEM	0.60	0.71	0.79	0.78	0.49
Main effect						
CP%	13.5	90.1^{a}	83.0	75.2	7.05^{d}	11.2^{a}
	14.5	88.5^{b}	83.3	76.3	9.22^{c}	10.8^{a}
	15.5	88.5^{b}	83.3	76.3	11.8^{b}	10.2^{a}
	16.5	88.6^{b}	84.5	77.4	14.9^{a}	9.18^{b}
	17.5	88.1^{b}	83.4	76.5	15.5^{a}	8.98^{b}
	SEM	0.43	0.5	0.56	0.55	0.35
Protease	-	88.91	83.63	76.51	11.43	10.34
	+	88.60	83.38	76.13	12.31	9.83
	SEM	0.27	0.32	0.35	0.35	0.22
Source of variation			Probability			
P-value	CP	<0.05	0.293	0.129	<0.05	<0.05
	Protease	0.411	0.583	0.444	<0.05	0.102
	CP*protease	0.886	0.808	0.622	<0.05	0.923

$^{a\text{–}d}$Values within a column with no common superscripts differ significantly ($P < 0.05$).

[1] Values are the means of 6 ducks per dietary treatment.

Table 5. Effects of dietary protein levels and protease supplementation on breast muscle quality of ducks at 49 D of age (Exp1).

			Breast muscle quality				
CP%	Protease supplementation	pH value	Lightness (L*)	Redness (a*)	Yellowness (b*)	Shear force (kgf/cm^2)	Drop loss,%
13.5	-	7.04^{1}	38.50	10.52	3.77	4.80	3.51
14.5	-	6.87	37.56	10.36	2.19	5.26	3.50
15.5	-	6.82	34.89	9.90	2.37	5.60	3.21
16.5	-	6.73	33.98	8.46	1.58	6.07	3.73
17.5	-	6.70	36.58	10.51	1.82	6.08	5.28
13.5	+	7.25	34.76	9.05	2.88	3.59	2.75
14.5	+	6.87	36.21	8.96	1.46	5.04	4.44
15.5	+	6.80	36.23	10.38	1.99	6.02	3.14
16.5	+	6.67	34.14	9.14	1.65	5.04	4.03
17.5	+	6.62	35.39	9.91	1.92	5.37	5.50
	SEM	0.07	1.64	0.67	0.45	0.43	0.69
Main effect							
CP%	13.5	7.14^{a}	36.63	9.79	3.33^{a}	4.19^{b}	3.13^{b}
	14.5	6.87^{b}	36.89	9.66	1.83^{b}	5.15^{a}	3.97^{b}
	15.5	$6.81^{b,c}$	35.56	10.14	2.18^{b}	5.81^{a}	3.17^{b}
	16.5	6.70^{c}	34.06	8.80	1.61^{b}	5.55^{a}	3.88^{b}
	17.5	6.66^{c}	35.98	10.21	1.87^{b}	5.73^{a}	5.39^{a}
	SEM	0.05	1.16	0.47	0.32	0.30	0.49
Protease	-	6.83	36.30	9.95	2.35	5.56	3.85
	+	6.84	35.35	9.49	1.98	5.01	3.97
	SEM	0.03	0.73	0.30	0.20	0.20	0.31
Source of variation				Probability			
P-value	CP	<0.01	0.454	0.237	<0.01	<0.01	<0.05
	Protease	0.828	0.360	0.280	0.200	<0.05	0.771
	CP*protease	0.319	0.611	0.343	0.732	0.321	0.807

$^{a–c}$Values within a column with no common superscripts differ significantly ($P < 0.05$).
[1] Values are the means of 6 ducks per dietary treatment.

Optimal Crude Protein Requirement of Ducks From 15 to 49 D of Age

As shown in Table 9, the optimal CP requirement for ducks with or without protease supplementation was 16.53 or 17.02% based on BW and BWG and was 16.75 or 16.64% based on FI, using linear broken-line regression. However, there was a lower R^2 value (0.267 [without protease] or 0.454 [with protease]) when used linear broken-line regression based on the F:G ratio.

Table 6. Effects of dietary protein levels and protease supplementation on DM, energy, and nitrogen and phosphorus retention of ducks aged from 28 to 34 D (Exp2).

CP%	Protease supplementation	DM (%)	Energy (%)	AME (Kcal/kg)	Nitrogen (%)	Phosphorus (%)
13.5	-	$73.45^{a,1}$	$78.49^{b,c}$	$2,964^{1,c,d}$	$59.68^{a,b}$	53.68
14.5	-	$70.10^{b,c}$	$78.03^{b,c,d}$	$3,040^{b,c}$	53.98^{b}	48.19
15.5	-	66.87^{d}	75.35^{e}	$2,887^{e}$	54.05^{b}	50.26
16.5	-	73.86^{a}	78.80^{b}	$3,047^{b}$	64.82^{a}	57.75
17.5	-	66.56^{d}	$76.01^{d,e}$	$2,978^{b,c,d}$	53.35^{b}	55.44
13.5	+	74.33^{a}	82.03^{a}	$3,231^{a}$	66.14^{a}	58.32
14.5	+	69.61^{c}	$75.95^{d,e}$	$2,845^{e}$	51.66^{b}	54.65
15.5	+	69.38^{c}	$76.62^{c,d,e}$	$2,916^{d,e}$	51.41^{b}	53.85
16.5	+	$72.08^{a,b}$	$77.86^{b,c,d}$	$2,991^{b,c,d}$	$57.96^{a,b}$	64.58
17.5	+	$70.69^{b,c}$	$78.05^{b,c,d}$	$3,032^{b,c}$	64.15^{a}	62.49
	SEM	0.78	0.67	25.51	3.20	1.75
Main effect						
CP%	13.5	73.89^{a}	80.26^{a}	$3,098^{a}$	62.91^{a}	56.00^{b}
	14.5	69.85^{b}	$76.99^{b,c}$	$2,942^{c}$	52.82^{b}	51.42^{c}
	15.5	68.13^{c}	75.98^{a}	$2,901^{c}$	52.73^{b}	52.05^{c}
	16.5	72.97^{a}	78.33^{b}	$3,019^{b}$	61.39^{a}	61.17^{a}
	17.5	$68.62^{b,c}$	$77.03^{b,c}$	$3,005^{b}$	$58.75^{a,b}$	$58.96^{a,b}$
	SEM	0.55	0.47	18.04	2.16	1.24
Protease	-	70.17	77.33	2,983	57.18	53.06
	+	71.22	78.10	3,003	58.26	58.78
	SEM	0.35	0.30	11.41	1.40	0.78
Source of variation			Probability			
P-value	CP	<0.05	<0.001	<0.001	<0.005	<0.001
	Protease	<0.001	0.075	0.224	0.581	<0.001
	CP*protease	<0.005	<0.005	<0.001	<0.05	0.824

$^{a–e}$Values within a column with no common superscripts differ significantly ($P < 0.05$).
[1] Values are the means of 6 replicates of 2 ducks each per treatment.

Table 7. Effects of dietary protein levels and protease supplementation on standardized ileal digestibility of essential amino acids of ducks at 34 d of age (Exp2).

CP%	Protease supplementation	Arg	His	Ile	Lys	Leu	Met	Phe	Val	Thr	Total EAA
13.5	-	82.20[1]	$73.21^{a,b}$	70.76	77.66	82.92	89.14	75.25^{a}	63.21	72.39^{a}	77.24
14.5	-	77.94	$66.53^{a,b,c}$	67.43	76.20	81.21	87.26	$69.00^{a,b}$	55.54	72.42^{a}	74.16
15.5	-	75.73	52.62^{d}	56.52	67.17	76.01	85.77	$64.39^{b,c}$	51.72	$61.60^{b,c,d}$	67.61
16.5	-	81.20	$58.96^{c,d}$	64.63	69.25	78.93	85.13	$70.31^{a,b}$	55.99	$66.92^{a,b}$	71.77
17.5	-	75.13	$58.82^{c,d}$	51.44	62.31	72.00	81.71	$64.94^{b,c}$	49.00	56.45^{d}	64.84
13.5	+	73.75	63.36^{c}	64.81	71.89	78.15	86.85	$65.08^{b,c}$	51.66	$66.53^{a,b}$	70.48
14.5	+	73.21	$64.39^{b,c}$	62.00	71.32	75.86	86.27	$63.06^{b,c}$	50.72	$65.01^{a,b,c}$	68.93
15.5	+	77.41	$64.87^{a,b,c}$	65.33	72.01	77.47	87.34	$67.99^{a,b,c}$	58.18	$68.86^{a,b}$	71.89
16.5	+	80.85	73.54^{a}	69.84	74.57	80.88	80.03	73.37^{a}	58.45	69.88^{a}	74.62
17.5	+	72.72	$60.37^{c,d}$	57.62	63.43	71.10	82.89	60.44^{c}	50.75	$57.73^{c,d}$	64.84
	SEM	2.577	2.889	3.288	3.019	1.879	2.364	2.623	3.565	2.540	2.516
Main effect											
CP%	13.5	77.98	68.29^{a}	67.79^{a}	74.77^{a}	80.53^{a}	88.00	$70.16^{a,b}$	57.43	69.46^{a}	73.86^{a}
	14.5	75.57	65.46^{a}	64.71^{a}	73.76^{a}	78.54^{a}	86.77	$66.03^{b,c}$	53.13	68.71^{a}	71.55^{a}
	15.5	76.57	58.75^{b}	$60.92^{a,b}$	69.59^{a}	76.74^{a}	86.55	$66.19^{b,c}$	54.95	65.23^{a}	$69.75^{a,b}$
	16.5	81.03	66.25^{a}	67.23^{a}	71.91^{a}	79.91^{a}	82.58	71.84^{a}	57.22	68.40^{a}	73.19^{a}
	17.5	73.92	59.59^{b}	54.53^{b}	62.87^{b}	71.55^{b}	82.30	62.69^{c}	49.87	57.09^{b}	64.84^{b}
	SEM	1.822	2.043	2.325	2.134	1.329	1.671	1.855	2.521	1.796	1.779
Protease	-	78.44	62.03	62.16	70.52	78.22	85.80	68.78	55.09	65.96	71.12
	+	75.59	65.31	63.92	70.64	76.69	84.68	65.99	53.95	65.60	70.15
	SEM	1.152	1.292	1.470	1.350	0.840	1.057	1.173	1.594	1.136	1.125
Source of variation					Probability						
P-value	CP	0.085	<0.005	<0.005	<0.005	<0.001	0.058	<0.01	0.201	<0.001	<0.01
	Protease	0.086	0.079	0.401	0.947	0.206	0.455	0.099	0.614	0.825	0.544
	CP*protease	0.338	<0.001	0.080	0.214	0.177	0.619	<0.05	0.112	<0.05	0.130

[a–d] Values within a column with no common superscripts differ significantly ($P < 0.05$).

Abbreviations: Arg, arginine; EAA, essential amino acid; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Thr, threonine; Val, valine.

[1] Values are the means of 6 replicates of 2 ducks each per treatment.

DISCUSSION

Attempts to decrease the CP content of broiler diets have been performed. Most researchers agree that reduction of dietary CP has some noxious effects on performance and appetite (Namroud et al., 2008). These reports were further validated by the data in the present study that LP diets (13.5, 14.5, and 15.5%) could depress the BW, BWG, and FI of ducks aged between15 to 49 D. These findings are in agreement with those of previous studies showing that feeding ducks with 15% CP diets had a lower BW and BWG than feeding with 17% and 19% CP diets, irrespective of dietary ME concentration (Zeng et al., 2015). Namroud et al. (2008) reported that decreasing dietary CP below a minimum level (<19%) was sufficient to maintain EAA levels, retard growth and feed intake, and increase the F:G ratio. Reduced growth performance in broilers fed LP diets and supplemented with EAA has been reported irrespective of their environmental condition. This reduction could be associated with lower levels of specific NEAA such as Gly and serine or Glu or with a nonspecific need for N (Namroud et al., 2008).

In the present study, we found the NEAA concentration in 13.5 and 14.5% CP diets was lower. In most of the previous similar studies, a limiting minimum level for CP reduction lower than this level could rapidly influence performance with or without EAA or NEAA supplementation (Baeza and Leclercq, 1998; Bregendahl et al., 2002; Yamazaki et al., 2006; Namroud et al., 2008).

In the present study, a lower digestibility of feed ingredients was used in the experimental diets. Widyaratne and Drew (2011) reported that LP diets could support growth performance of broiler chickens at similar levels to high-protein diets when highly digestible feed ingredients were used. Consistently, we found the N utilization of diets ranged from 51.51 to 66.16%. These values were lower than the ranges of 73.48 to 81.7% reported by Zeng et al. (2015) when ducks were fed a typical corn-soybean meal diet. In addition, we found that 16.5% and 17.5% CP had a higher energy, and N and P utilization than 14.5% and 15.5% CP, which may explain why ducks fed diets containing 16.5 and 17.5% CP had a better growth performance. Furthermore, by the linear broken-line regression model, the optimal dietary CP levels ranged from 16.64 to 17.02% based on FI and BWG regardless of protease supplementation.

In contrast, we observed that 13.5 and 17.5% CP had the highest and lowest SIDAA, respectively. Similar effects have been recently observed in pigs (Wang et al., 2017). This observation raises the interesting possibility that LP diets may have elevated amino acid digestibility and/or reduced endogenous amino acid flow to compensate intestinal AA deficiency. The other reason is thought to be related to the higher-protein diet (17.5%) contains increased levels of lower digestible ingredients, namely, cottonseed meal, rapeseed meal, wheat middlings, feather meal, and dried distillers grains with solubles and it contains 20% less higher digestible corn, which indicates that lower digestible ingredients in diets with 17.5% CP maybe lead to a lower SIDAA.

In the present study, although a numerical improvement in growth performance by protease supplementation was observed, it was also found that protease

Table 8. Effects of dietary protein levels and protease supplementation on standardized ileal digestibility of NEAA of ducks at 34 D of age (Exp2).

CP%	Protease supplementation	Ala	Asp	Cys	Gly	Glu	Pro	Tyr	Ser	Total NEAA	Total AA
13.5	-	69.02[1]	65.56	$90.47^{a,b,c}$	$70.05^{a,b}$	79.21	72.13^{a}	74.22^{a}	62.97	73.43	75.27
14.5	-	63.24	63.66	$85.21^{d,e}$	$63.66^{a,b,c}$	75.05	$64.98^{b,c}$	$71.07^{a,b}$	64.47	69.14	71.59
15.5	-	63.58	55.82	$86.44^{c,d,e}$	57.62^{c}	71.53	60.64^{c}	$63.30^{b,c}$	56.16	64.50	65.98
16.5	-	66.60	62.52	$89.70^{a,b,c}$	$65.50^{a,b,c}$	75.88	$65.08^{b,c}$	$64.95^{a,b,c}$	65.49	69.64	70.65
17.5	-	61.81	53.98	83.12^{e}	61.06^{c}	71.60	60.41^{c}	50.56^{e}	56.06	63.62	64.18
13.5	+	65.87	55.74	$88.31^{b,c,d}$	60.53^{c}	71.47	$59.43^{c,d}$	$65.45^{a,b,c}$	54.41	64.84	67.63
14.5	+	62.12	55.49	$89.59^{a,b,c}$	$61.95^{b,c}$	70.93	$59.12^{c,d}$	$56.13^{c,d,e}$	54.97	63.96	66.41
15.5	+	69.24	59.60	$91.18^{a,b}$	$65.54^{a,b,c}$	74.00	63.21^{c}	$61.50^{b,c,d}$	61.36	68.12	69.95
16.5	+	70.97	64.19	92.88^{a}	70.79^{a}	78.59	$70.10^{a,b}$	$68.78^{a,b}$	65.49	72.66	73.60
17.5	+	63.51	53.60	$90.85^{a,b,c}$	60.93^{c}	70.47	53.52^{d}	$53.06^{d,e}$	51.89	62.46	63.59
	SEM	2.978	3.190	1.377	2.594	2.097	2.068	3.199	3.029	2.373	2.431
Main effect											
CP%	13.5	67.45	60.65	$89.39^{a,b}$	$65.29^{a,b}$	$75.34^{a,b}$	$65.78^{a,b}$	69.84^{a}	58.69^{b}	69.13^{a}	71.45^{a}
	14.5	62.68	59.57	87.40^{b}	$62.81^{a,b}$	$72.99^{a,b}$	62.05^{b}	$63.60^{a,b}$	$59.72^{a,b}$	$66.55^{a,b}$	$69.00^{a,b}$
	15.5	66.41	57.71	$88.81^{a,b}$	61.58^{b}	$72.77^{a,b}$	61.93^{b}	62.40^{b}	58.76^{b}	$66.31^{a,b}$	$67.96^{a,b}$
	16.5	68.78	63.36	91.29^{a}	68.15^{a}	77.23^{a}	67.59^{a}	$66.86^{a,b}$	65.49^{a}	71.15^{a}	72.12^{a}
	17.5	62.66	53.79	86.98^{b}	60.99^{b}	71.04^{b}	56.97^{c}	51.81^{c}	53.97^{b}	63.04^{b}	63.88^{b}
	SEM	2.106	2.256	0.974	1.834	1.483	1.462	2.262	2.142	1.678	1.719
Protease	-	64.85	60.31	86.99	63.58	74.66	64.65	64.82	61.03	68.06	69.53
	+	66.34	57.72	90.56	63.95	73.09	61.08	60.98	57.62	66.41	68.23
	SEM	1.332	1.427	0.616	1.160	0.938	0.925	1.431	1.355	1.061	1.087
Source of variation		Probability									
P-value	CP	0.150	0.054	<0.05	<0.05	<0.05	<0.001	<0.001	<0.05	<0.05	<0.05
	Protease	0.432	0.206	<0.001	0.821	0.244	<0.01	0.064	0.082	0.275	0.402
	CP*protease	0.556	0.142	<0.05	<0.05	0.076	<0.001	<0.05	0.102	0.058	0.088

$^{a–d}$Values within a column with no common superscripts differ significantly ($P < 0.05$).

Abbreviations: AA, amino acid; Ala, alanine; Asp, aspartic acid; Cys, cysteine; Gly, glycine; Glu, glutamic acid; NEAA, nonessential amino acids; Pro, proline; Ser, serine; Tyr, tyrosine.

[1] Values are the means of 6 replicates of 2 ducks each per treatment.

supplementation could significantly improve dietary energy, N and P utilization, and SID of His, Phe, Thr, Cys, Gly, Pro, and Tyr. These results agreed with those of the study of Freitase et al. (2011), which reported no effect of supplemental protease on growth performance, with a significant increase in apparent AA, N, or energy digestibility in broilers. Yuan et al. (2015) showed that supplementation of protease enzyme had no impact on the F:G ratio in broilers. Furthermore, Angel et al. (2011) also observed that the performance index of broilers was not significantly affected by enzyme supplementation in comparison with that of control broilers. Similarly, many reports have verified that exogenous protease supplementation could increase dietary SIDAA (Cowieson and Roos, 2014), N, energy, starch, and fat digestibility (Fru-Nji et al., 2011; Kalmendal and Tauson, 2012; Amerah et al., 2017). Freitas et al. (2011) reported that exogenous protease could improve the digestibility of protein to a greater extent in high-protein/high-energy diets than in diets with LP/low-energy. A recent meta-analysis of the effects of a monocomponent protease on apparent ileal AA digestibility in pigs and poultry revealed that the mean response was approximately +4% over a range of control diets and single feed ingredients (Cowieson and Roos, 2014). These results suggested that protease supplementation changes the dietary energy, CP and AA concentration, and further alters the pattern of AA balance by increasing nutrient utilization. These factors consequently result in the inconsistent growth performance

Table 9. Summary of CP requirement for Pekin ducks from 15 to 49 D age (Linear broken-line regression[1]).

	CP requirement			
Items	Estimated requirement (%)	R^2	95% CI[2]	*P*-value
Without protease supplementation				
BW, (g)	17.02	0.790	16.177 to 17.860	<0.001
BW gain, BWG (g)	17.02	0.793	16.187 to 17.857	<0.001
Feed intake, FI (g)	16.64	0.842	16.026 to 17.247	<0.001
Feed-to-gain ratio, F:G (g: g)	15.04	0.267	13.526 to 16.547	0.015
With protease supplementation				
BW, (g)	16.53	0.818	15.905 to 17.173	<0.0001
BWG, (g)	16.53	0.822	15.902 to 17.164	<0.001
FI, (g)	16.75	0.844	16.126 to 17.381	<0.001
F:G, (g: g)	14.90	0.454	14.077 to 15.726	<0.001

[1] Linear broken-line is $Y = L + V \times (R\text{-}X)$, where L is the ordinate, V is the random component of the slope, R is the abscissa of the breakpoint, and the value R-X is zero at values of $X > R$.Y = response index, X = dietary CP levels (%), R = breakpoint (the optimal level), L = the response at X, R. 95% CI = 95% confidence interval of the estimated CP requirement.

observed in different studies of pigs or poultry (Cowieson and Roos, 2014). Walk et al. (2018) evaluated 8 neutral and 6 acid proteases and found that protease supplementation improved the apparent ileal digestibility of AA, but this was not reflected in improvements in growth performance of poultry or chicks.

As expected, breast meat yield decreased in ducks fed LP diets in the present study. Previous studies have reported the same phenomenon in ducks. Farhat and Chavez (1999) observed changes in breast muscle thickness using ultrasound showing values of 8.42, 7.26, and 6.93 mm for high (23%), medium (19%), and low protein (17%) programs, respectively. Mohammadigheisar et al. (2018) found that feeding broiler chickens with LP diets had no effect on breast meat yield. Nevertheless, ducks fed 13.5% CP had better carcass and leg meat yields. This may be due to that birds being fed low CP increased the deposition of abdominal fat (Rosebrough and McMurtry, 1993) because low-CP diet had a higher calorie: protein ratio. It appears that the excess available energy beyond that required for protein deposition is diverted to abdominal fat deposition (Zeng et al., 2014). Moreover, this effect was, at least partially, due to depressed growth performance by low-CP diets. We also found that protease supplementation significantly increased the yield of breast meat. Mahmood et al. (2018) reported an increase in the carcass and breast meat yield of broilers associated with protease supplementation could be attributed to enhanced utilization and deposition of protein (Xu et al., 2017).

Meat quality is a very important features for producers and consumers. The pH of a meat has a direct bearing on the quality attributes such as tenderness, water holding capacity, color, juiciness, and shelf life. Broiler breast meat with high pH has a higher water binding capacity than meat with a lower pH (Mir et al., 2017). Calvo et al. (2017) also observed a positive correlation between higher pH values and the lower drop loss for pork. Low water holding capacity results in increased cooking and drip losses, low shelf life, and decreased tenderness or increased shear force and was found to be associated with low pH (Barbut, 1993). Identification of color is an easy way to determine the pH of meat: very dark meat will have a high pH, whereas light meat will have a low pH (Fernandez et al., 2002). Similarly, we also found that breast meat from 13.5% CP group had a higher pH with a lower drop loss and shear force and a higher level of b* in this study. These results indicated that ducks fed 13.5% CP had a positive effect on meat quality. The reason may be owing to the low-CP (13.5%) diet decreasing N deposition and increasing fat retention in breast meat. Bregendahl et al. (2002) reported that chicks fed low-CP diets retained less N and more ether extract compared with chicks fed control diets. Essén-Gustavsson et al. (1994) found that intramuscular fat was higher in both *M. longissimus* and *M. biceps* fermorsi from pigs on the LP diet (13.5%) than the same muscles from pigs on a high-protein diet (18.5%). They also showed the shear force in pigs fed a high-protein diet was higher than those fed a LP diet (4.7 and 4.0 kg/cm^2, respectively). This may explain why protease supplementation significantly increased the shear force of breast meat in our study.

CONCLUSIONS

In summary, LP (13.5, 14.5, and 15.5%) diets containing many complex meals could depress FI, BW, and BWG, as well as decrease the breast meat yield of ducks during 15 to 49 D of age. Although having the poorest growth performance, 13.5% CP had a positive effect on meat quality, nutrient utilization, and SIDAA. Protease supplementation increased P retention regardless of CP level; when CP = 14.5%, protease could significantly increase breast muscle yield. Ducks fed diets containing many complex meals should keep the dietary CP levels ≥16.53% based on BWG and FI regardless of protease supplementation.

ACKNOWLEDGMENTS

This research was supported by grants from National Key R & D Program of China (2017YFD0502004); Modern Agri-industry Technology Research System of China (CARS-42-10); “111” project of Foreign Experts Affairs of China; Sichuan Agricultural University 211 Foundation.

Conflict of Interest Statement: The authors did not provide a conflict of interest statement.

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