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Effects of Dietary Crude Protein Level and Stocking Density on Growth Performance, Nutrient Retention, Blood Profiles, and Carcass Weight of Growing-Meat Quails

Research Article

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ABSTRACT

Two experiments were conducted to evaluate the effect of stocking density on growing-meat quail performance (experiment I) and the effect of dietary crude protein (CP) levels and stocking density on growth performance, nutrient retention, blood metabolites, and carcass weight of growing-meat quails under high ambient temperature (experiment II). Three hundred and eighty male growing-meat quails were raised under 4 different stocking densities of 13, 17, 21, and 25 quails per cage (experiment I). Each treatment was performed in 5 replicates using a completely randomized design. In experiment II, 600 male growing-meat quails were assigned to 6 treatments (5 replicates) with 2 stocking densities (17 and 23 quails per cage) and 3 levels of CP (20, 22, and 24%) in a 2 × 3 factorial arrangement. In experiment I, the growing quails raised at 25 birds per cage had lighter body weight (BW) and body weight gain (BWG) than those at 13 and 17 quails per cage ($P<0.05$). Linear reductions in BW ($P=0.008$), feed intake ($P=0.033$), and BWG ($P=0.012$) as increasing stocking density were detected. In experiment II, CP levels and stocking density had no effect on growth performance, nutrient retention, blood profiles, and relative carcass weight ($P>0.05$). However, a 20% CP level significantly increased CP digestibility and decreased uric acid concentration compared to 24% CP ($P<0.05$). Furthermore, increased relative breast weight was detected in the quails raised under a high stocking density ($P<0.05$). The growing-meat quails have enhanced growth performance at the density of 32.10 to 51.85 birds/m^2. With a 20% CP diet, increased growth performance and CP digestibility were observed.

KEY WORDS crude protein, growing-meat quails, growth performance, stocking density.

INTRODUCTION

Greenhouse gas emissions have become a critical issue in the livestock industry. The production of ammonia originating from poultry manure negatively impacts animal health and well-being as well as source of greenhouse gas (Ritz *et al.* 2004; Steinfield *et al.* 2006). Numerous approaches have been used in feed formulation for reducing N excretion (Namroud *et al.* 2008; Boontiam *et al.* 2016). For instance, low protein diet positively reduced N excretion when used in broiler diets (Hernández *et al.* 2012). In quails, the dietary supplementation of crude protein (CP) ranging from 20 to 22% had significantly reduced N excretion without growth retardation (Omidiwura *et al.* 2016). In contrast, reduced CP diet had minimal effect on the growth performance and feed efficiency of growing-meat quails (Soares *et al.* 2003). The difference might be due to the environment, nutrition, and genetic factors.

High stocking density is an economical approach in commercial farms. A previous report showed that it decreased fix cost of production, resulting in high profitability because of the increasing kg of bird per area (Puron *et al.* 1995). However, this strategy caused decreased feed intake (FI) and immunity in response to high stocking density of 45 quails/m^2 (Camci *et al.* 2004; Faitarone *et al.* 2005; El-Tarabany, 2016). Additionally, severe problems can occur when birds are raised in open-house systems. We hypothesized that the addition of proper CP levels may affect these factors, and a lower CP diet may reduce N excretion. Consequently, two experiments were performed to investigate the effect of different stocking densities on growing meat quail performance (experiment I) and the effect of different CP levels and stocking densities on growth performance, nutrient retention, blood metabolites, and carcass traits of growing quails under high ambient temperature (experiment II).

MATERIALS AND METHODS

Experimental design and quail husbandry

Two experiments were carried out at a university research farm (Ladkrabang, Bangkok, Thailand). During the experimental period, the growing-meat quails (*Coturnix japonica*) were placed in cages (0.45 min width×0.90 m in length). They were fed an experimental diet for 3 weeks, beginning at 3 weeks of age and ending at 6 weeks of age.

In experiment I, three hundred and eighty male growing-meat quails were individually weighted at 21 days of age. The initial mean body weight (BW) was 72.09 g. The experimental birds were then allotted to 4 treatments based on different stocking densities of 13, 17, 21, and 25 quails per cage, which gave a space allowance of 32.10, 41.98, 51.85, and 61.73 birds/m^2, respectively. Each treatment was performed in 5 replicates using a completely randomized design.

In experiment II, 600 male growing-meat quails (mean initial BW=73.58 g) were used. The quails were assigned to 6 dietary treatments with 2 levels of stocking density (41.98 quails/m^2 of 17 quails per cage and 56.79 quails/m^2 of 23 quails per cage indicated as low and high stocking density, respectively) and 3 levels of CP (20, 22, and 24%) in a 2 × 3 factorial arrangement. Each treatment was performed in 5 replicates, with 85 and 115 growing-meat quails per treatment in the low and high stocking density treatments, respectively.

During the course of the experiments, the quails were raised in an open-house system, and the temperature was ranged from 33 to 38 ˚C. The trial was conducted in April, which the temperature ranged from 33 ˚C to 38 ˚C in the morning (from 10 to 12 am) and 37 ˚C to 38 ˚C in the afternoon (from 1 to 4 p.m.). Lighting regimen was artificially provided for a 23-h photoperiod (23L:1D). The quails had free access to experimental diets and fresh water through an automatic nipple drinker, whereas feed was manually given twice daily at 08:00 and 15:00 throughout the entire period to avoid deprivation of feed. Diets were formulated to meet or exceed the recommendations provided by NRC (1994) as shown in Table 1. The procedures of care and handling of experimental quails were performed under the approval of the National Research Committee of the National Research Council of Thailand.

Quail performance

The quails were weighed after 21 and 42 days. Feed intake (FI), final BW, body weight gain (BWG), and feed conversion ratio (FCR) were measured. Mortality was daily recorded for each cage at 08:00 and 18:00, and used for correcting the FCR value.

Nutrient retention

Six hundred male growing-meat quails that were not used in the feeding trial were used for determining nutrient retention. At 21 days of age, the quails were raised in metabolic cages with similar stocking density as described above. Experimental diets were mixed with ferric oxide (Fe_2O_3) at 10 g/kg of diet used as an indigestible marker at the beginning and end of the experiment. The quails were fed the diets from 22 to 25 days of age as an adjustment period. The collecting period ranged from 26 to 28 days of age. Fecal samples without the contamination of white feathers, scales, filoplumes, and other contaminants were collected every 12 h and immediately kept at –20 ˚C. Pooled samples were dried in an oven at 60 ˚C for 72 h and ground to 1.0-mm-sized particles. Ground fecal samples were analyzed for dry matter (procedure # 930.15), ash (procedure # 942.05), CP (procedure # 984.13), and ether extract (procedure # 920.39) using the protocols described by AOAC (2000). Each representative sample and diet was analyzed in triplicate.

Blood profiles

At the end of the experiment (6 weeks of age), 10 quails from each treatment (2 from each replicate) were randomly selected for blood collection. Blood samples were collected from a left wing vein using 1.5 mL sterilized syringes with needles. The samples were immediately transferred into non-heparinized tubes, placed at room temperature for 2 h, and centrifuged at 3000 rpm for 15 min. The sera were kept in 2 mL eppendorf tubes at –40 ˚C. The representative samples were analyzed for blood glucose, aspartate aminotransferase (AST), and uric acid concentrations using enzymatic kinetic methods.

Table 1 Ingredients and nutrient composition of the experimental diets (%, as-fed basis)

Ingredient (%)	**Crude protein level (%)**		
	20	22	24
Corn	58.44	54.36	49.55
Soybean meal (45%)	32.35	32.37	31.72
Corn gluten meal (42%)	0.85	4.11	7.92
Wheat bran (14.2%)	2.59	3.39	5.04
Soybean oil	2.00	2.00	2.00
L-lysine sulfate (78%)	0.31	0.31	0.31
DL-methionine (99%)	0.20	0.20	0.20
Monodicalcium phosphate	1.34	1.34	1.34
Limestone	1.30	1.30	1.30
Vitamin and mineral premix[1]	0.25	0.25	0.25
Salt	0.37	0.37	0.37
Calculated values[2]			
Metabolizable energy (kcal/kg)	2,990	2,990	2,990
Crude protein (%)	20.00	22.00	24.00
Lysine (%)	1.30	1.30	1.30
Methionine (%)	0.75	0.75	0.75
Calcium (%)	0.80	0.80	0.80
Available phosphorus (P) (%)	0.30	0.30	0.30
Analyzed values (%)			
Moisture (%)	9.78	9.97	9.29
Metabolizable energy (kcal/kg)	2,975	2,982	2,897
Crude protein (%)	19.91	21.92	23.96
Crude fat (%)	5.13	5.10	5.11
Ash (%)	4.86	4.64	4.78

[1] The composition of vitamin-mineral premix per kg of diet: vitamin A: 8000 IU; vitamin D_3: 1600 IU; vitamin E: 32 IU; vitamin B_{12}: 0.02 mg; vitamin K: 3 mg; vitamin B_2: 8 mg; vitamin B_6: 4 mg; vitamin B_3: 60 mg; Folic acid: 2 mg; Calcium-pantothenic acid: 20 mg; $ZnSO_4$: 118 mg; $MnSO_4.H_2O$: 116 mg; $CuSO_4$: 33 mg; $FeSO_4.H_2O$: 60 mg; and Na_2SeO_3: 0.8 mg.

[2] Calculated values presented as %, as-fed basis (AOAC, 2000).

The protocols followed the manufacturer guidelines (Antrim, United Kingdom). Each sample was run in triplicate under the same conditions to avoid assay variation.

Relative carcass weights

The quails (5 birds from each replicate) that were used for blood collection were weighed and sacrificed by cervical dislocation. The quails were cut longitudinally, and the intestinal organs were carefully removed. Eviscerated carcass, leg muscle, breast, and liver were collected and weighed for later calculation of relative organ weights.

Statistical analysis

Data were analyzed by one-way (experiment I) and two-way ANOVA (experiment II). In Experiment II, the main effects of CP level and stocking density, and their interaction, were determined by the general linear model procedure of SAS (2004).

Each cage was defined as the experimental unit for growth performance (experiments I and II) and nutrient retention, whereas in the analyses of nutrient retention, blood profiles, and carcass traits the quails were used as the experimental unit (experiment II). Significant differences were determined at $P < 0.05$.

Significant differences among treatments were separated by Duncan's new multiple range test in both experiments, whereas the tendency of quail performance was determined in experiment I by detecting at the probability range from $P > 0.05$ to $P < 0.10$.

RESULTS AND DISCUSSION

Experiment I

Growth performance

The cumulative growth performance of the quails is presented in Table 2. The results show that final BW and BWG were reduced in the quails at the density of 61.73 birds/m^2 (25 quails/m^2) compared to those at the density of 32.10 (13 quails/m^2) and 41.98 birds/m^2 (17 quails/m^2) ($P<0.05$). Furthermore, lower BW ($P=0.008$), FI ($P=0.33$), and BWG ($P=0.012$) were observed as the stocking density increased. These results are consistent with those of previous studies (Camci *et al.* 2004; Attia *et al.* 2012; El-Tarabany, 2016) that showed reductions in the growth performance and FI in quails at high stocking densities. It has been reported that high stocking density limit the movement of the birds to a confine area within the pen (Cengiz *et al.* 2015).

Table 2 Effects of different stocking densities on growth performance of growing-meat quails from 21 to 42 days of age

Item	Stocking density (quails/cage)				SEM	P-value	
	13	17	21	25		Linear	Quadratic
Initial BW (g/bird)[1]	72.36	71.30	72.36	72.33	0.045	0.131	0.234
Final BW (g/bird)	156.42^{a}	153.84^{a}	150.83^{ab}	139.92^{b}	2.153	0.008	0.091
FI (g/bird)	387	349	333	330	11.385	0.033	0.259
BWG (g/bird)	84.06^{a}	82.54^{a}	78.47^{ab}	67.59^{b}	2.267	0.012	0.151
FCR (feed: gain)	4.61	4.23	4.24	4.90	0.174	0.583	0.637

[1] A total of 380 male growing-meat quails (n=5 replicates per treatment) with the average initial BW of 72.09 g.
BW: body weight; FI: feed intake; BWG: body weight gain and FCR: feed conversion ratio.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

It might be difficult for the birds to easier access to feeders and drinkers than those reared under low stocking density. Furthermore, growth retardation of the birds under high stocking density may decrease in gaseous and heat exchange within the pen (Banhazi *et al.* 2008), resulting in lower BW, FI, and BWG. Our study indicated that an adequate stocking density for growing-meat quails should not exceed 51.85 birds/m^2 for minimizing production loss during high ambient temperature. However, the results of FCR and mortality (not detected; data not shown) were inconsistent with a previous study (Attia *et al.* 2012), possibly because the experimental period was too short so that any detrimental effect was minimal. Hence, further studies are needed to elucidate this aspect.

Experiment II

Growth performance and feed conversion ratio

There was no effect of CP level and stocking density on final BW, BWG, FI, and FCR (Table 3). Recently, a published report showed that the growing Japanese quails fed diet varying from 20 to 26% CP had no significant effect on growth performance during the rearing period (Omidiwura *et al.* 2016).

This observation was in agreement with (Whyte *et al.* 2000), who demonstrated that growing-meat quails were able to physiologically adapt when offered lower nitrogenous diets.

Our research also observed that a lower CP diet was suitable during the growing period of the quails raised in the high stocking density and under high ambient temperature. However, a previous report revealed that the growth performance of the quails significantly improved when the CP level was higher than 26% compared to that at 22 and 24% (Jahanian and Edriss, 2015). These different results might be due to genetics, environmental condition, quail age, and diet composition.

Nutrient retention

The effect of CP, stocking density, and their interaction resulted in no significant differences in dry matter, ash, and crude fat digestibility (Table 4).

These findings were partly in contrast with those described by Omidiwura *et al.* (2016), who found that growing-meat quails fed diets containing 24% CP significantly improved ash digestibility. This contradiction may be due to difference in diet composition, especially in the metabolizable energy content at 3100 kcal/kg (Omidiwura *et al.* 2016). However, the inclusion level of 20% CP improved CP digestibility more efficiently than 24% CP. This indicates that the growing quails were able to utilize a diet low in CP without any detrimental effect on their growth performance. The result was consistent with a lower production of uric acid observed in this study. However, some studies demonstrated that a level of dietary CP ranging from 24 to 25% positively influenced the digestibility of CP in meat-type quails (Dowarah and Sethi, 2014; Omidiwura *et al.* 2016). The inconsistent results could be explained by the different experimental diets, quail breeds, and collecting fecal period. This result may be of interest for the quail producers wanting to reduce production costs using a suboptimal dietary CP level for growing-meat quails under high stocking density conditions.

Blood profiles

No interaction effects of dietary treatment on glucose, AST, and uric acid concentrations were observed in this study (Table 5). AST is used as an effective biomarker to identify tissue damages, especially in the liver. In this study, a reduction of CP up to 20% and high stocking density and high ambient temperature had no effect on AST activity, indicating that treatments were not harmful to the liver. However, the main effect of stocking density was elevated glucose concentration (P=0.099), suggesting that raising the quails at a high stocking density activated glucose metabolism. An increasing level of glucose is used as an indicator of glucose availability, which activates metabolic function in the liver (Eiler, 2004). This effect modulates glucose uptake to supply nutrients for quails, which can further use glucose for their metabolism. However, some studies have showed that the increased level of glucose induced the secretion of hormonal stress via the activation of the hypothalamic-pituitary-adrenal axis (Siegel, 1980; Li *et al.* 2009).

Table 3 Effects of crude protein level and stocking density on growth performance of growing-meat quails from 21 to 42 days of age

Experimental treatments		**Growth perfomance[1]**			
		Final BW (g)	BW gain (g/bird)	FI (g/bird)	FCR
Crude protein (%)	Stocking density				
20	Low	159.05	84.49	375	4.45
	High	160.00	85.16	375	4.40
22	Low	157.86	83.43	406	4.87
	High	157.89	81.91	372	4.54
24	Low	152.62	79.79	388	4.86
	High	161.05	84.68	371	4.38
SEM		1.340	62.505	6.596	1.242
Crude protein (%)					
20		159.52	84.83	375	4.43
22		157.88	82.67	389	4.71
24		156.84	82.23	379	4.61
Stocking density					
Low		82.57	82.57	390	4.73
High		83.92	83.92	372	4.43
Probability (P-value)					
Crude protein		0.769	0.663	0.819	0.693
Stocking density		0.325	0.595	0.379	0.316
Crude protein × stocking density		0.488	0.574	0.756	0.819

[1] A total of 600 male growing-meat quails (n=5 replicates per treatment) with the average initial BW of 73.58 g.
BW: body weight; FI: feed intake and FCR: feed conversion ratio.
SEM: standard error of the means.

Table 4 Effects of crude protein level and stocking density on nutrient retention of growing-meat quails

Experimental treatments[1]		**Dry matter (%)**	**Ash (%)**	**Crude protein (%)**	**Crude fat (%)**
Crude protein (%)	Stocking density				
20	Low	55.83	26.95	43.66	61.14
	High	61.79	28.33	50.10	62.84
22	Low	46.94	21.69	41.06	62.13
	High	51.91	27.41	44.23	64.79
24	Low	61.58	23.66	31.01	65.92
	High	52.86	29.62	36.52	65.05
SEM		2.119	0.856	1.731	1.662
Crude protein (%)					
20		58.81	27.64	46.88^{a}	61.99
22		49.23	24.55	42.64^{ab}	63.46
24		57.22	26.64	33.77^{b}	65.49
Stocking density					
Low		54.78	24.56	38.58	64.23
High		55.52	26.99	43.62	63.06
Probability (P-value)					
Crude protein		0.500	0.728	0.055	0.821
Stocking density		0.915	0.307	0.221	0.803
Crude protein × stocking density		0.623	0.581	0.938	0.948

[1] A total of 600 male growing-meat quails (n=5 replicates per treatment) with the average initial body weight of 74.29 g.
Low: low stocking density (17 quails/cage, 41.98 quails/m^2) and High= high stocking density (23 quails/cage, 56.79 quails/m^2).
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Our results showed that the main effect of dietary CP level at 20% was to significantly lower uric acid concentration compared to high inclusion of dietary CP at 24% (P=0.037). In poultry, uric acid originating from the catabolism of amino acids is an effective marker to identify protein turnover in the tissue (Hernández *et al.* 2012).

The imbalance or excess amino acid is converted into C-skeleton and nitrogen excretion (Kriseldi *et al.* 2018). In our study, the decrease in uric acid concentration may have been affected by the reduction of dietary CP content. This reduced uric acid concentration in the blood as well as in the excreta as confirmed by the CP digestibility.

Table 5 Effects of crude protein level and stocking density on blood metabolites of growing-meat quails

Experimental treatments[1]		Glucose (mg/dL)	Aspartate aminotransferase (mg/dL)	Uric acid (mg/dL)
Crude protein (%)	Stocking density			
20	Low	241.67	401.00	4.40
	High	283.50	439.25	4.35
22	Low	265.75	377.50	5.55
	High	264.17	494.67	5.97
24	Low	264.83	643.33	6.49
	High	275.75	611.25	7.04
SEM		3.342	62.505	0.284
Crude protein (%)				
20		262.58	420.13	4.37^{b}
22		264.96	636.08	5.76^{ab}
24		270.29	627.29	6.77^{a}
Stocking density				
Low		257.42	473.94	5.48
High		274.47	648.39	5.78
Probability (P-value)				
Crude protein		0.790	0.619	0.037
Stocking density		0.099	0.402	0.638
Crude protein × stocking density		0.199	0.497	0.923

[1] Values are represented as the means of 10 growing-meat quails in each treatment.
Low: low stocking density (17 quails/cage, 41.98 quails/m^2) and High= high stocking density (23 quails/cage, 56.79 quails/m^2).
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 6 Effects of crude protein level and stocking density on carcass traits of growing-meat quails

Experimental treatments[1]		Eviscerated carcass (%)	Leg muscle (%)	Breast (%)	Liver (%)
Crude protein (%)	Stocking density				
20	Low	64.92	14.90	29.54	1.59
	High	71.39	15.08	24.44	1.67
22	Low	71.52	15.13	21.01	1.34
	High	71.74	14.99	27.19	1.66
24	Low	71.43	14.69	25.07	1.31
	High	70.09	16.19	26.14	1.32
SEM		1.142	0.116	0.249	0.045
Crude protein (%)					
20		68.15	14.99	26.89	1.63
22		71.63	15.06	24.10	1.50
24		70.76	15.44	25.61	1.31
Stocking density					
Low		69.29	14.91	23.51^{b}	1.41
High		71.07	15.42	27.56^{a}	1.54
Probability (P-value)					
Crude protein		0.567	0.589	0.491	0.121
Stocking density		0.524	0.209	0.055	0.267
Crude protein × stocking density		0.484	0.224	0.524	0.516

[1] Values are represented as the means of 25 growing-meat quails in each treatment.
Low: low stocking density (17 quails/cage, 41.98 quails/m^2) and High= high stocking density (23 quails/cage, 56.79 quails/m^2).
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Relative carcass weights

The relative weights of eviscerated carcass, leg muscle, and liver were not significantly influenced by CP inclusion, stocking density, and their interaction (P>0.05; Table 6). This is consistent with the findings of researchers who have revealed that the relative carcass weight of broiler chickens was not influenced by stocking density (Tong *et al.* 2012; Vargas-Rodríguez *et al.* 2013).

Analyzing the main effect of CP level on the relative weights of eviscerated carcass and leg muscle, no difference was observed. This result was in contrast to that described by Jahanian and Edriss (2015) who demonstrated that lighter weights of eviscerated carcass and leg muscle were found in quails fed 22% CP than those fed 24% CP. The inconsistent results may be due to no significant improvements in growth performance and FI.

Additionally, our study showed the improvement in the percentage of breast muscle in the quails raised at high stocking density, in contrast to a previous work (Jahanian and Edriss, 2015). The difference may be associated with the short experimental period, which did not observe the negative impact of stocking density on carcass composition. A recent review showed that external stressors such as stocking density potentially induces the hypothalamic-pituitary-adrenal axis (Mormede *et al*. 2007), which further releases hormonal stress and subsequently decreases the percentage of breast muscle. Therefore, it is important to study the acute and chronic stress parameters in meat-type quails.

CONCLUSION

Growth performance of growing-meat quails increased at the stocking density of 32.10 to 51.85 birds/m2. Furthermore, the inclusion of 20% CP enhanced growth performance, protein utilization, and CP digestibility.

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