INTERACTION OF CALCIUM, PHOSPHORUS AND PROTEIN IN BROILERS

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Summary

In order to study the interrelationships of calcium (0.45 vs 0.90%), phosphorus (0.40 vs. 0.70%) and protein (17, 20, 23%), 2 × 2 × 3 factorial design was employed. A total of 480 broilers (Hisex-Hibro) aged 3 days were fed the expermental diets for a period of 28 days. Body weight gain, daily feed intake and feed efficiency were investigated for the simple effects, first order interaction and second order interaction of the dietary factors. These effects were also applied to bone ash retention, percent Ca in bone & ash, percent P in bone & ash, and protein utilizability. Results were as follows. 1) For body weight gain, simple effects of dietary levels of Ca, P, CP were found to be significant ($p < 0.05$). Body weight gain at 0.90% Ca level was improved as the dietary CP levels increased. For the feed intake, single effects of dietary levels of both P and CP were found ($p < 0.05$). Feed efficiency was improved as the dietary **CP** and P levels increased. Ca × P interaction was found to be significant for body weight gain, feed intake and feed efficiency ($p < 0.05$), however, Ca × P × CP interaction effect was not found. 2) Protein utilizability decreased as the dietary CP level increased ($p < 0.01$). 3) 0.90% Ca in diet showed less bone ash retention than 0.45% Ca level. And, increasing the dietary P level resulted in increased bone ash retention. Increasing the dietary P level resulted in increased bone Ca retention ($p < 0.01$) and increased bone P retention ($p < 0.05$). Dietary CP levels had significant ($p < 0.01$) effect on bone Ca retention except for 23% CP level. Increasing the dietary Ca level resulted in wider Ca:P ratio of bone, but increasing the dietary P level resulted in narrower Ca:P ratio of bone. 4. Ca × P interaction effects were found to be significant ($p < 0.01$) for bone ash, bone Ca & P, ash P content, and bone Ca:P ratio. Ca × P × CP interaction effects were found for bone ash ($p < 0.01$), bone Ca ($p < 0.05$) and bone P content ($p < 0.01$).

(**Key Words** : Calcium, Phosphorus, Protein, Interaction, Broilers)

Introduction

Studies on calcium, phosphorus and vitamin D in animal nutrition have been conducted by many scientist since early 1920's. Calcium, phosphorus and vitamin D are discussed together because they are closely associated with metabolism, particulary, the formation of bone and satisfactory growth (Scott et al., 1976; Bethke et al., 1928; Hart et al., 1930; Wilgus, 1931; McChesney and Giacomino, 1945; Carver et al., 1946; Migicovsky and Emslie, 1947; Kim and Han, 1982a, b).

The most striking factors which affect calcium and phosphorus nutrition were found to be calcium and phosphorus themselves and vitamin D (Bethke et al., 1923; Nowotarski and Bird, 1943; Itoh and Hanato, 1960; Ohh et al., 1982; Nelson et al., 1965a, b; Fritz et al., 1969; Word and Nelson, 1977; Woodard et al., 1979). Protein was also known to affect calcium metabolism (Fammatre et al., 1977). Many studies on the calcium and protein interrelationships have been conducted (Hawks et al., 1942; McCanc et al., 1942; Reinhard et al., 1976). Also, studies on the phosphorus and protein interrelationships have been conducted by other scientists (Howe and Beecher, 1981; Desikachar and Subrahmanyan, 1949).

In studies with growing and finishing swine, Fammatre et al. (1977) reported significant interaction of protein with calcium was found in feed conversion. And, in studies with young rat, Howe and Beecher (1981) reported that increasing dietary protein at the 0.35% phosphorus level slightly increased phosphorus absorption. However, when 0.8% phosphorus was included, increasing the protein level of the diet decreased the calcium and phosphorus absorption. There was an evidence that the requirement for calcium increased as the levels of energy and protein of the diet were increased (Edwards et al., 1960).

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This study was performed to investigate; 1) calcium and protein interaction, 2) phosphorus and protein interaction, and 3) calcium × phosphorus × protein interaction in broiler broilerss.

Materials and Methods

Experimental design

To investigate the interrelationship of dietary calcium (Ca), phosphorus (P) and protein (CP) in the broilers, an experiment was carried out in 2 × 2 × 3 factorial design where 2 levels of dietary Ca (0.45, 0.90%), 2 levels of dietary P (0.40, 0.70%), and 3 levels of CP (17, 20, 23%) were involved. Treatments were composed of 12 possible combinations of 3 factors with 4 replications for each treatment.

Experimental animal, period and location

Animals used in the present study were 3 days old broilers (Hisex-Hibro strain) purchased from Chunbo Hatchery Co. Feeding trial, chemical analysis of experimental feeds and bones were conducted in the Laboratory of Nutrition, College of Agriculture and Life Science, Seoul National University. Feeding trial lasted for 6 weeks.

Experimental diets

Isocaloric basal diets were formulated to include 17, 20, 23% of CP, respectively (table 1). Dietary Ca and P levels were adjusted with mineral mixture containing limestone, phosphoric acid and sand.

TABLE 1. FORMULA AND CHEMICAL COMPOSITION OF BASAL DIETS CONTAINING DIFFERENT LEVELS OF CRUDE PROTEIN

	Dietary protein (%)		
	17	20	23
Ingredients (%):			
Corn, yellow	72.48	60.69	48.86
Soybean meal	12.18	22.63	33.08
Corn gluten meal	5.00	5.00	5.00
Soybean oil	2.11	4.47	6.85
Fish meal	3.94	2.84	1.75
Limestone, pulverized	0.63	0.67	0.70
Methionine (50%)	0.20	0.26	0.34
Lysine	0.16	0.14	0.12
Vit.-min. mixture[1]	0.40	0.40	0.40
Antibiotics[2]	0.10	0.10	0.10
Salt	0.30	0.30	0.30
Variables[3]	2.50	2.50	2.50
Total	100.00	100.00	100.00
Chemical composition:			
Energy (ME kcal/kg)	3,243	3,209	3,225
Crude protein (%)	16.87	20.23	22.91
Calcium (%)	0.47	0.46	0.46
Phosphorus (%)	0.39	0.41	0.42

[1] Vit.-min. mixture contains following in a kg; vitamine A, 2,000,000 IU; Vitamin D_3, 400,000 IU; Vitamin E, 900 IU; Vitamin K_3, 200 mg; Thiamin, 100 mg; Riboflavin, 2,000 mg; Vitamin B_6, 200 mg; Vitamin B_{12}, 1,500 mg; Pantothenate, 1,500 mg; Niacin, 2,000 mg; Folacin, 60 mg; Choline, 30,000 mg; Iron, 4,000 mg; Copper, 500 mg; Zinc, 9,000 mg; Iodine, 250 mg; Cobalt, 100 mg; Dried yeast, 20,000 mg.

[2] Zinc-bacitracin was used.

[3] Limestone, phosphoric acid and washed sand were mixed to adjust Ca and P levels.

Methods of experiments

Feeding trial; A day old broilers had been fed *ad-libitum* a preliminary diet and tap water until experimental diets were allowed. Three days old broilers were allotted into 48 groups of almost equal average body weights (59 g); each of 48 groups were composed of 10 broilers. All the broilers were fed *ad-libitum* the experimental diets and tap water for a period of 28 days after overnight deprivation of feed, and were raised in steel wire batteries kept in the 24-hours lighted and air ventilated room. Body weight and feed intake were recorded weekly to calculate body weight gain and feed efficiency.

Metabolizability trial; To determine protein utilizability of experimental diets at the end of the feeding trial, a metabolic trial was carried out for 7 days in which the first 4 days were preliminary and the last 3 days were set for excreta collection. Each broiler was individually housed in metabolic cages and then the experimental diet and water were fed *ad-libitum*. Excreta were collected three times a day avoiding the contamination of foreign materials such as feeds, feathers and then pooled and dried in an air-forced drying oven at 65±2℃ for 24 hours to gain constant dried weight. All the excreta prepared in this manner were pooled, ground and analyzed for nitrogen. The protein utilizability were calculated as follows;

Protein utilizability (%)

$$= 100 - \frac{\text{amount of excreta (g)} \times \text{protein content of excreta (\%)}}{\text{feed intake (g)} \times \text{protein content of diet (\%)}} \times 100$$

Bone sample preparation; Four broilers per treatment were sacrificed for bone sample collection. Tibia and femur were removed by soaking in hot water for 3 to 5 minutes in order to facilitate scaling, and dried in the air-forced drying oven for 48 hours at 60℃. Dried bone were ground with Wiley Mill and conserved at freezing temperature for further analysis.

Chemical analysis

Ash, Ca and P contents of bones and the proximate composition of the basal diet were analyzed according to AOAC (1980) methods. Nitrogen content of excreta was undertaken by Kjeldahl method.

Statistical analysis

Analysis of variance for the results obtained from the present study were undertaken by PDP 11/70 computer system in order to evaluate the effects of single factor, two-way interaction, or three-way interaction on the response variables (Steel and torrie, 1960).

Results and Discussion

Body weight gain, feed intake and feed efficiency

Analysis of variances for body weighth gain, feed intake and feed efficiency were presented in table 2. Significant increase in body weight gain was observed as the level of either dietary P or CP was increased. However, broilers received the diet containing 0.90% Ca × 0.70% P showed the highest weight gain whereas broilers fed 0.90% Ca × 0.40% P showed the lowest weight gain. Low P seemed to cause more severe growth retardation than low Ca. There was Ca × P effect ($p < 0.01$) for weight gain, which implied that optimum Ca:P ratio is important for normal growth as pointed out by Woodard et al. (1979).

At 0.45% Ca × 0.40% P or 0.45% Ca × 0.70% P, weight gain was better for 20% CP than 23% CP. From this result, it could be inferred that Ca and P requirements increase as the level of dietary CP increased. There was an evidence that the requirement for Ca increased as the levels of energy and CP of the diet are increased (Edwards et al., 1960). But at 0.90% Ca × 0.40% P or 0.90% Ca × 0.70% P, body weight gain increased as CP increased. Wassermen et al. (1956) reported that dietary CP increased intestinal Ca absorption, and this increase might be due to an interaction of Ca with peptides or amino acids in the intestine (Wassermen et al., 1956). Ca × P × CP interaction for body weight gain was not found to be significant.

Either dietary P or CP had affect on feed intake ($p < 0.05$) On the whole, Ca levels failed to affect feed intake. Broilers received the diets containing 0.45% Ca × 0.40% P showed the highest feed intake. While broilers received the diets containing 0.90% Ca × 0.40% P showed the lowest feed intake. Dietary P levels had an influence on feed intake ($p < 0.05$). These results were in good agreement with those obtained by Nelson et al. (1965a) in which addition of Ca or P increased feed consumption of animals offered insufficient Ca and P. Feed intake tended to decrease as P level increased at 0.45% Ca. While feed intake increased as P level increased at 0.90% Ca. There was a tendency that feed intake decreased at all Ca and P level as CP level increased. Ca × P interaction on feed intake was found to be significant ($p < 0.01$), but Ca × P × CP interaction failed to show significances.

Feed efficiency of the broilers fed on 0.45% Ca was better than that of broilers fed on 0.90% Ca. But feed efficiency tended to improve as P level increased regardless of Ca level ($p < 0.05$). Vandepopuliere et al. (1961) reported that feed efficiency of chicks was improved as the P level was increased from 0.44% to 0.58%. Also, Waldroup et al. (1963) reported that feed utilization was improved with increases in body weight gain. Present results were in good agreement with those reported by Vandepopuliere et al. (1961) and Waldroup et al. (1963). While Nelson et al. (1965a, b) reported that deficient or excessive Ca level depressed feed consumption. Feed efficiency was not affected by dietary Ca levels, but feed efficiency tended to improve as P level increased ($p < 0.05$). Moreover, feed efficiency improved as CP level increased regardless of Ca and P levels ($p < 0.05$).

TABLE 2. BODY WEIGHT GAIN, FEED INTAKE, AND FEED EFFICIENCY OF BROILERS (28 DAYS)[1]

Treatments			Body weight	Feed intake	Feed efficiency
Ca	P	CP	gain (g/broiler)	/broiler (g)	(feed/gain)
0.45	0.40	17	835.25	1,556.48	1.86
		20	895.57	1,489.60	1.66
		23	877.60	1,372.28	1.56
	0.70	17	836.07	1,516.70	1.81
		20	908.30	1,477.43	1.63
		23	879.10	1,361.38	1.55
0.90	0.40	17	686.82	1,325.53	1.93
		20	692.50	1,279.18	1.85
		23	709.90	1,139.35	1.60
	0.70	17	854.27	1,510.28	1.77
		20	891.32	1,462.83	1.64
		23	921.87	1,438.35	1.56
SEM[2]			25.99	38.24	0.04
Analysis of variance:					
Ca			**	**	*
P			**	**	**
Ca × P			**	**	**
CP			**	**	**
Ca × CP			NS	NS	NS
P × CP			NS	NS	NS
Ca × P × CP			NS	NS	NS

[1] Average initial body weight was 59 ± 1.0 g.

[2] Standard error of mean.

* $p < 0.05$, ** $p < 0.01$, NS nonsignificant.

Protein utilizability and bone composition

Analysis of variances for protein utilizability and bone composition were depicted in table 3. Protein utilizability was decreased as the dietary Ca level was increased or as the dietary P level increased. Protein utilizability at both Ca levels was decreased as the dietary P level was increased. But broilers fed 0.90% Ca × 17% CP showed slightly improved protein utilizability as the dietary P was increased. Vippermen et al. (1974) reported that nitrogen retention was more affected by dietary P level than by dietary Ca level, and that increasing dietary Ca level without increasing dietary P level resulted in the decrease of nitrogen retention. Increasing the dietary CP level resulted in decreased protein utilizability ($p < 0.01$). Protein utilizability at the 0.45% Ca × 0.40% P × 17% CP was highest.

TABLE 3. PROTEIN UTILIZABILITY, ASH CONTENT OF BONE, BONE CA CONTENT, CA CONTENT OF BONE ASH, BONE P CONTENT, P CONTENT OF BONE ASH AND BONE CA:P RATIO OF BROILERS[1]

Treatments Ca	 P	 CP	Protein utilizability (%)	Ash content of bone (%)	Bone Ca content (%)	Ca content of bone ash (%)	Bone P content (%)	P content of bone ash (%)	Bone Ca:P ratio
0.45	0.40	17	64.67	39.20	15.72	40.09	6.06	15.46	2.59
		20	57.83	36.93	14.98	40.60	5.54	14.99	2.71
		23	53.64	35.74	14.07	39.36	5.33	14.92	2.65
	0.70	17	61.67	41.94	16.62	39.65	6.01	14.32	2.78
		20	55.02	38.37	14.74	38.41	5.60	14.60	2.63
		23	52.46	36.10	14.13	39.16	5.27	14.61	2.68
0.90	0.40	17	59.96	39.79	15.41	38.74	5.58	14.04	2.76
		20	59.75	33.22	13.71	41.26	4.60	13.83	2.99
		23	57.54	32.37	12.81	39.55	4.41	13.60	2.91
	0.70	17	60.83	40.58	16.16	39.91	5.63	13.88	2.87
		20	54.62	40.92	15.94	38.98	6.18	15.14	2.59
		23	48.31	41.33	16.41	39.69	6.44	15.58	2.55
SEM[1]			1.38	0.96	0.36	0.24	0.18	0.20	0.04
Analysis of variance:									
Ca			NS	NS	NS	NS	NS	**	*
P			**	**	**	NS	**	NS	NS
Ca × P			NS	**	**	NS	**	**	**
CP			**	**	**	NS	**	**	NS
Ca × CP			NS	**	NS	NS	**	NS	NS
P × CP			NS	**	NS	NS	**	*	NS
Ca × P × CP			NS	**	*	NS	**	NS	NS

[1] Standard error of mean.

* $p < 0.05$, ** $p < 0.01$, NS nonsignificant.

Bone ash retention was not affected by dietary Ca level, which was similiar to the reports by Jensen and Marz (1966) and Vandepopuliere et al. (1961) where adding Ca or P alone failed to improve bone calcification. However, dietary P levels affected bone ash retention ($p < 0.01$). Waldroup et al. (1963) reported that percent bone ash retention was significantly increased when the P level of the diet was increased from 0.48% to 0.70%. The highest ash retention was observed at the 0.70% P level. This reveals that increasing dietary P level accelerated bone ash retention rate. Similar trend was found by Formica et al. (1962) who indicated positive correlationship with dietary Ca and P levels at the adequate Ca:P ratio. Increasing P levels increased bone ash content at both high and low Ca level. This suggested that the P level might be more critical than the Ca level. Dietary CP level affected bone ash retention ($p < 0.01$). Bone ash retention decreased as the dietary CP level increased. Ca × P × CP interaction for bone ash retention was not found to be significant ($p > 0.05$).

Ca retention in bone and Ca percentage in ash were not significantly affected by the dietary Ca level, although 0.90% Ca level slightly increased Ca retention in bone and Ca content in ash. Dietary P levels affected bone Ca retention in bone but not P content in ash ($p < 0.01$). Increasing the dietary P level resulted in increased Ca retention in bone, and resulted in decreased Ca percentage in ash. Dietary CP levels affected Ca retention in bone ($p < 0.01$), but not Ca content in ash. Increasing the CP level with either 0.45% Ca level or 0.90% Ca level resulted in decreased Ca retention in bone. Interaction between dietary Ca and P was found to be significant for Ca retention in bone ($p < 0.01$). But Ca content in ash was not found to be influenced by any factors. Interaction of Ca, P and CP levels with the Ca retention in bone was found to be significant ($p < 0.05$), but not with the Ca percentages in ash. At 0.40 and 0.70% P level with 0.45% Ca level, the dietary CP failed to influence Ca retention in bone.

As the dietary P level was increased, the bone P content was increased regardless of Ca levels ($p < 0.05$). While, P percentage in ash was increased as the dietary P level was increased ($p < 0.05$). At the 0.45% Ca, bone P content slightly increased only at the 20% CP level as the dietary P level increased. And ash P percentage was decreased as the dietary P level was increased. At the 0.90% Ca level, bone P content was increased as the dietary P level was increased. Dilworth and Day (1965) reported that increasing the dietary P level resulted in increased P retention of tibia and femur. Increasing the Ca level resulted in decreased bone P content ($p < 0.05$). Dietary CP levels affected bone P retention, and P percentage in ash ($p < 0.05$). Ca × P interaction was found to be highly significant for the bone P retention and the P percentage in ash ($p < 0.01$). Ca × P × CP interaction was found to be significant ($p < 0.01$) for the bone P retention, but not for the ash P percentage.

0.90% Ca widened Ca:P ratio of bone. However, the dietary CP levels did not affect this ratio. The Ca:P ratio was narrowed as the dietary CP level was increased. The widest Ca:P ratio was observed with 20% CP diet at 0.90% Ca. But this ratio was slightly narrowed as the dietary CP level increased. With 0.90% Ca, narrow ratio was observed at the 20 and 23% of dietary CP. This ratio was widened at the 0.40% P level as the dietary Ca level increased. But this ratio was narrowed at 0.70% P levels as the dietary Ca level increased except for the 17% CP diet. The most narrow ratio was found at 0.90% Ca and 0.70% P. These results suggest that the ratio of Ca:P in bone was affected by dietary ratio of Ca:P or modified by dietary CP level. And the present results probably revealed that dietary CP level influenced the rate of P absorption and consequently increases the rate of P retention more than that of Ca retention in bone.

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