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Original Article

Growth Performance, Tibia Characteristics, Immune Response and Blood Metabolites of Broiler Chickens Fed Diets Containing Citrus Brown Snail (*Caucasotachea Lencoranea*) Powder as a Source of Calcium

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ABSTRACT

Objective: An experiment was conducted to investigate the effects of citrus brown snail powder as a source of calcium on growth performance, tibia characteristics, immune response and blood metabolites in broiler chickens. **Methods:** One hundred and eighty 1-day old chicks were randomly distributed into four treatments, each of which had three replicates. Each replicate contains 15 chicks. The treatments were included four inclusion rates of citrus brown snail powder (0, 0.5, 1 and 1.5%) in diets. **Results:** The results showed that diets supplemented with 2% snail powder improved feed conversion ratio of broilers at 42 days of age (P <0.05). Relative weights of organs, except for liver and digestive tract, were not affected by dietary treatments. The dietary treatments had a significant effect on blood triglyceride, alanine aminotransferase (ALT) and lymphocyte of broilers (P <0.05). The others blood metabolites such as calcium, phosphorous, total protein, glucose, cholesterol, aspartate aminotransferase (AST), hemoglobin and heterophil to lymphocyte ratio were not influenced by dietary treatments. The results of this experiment showed that use of citrus brown snail powder in diets had no significant effect on the immune response (antibody titer) against Newcastle (NDV) and infectious bronchitis (IBV) disease viruses in broilers. Tibia calcium and phosphorous content was increased in broilers fed diets supplementation with citrus brown snail powder (P <0.05). It was concluded that the addition of citrus brown snail powder to diet can improved growth performance and tibia characteristics in broilers.

1.INTRODUCTION

Calcium is the mineral with the highest concentration in the body of poultry, consisting of 1.5% of its body weight (Pelicia et al 2011). Calcium plays two important physiological roles in the poultry. First, it provides the structural strength of the poultry skeleton by the formation of calcium salts. Second, it plays vital roles in many of the biochemical reactions within the body via its concentration in the extracellular fluid. Many studies have been conducted to investigate the effects of calcium and phosphorous levels in diets on poultry tibia bone abnormalities (Pelicia et al 2011, Onyango et al, 2003 and Walk et al, 2012). Abnormalities of calcium metabolism

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are common in the poultry industry, leading to poor production and tibia dyschondroplasia in broiler chickens. Calcium requirements of broiler chickens may be supplied by different sources such as limestone, calcium sulfate, oyster shell, bone meal and eggshells. Limestone and oyster shell are used extensively in broiler diets in Iran. However, there are some biological sources of calcium which may be used in broiler diets. Citrus brown snail (*Caucasotachea lencoranea*) is one of these biological sources of calcium that are found in north part of Iran, extensively. It is considered as the main pest of the citrus orchards in Iran. The shell of citrus brown snail reaches about 32 mm in diameter and has 5 whorls, with dark brown 5 spiral tapes (Kherodin, et al 2012) which may be a suitable source of calcium for broiler chickens. Investigations has been done on the use of many sources of calcium such as limestone and oyster shell in layers and broilers diets. In addition, some researchers have been studied the utilization of giant snails (*Archachatina achatina* and *Achatina fulica*) as a source of protein and calcium in poultry diets (Ahaotu, et al 2013, Diomande et al, 2008). But there is no information on the use of citrus brown snail (*Caucasotachea lencoranea*) powder in poultry feeding. Ogunsola (2011, thesis) reported that the inclusion of snail shells as a replacement for oyster shell in diets for broiler chickens improved feed conversion ratio, carcass yield and bone development. In this regard, Diamonde et al (2008) investigated the effects of snail (*Achatina fulica*) meal on broilers production and meat sensorial quality, who found that use of snail (*Achatina fulica*) meal as a replacement for fish meal had no negative effect on broiler performance and meat quality.

There is no report about the effects of citrus brown snail (*Caucasotachea lencoranea*) powder on broiler performance. So the aim of this study was to investigate the effects of citrus brown snail powder as a source of calcium on growth performance, blood metabolites, tibia characteristics and immune response of broiler chickens.

2. MATERIALS AND METHODS

2.1. Citrus brown snail powder preparation

Citrus brown snails were collected from a local citrus garden in Mazandaran province (north part of Iran). The fresh snails were washed and boiled for 15 minutes. Then, snails were oven dried at 60 °C for 24 hours and ground to powder by a hammer mill. Duplicate samples of citrus brown snail powder were analyzed for chemical composition in our animal nutrition laboratory. Briefly, the samples were oven dried and chemical composition of samples for dry matter, crude protein, ether extract, calcium, total phosphorous, total ash and sodium were determined by association of official analytical chemist's methods (AOAC, 2000). The citrus brown snail powder used in this experiment had 92% dry matter, 12.5% crude protein, 1% ether extract, 31.7% calcium, 0.15% phosphorous and 0.54% sodium.

2.2. Birds and Treatments

One hundred and eighty 1 day-old Ross 308 broiler chicks (Mixed sex) were obtained from a local hatchery. Birds were weighed and randomly assigned to 12 straw/wood shavings mix floor pens with 15 birds per pen. The chicks were raised on floor pens for 42d and had free access to feed and water during the whole period of experiment. The ambient temperature was maintained at 32 °C for the first 3d and then gradually decreased until 24 °C was reached by 21d. Experimental procedures were approved by department of animal science, Islamic Azad University, Qaemshahr branch.

Birds were fed with one of four dietary treatments consisted of a control corn-soybean meal diet and three diets incorporated with 0.5, 1 and 1.5 % of citrus brown snail powder as a source of calcium. Dietary treatments were formulated with equal nutrients to energy ratio to meet or exceed the minimum ROSS broiler chickens requirements (2009) for starter (1-14d), grower (15-25d) and finisher (26-42d) periods. Compositions of the experimental diets are shown in Table 1.

2.3. Sample Collection and Procedures

Feed intake and body weight gain of each pen was measured at the end of each week. Feed conversion ratio (FCR) for each pen was calculated by dividing feed intake by body weight gain. No mortality was observed in this experiment. The birds were vaccinated against Newcastle (NDV) and infectious bronchitis (IBV) disease viruses at days 8 and 12 of age, respectively. Serum samples from two birds per pen were taken at day 10 after NDV and IBV inoculations to measure the antibody response. The hemagglutination inhibition test was set up to determine the antibody production against NDV. The antibody titer against IBV was measured according to Kidd et al., (2001). In addition, two birds per replicate were bled through the wing vein at 35 d of age to determine the blood biochemical metabolites, cell blood count (CBC) parameters and activities of serum alanine and aspartate aminotransferases (ALT and AST) as the indicators of

liver health. A part of blood sample (2 mL) was drawn and allowed to clot at room temperature (18 ºC) for 2 h prior to serum collection. Serum was separated by centrifugation and stored at -20 ºC for further analysis. These sera samples were used to measure blood biochemical metabolites and enzymes activities. The remaining part of blood sample (2 mL) was collected in tubes containing EDTA, and then analyzed for cell blood count (CBC) parameters. At the end of the experiment (on day 42), after overnight fasting , two birds from each pen with a body weight close to pen mean were selected and killed by cervical dislocation. Viscera were manually removed and carcass characteristics (carcass yield, breast, thigh and internal organs) determined. Then, weight of liver (without gallbladder), pancreas, heart and spleen were measured. All carcass data are presented based on percent of live weight of each bird.The left tibia of each slaughtered bird was taken to measure the tibia characteristics and minerals (Calcium and Phosphorous) concentrations. Briefly, Tibiae were defleshed, and cartilaginous caps were removed immediately after collection. Then, tibiae bones were weighed and their length and diameter were measured using a caliper. The weight/length index (WLI) was also calculated by dividing bone weight (in mg) by its length (in mm). In order to determine the minerals concentrations, the tibiae were kept frozen in plastic bags at −20°C to maintain wetness until analysis. Then, frozen tibiae were thawed by leaving them in plastic bags at room temperature for 1 h and oven-dried at 105°C for 12 h. Subsequently, Samples were then grinded in a mill, weighed and put in a muffle furnace overnight at 550° C for 3 h. The ash was used to quantify calcium and phosphorous amount in the tibia. The calcium and phosphorous contents (% of ash) were measured by atomic absorption and spectrophotometer methods, respectively.

2.4. Statistical analysis

Statistical analysis was carried out to evaluate the effects of treatments on performance, tibia characteristics, blood metabolites and immune response of broilers using SAS (2001). Statistical significance of differences among treatments was done using the Duncan's multiple range test at ($P < 0.05$).

3. RESULTS

Table 2 displays the effects of citrus brown snail powder on broiler performances. During all phases of the experiment, diets supplemented with 0 and 0.5% citrus brown snail powder could improve the growth performance of broilers, and significant differences ($P<0.05$) were observed among treatments except FCR for 26-42 d. The greatest weight gain and feed intake were observed in broilers fed 0.5% citrus brown snail powder and the best FCR was belong to this group during the whole growth period of 42 days of age. The results of effects of citrus brown snail powder on the relative weights of carcass parts and internal organs are given in Table 3. The results of our present study showed that the relative weights of carcass parts including thigh and breast were not influenced by dietary treatments. In addition, no differences were observed for the relative weights of internal organs heart, pancreas and spleen, except for liver. The relative weight of liver increased linearly with increasing dietary citrus brown snail powder levels in broilers diets.

The data of blood metabolites and hepatic enzymes activities in broiler chickens are presented in Table 4. The results of blood metabolites indicated that blood triglycerides were influenced by dietary treatments ($P<0.05$), whereas no significant differences were observed for blood glucose, cholesterol, LDL, HDL, calcium, phosphorous and protein in broilers. In addition, the results of our present study showed that dietary citrus brown snail powder had no significant effects on activities of ALT and AST enzymes as indicators of liver health. Effects of dietary citrus brown snail powder on cell blood count (CBC) parameters and immune response against Newcastle disease (NDV) are presented in Table 5. The results showed that lymphocyte and MCH indices were influenced by dietary treatments ($P<0.05$). However, no significant differences were observed for the other CBC parameters including heterophil, H/L ratio, Hb and MCV. The greatest values for lymphocyte and MCH were belonged to diets with 0.5 and 1% citrus brown snail powder, respectively. Our results indicated that adding citrus brown snail powder to broiler diets did not alter antibody production against Newcastle (NDV) and IBV diseases. The results of effects of dietary citrus brown snail powder on tibia mineralization are shown in Table 6. Calcium and phosphorous content of tibia were influenced by dietary treatments in broilers ($P<0.05$). Calcium content of tibia increased linearly with increasing of dietary citrus brown snail powder. The greatest phosphorous tibia concentration was observed in broilers fed diet with 1% citrus brown snail powder supplement.

4. DISCUSSION

The results of present study showed that adding citrus brown snail powder improved growth performance of broiler chickens. Ahaotu et al., (2012) reported that dried giant snail (*Archachatina achatina*) meal up to 2% in diets of broilers did not have adverse effects on growth performance. In this regard, it is reported that meals from snails (*Achatina fulica*) can be included in diets for

broilers at up to 3% replacing fish meal (starter phase) and at 10% in the grower phase replacing fish meal completely, with no reduction in growth performance (Diomande et al., 2008). Similar nutritive values for snail meat and fish meal in pig diets were reported by Kaensombath Lampheuy and Ogle (2005). These authors replaced fish meal with ensiled or fresh meat from Golden Apple Snails in the diets of growing - finishing pigs and reported no differences in growth rate. The previous studies investigate the effects of snail meal as a source of protein in broiler diets, predominantly. Anyway, they did not state that the positive response of growth performance was due to the effects of calcium or protein in the diets. Broilers fed diets with high amount of citrus brown snail powder (1 and 1.5%) had lower growth rate in our present study. It is reported that low availability of nutrients in snail meal decreased growth performance of broiler chickens (Maurice *et al*., 1984), due to the negative effects of the higher levels of calcium and ash on nutrients and ME utilization (Carre, 1990). However, all diets in our experiment had similar calcium content and it does not seem that calcium content of diets with high inclusion rate of citrus brown snail powder decline growth performance of broilers. Our present results showed that the relative weight of liver increased linearly with increase of citrus brown snail powder in broiler diets. Arockiam et al. (1992) indicated that serum GPT levels was higher in broilers fed diets supplemented with 6% snail meal, which may reflect toxic effects of snail meal on liver. These results are in accordance with our findings for relative weight of liver. In contrast, Creswell and Kompiang (1981) reported that an inhibitor was present in the raw snails that depressed growth rate of broiler chickens. However, these researchers mentioned that boiling the snails for 15-20 min completely overcame the negative impact. We could not to find information about toxic effects of citrus brown snail in animal health. So, it is suggested that more investigations should be conducted on chemical composition of citrus brown snail and its effects on animal production and health.

Liver as a main organ in avian metabolism is sensitive to nutritional modifications and activities of ALT and AST in serum are usually considered as an important index for understanding the liver health. It is cleared that when liver works healthy, the activity of these two enzymes in serum will reduce (Corduk et al, 2007). Our present results indicated that adding high levels of citrus brown snail powder to broiler diets reduced ALT significantly. In addition, the serum AST concentration was decreased numerically. So, it is suggested that citrus brown snail powder may be have chemical compositions which affect function of liver cells. In this regard, some researchers indicated that snails have capability for absorption of heavy metal such as lead, chromium and copper from their life environment (Ikhuoria and Uymmadu, 2001; Cui et al., 2012).

The effects of citrus brown snail powder on antibody titer against Newcastle (NDV), infectious bronchitis (IBV) disease viruses and H/L ratio were not significant. In literature review, we did not find data on animal immune response to dietary snail meal or powder. So, direct comparisons were not made.

All of the tibia characteristics were not influenced by citrus brown snail powder in broiler chickens. However, Calcium and phosphorous content of broiler tibia were increased with increase of dietary citrus brown snail powder in diets. There are several factors that affected calcium bioavailability such as physiological and dietary factors. The dietary factors help to achieve an appropriate status of calcium for a correct bone mineralization (Camara et al., 2002). In addition, Calcium absorption is related to several factors such as vitamin D, protein, phosphates and free fatty acids levels (Pelicia et al., 2011). Some studies have been conducted to evaluate the effects of calcium source on tibia mineralization of broilers (Ajakaiye et al., 2003; Pelicia et al., 2011). Calcium availability in dietary calcium sources present great variability as a function of their chemical composition and their capacity to physical bind to other dietary components, forming compounds that present low solubility and availability. According to the present results, it is suggested that the calcium content of citrus brown snail powder has a good availability to absorption for broiler chickens.

CONCLUSION

It was concluded that the addition of citrus brown snail powder to diet can improved growth performance and tibia characteristics in broilers, although its effects on immune response, carcass traits and some blood metabolites was negligible.

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Table 1.

the ingredients and chemical composition of experimental diets

Ingredients	**Basal diets**		
	Starter	Grower	Finisher
Corn grain	60.38	61.78	63.36
Soybean meal	34.25	32.30	28.90
Soybean oil	0.50	1.76	3.80
DCP	1.83	1.63	1.49
Oyster shell	1.37	1.24	1.25
Common salt	0.45	0.41	0.37
Mineral-premix	0.25	0.25	0.25
Vitamin-premix	0.25	0.25	0.25
DL-Methionine	0.34	0.25	0.23
L-Lysine	0.28	0.11	0.08
L-threonine	0.10	0.02	0.02
Chemical composition			
AME (kcal/kg)	2920	3020	3150
CP (%)	22.20	21.16	19.60
Calcium (%)	1.01	0.92	0.88
Available phosphorous (%)	0.48	0.44	0.41
Sodium (%)	0.20	0.19	0.17
TSAA (%)	1.03	0.93	0.85
Lysine (%)	1.38	1.19	1.07
Threonine (%)	0.91	0.80	0.74

Table 2.

effects of dietary treatments on broiler growth performance (gr/pen)

	Citrus brown snail powder levels (%)				
Growth performance	**0**	**0.5**	**1**	**1.5**	SEM
1 – 14 d					
Weight gain	287.1a	299.8a	266.3b	225.2c	5.9
Feed intake	486.5a	503.0a	442.1bc	418.4c	14.6
FCR	1.69ab	1.68b	1.66b	1.85a	0.05
15 -25 d					
Weight gain	398.4a	405.7a	343.4b	319.1b	9.0
Feed intake	592.5ab	559.7a	584.1a	516.2b	15.7
FCR	1.48bc	1.38c	1.70a	1.62ab	0.05
26 – 42 d					
Weight gain	379.3a	410.5a	350.2a	261.6b	18.8
Feed intake	973.9ab	1031.5a	909.7bc	745.3c	29.9
FCR	2.57	2.53	2.61	2.86	0.15
1 42 d					
Wight gain	354.9a	372.0a	320.2b	268.6c	7.8
Feed intake	684.3a	698.1a	645.3b	559.9c	15.7
FCR	1.99b	1.86b	2.00ab	2.11a	0.05

Table 3.

effects of dietary treatments on carcass characteristics of broilers (% of live weight)

	Citrus brown snail powder levels (%)				
Carcass traits	**0**	**0.5**	**1**	**1.5**	SEM
Thigh	19.32	19.18	20.41	18.52	0.59
Breast	18.41	16.89	14.98	15.57	1.13
Liver	2.22b	2.32b	2.60ab	2.87a	0.14
Heart	0.74	0.68	0.87	0.90	0.09
Pancreas	0.33	0.21	0.27	0.27	0.03
Spleen	0.10	0.11	0.08	0.10	0.02

Table 4.

effects of dietary treatments on blood metabolites and liver enzymes activities of broiler chickens

	Citrus brown snail powder levels (%)				
Blood metabolites	**0**	**0.5**	**1**	**1.5**	SEM
Glucose	232.0	245.1	239.3	239.6	9.8
Triglycerides	46.6ab	66.3a	42.4b	45.0ab	6.3
LDL	20.1	19.1	19.5	20.4	2.3
HDL	85.3	79.2	64.1	82.0	10.3
Calcium	10.4	10.7	10.2	11.1	0.56
Phosphorous	3.81	4.36	2.85	3.06	0.66
Protein	4.13	4.14	4.80	4.63	0.35
Enzymes activities					
ALT	4.16ab	4.50a	4.17ab	4.00b	0.12
AST	305.6	255.6	254.7	260.0	15.7

Table 5.

effects of dietary treatments on cell blood count (CBC) parameters (%) and immune response against Newcastle (NDV) disease

	Citrus brown snail powder levels (%)				
CBC parameters	**0**	**0.5**	**1**	**1.5**	SEM
Heterophil	25.66	27.01	24.65	26.66	0.82
Lymphocyte	70.01ab	69.11ab	71.12a	67.18b	1.00
H/L	0.36	0.39	0.35	0.40	0.02
Hb	9.35	9.45	9.44	10.49	0.65
MCV	145.2	139.9	159.1	150.9	9.7
MCH	41.5ab	39.5b	49.19a	45.7ab	2.46
Antibody titer					
NDV (log_2)	3.44	3.25	3.18	3.34	0.33
IBV (log_{10})	2.55	2.52	2.61	2.59	0.24

Table 6.

effects of dietary treatments on tibia characteristics of broiler chickens

	Citrus brown snail powder levels (%)				
Minerals	**0**	**0.5**	**1**	**1.5**	SEM
Calcium (% of ash)	26.71b	27.34b	31.60a	33.65a	0.83
Phosphorous (% of ash)	16.69b	17.43ab	18.00a	17.98a	0.28
Tibia length (mm)	68.11	68.21	68.16	68.22	1.51
Tibia diameter (mm)	5.76	5.59	5.65	5.61	0.18
Weight (gr)	4.79	4.81	4.77	4.82	0.13
WLI(mg/mm)	70.31	70.50	69.97	70.64	1.62