ブロイラー飼料へのプロピオン酸添加が中足骨の強度に及ぼす影響

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Effects of Dietary Propionic Acid on Breaking Strength and Calcium Content of the Tarsometatarsal Bone in Broiler Chickens

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Five-day-old broiler chicks of both sexes were fed the conventional diets containing 0, 1, 2, or 3% propionic acid (PA) and water *ad libitum* until 54 days of age, then the tarsometatarsal bone (TMT) was removed after the feeding experiment. The size, fracture and crash strength, crude ash and calcium contents of TMT were measured.

1. In male chicks, no effects of dietary PA on the size of TMT were obtained but the dry weight/fresh weight of TMT was increased. In females, the diameter, dry weight and dry weight/fresh weight of TMT were increased.

2. In both sexes, the elasticity, and fracture and crash strength of TMT were significantly improved by the dietary PA, in which 3% PA-fed birds showed the highest improvement in TMT strength.

3. Crude ash and calcium contents of TMT also showed a positive correlation with increasing dietary PA level in both sexes.

These results suggest that the dietary PA in broilers increased the breaking strength of the bone due to the elevated calcium content in the bone by activated absorption of mineral such as calcium.

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Key words : propionic acid, tarsometatarsal strength, tarsometatarsal Ca

Introduction

It has been known that an ossification of the chicken bone is accomplished by a decrease of the epiphyseal growth plate in the bone due to activated calcification of the hypertrophic cartilage cell zone in the growth plate (REILAND *et al.*, 1978). Recently, the market age of broiler (BR) has been reduced to less than 8 weeks. However, as the bone ossification is incomplete at this age, BR has fragile bones. Consequently, the bone breakage has became an important problem during picking through the pickers in BR.

Feeding program is also thought to be one of the factors to induce such a bone breakage. In mammals, calcium absorption is faster at younger stage than adult (HENRY and KON, 1953), while in chicks of BR an accumulation of minerals such as calcium was reduced by feeding the high energy diets (WHITEHEAD *et al.*, 1971). However, WATANABE and MUTAI (1975) suggested an increased calcium absorption in the intestine showing acidic condition. ISSHIKI (1979) reported an elevated blood

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calcium level in chickens fed the dietary lactic acid bacteria.

Among volatile fatty acids produced in the digestive tract of chickens (WATANABE and CHIBA, 1968), propionic acid (PA) is known to contain high energy and be easily absorbed at the proximal part of the intestine (BOLTON and DEWAR, 1965). Therefore, in this study, effects of dietary propionic acid on size, elasticity, fracture strength, crash strength and calcium contents of the tarsometatarsal bone (TMT) were investigated in BR chickens.

Materials and Methods

One hundred newly-hatched BR chicks, 50 of each sex, were obtained from a

Table 1. Composition of experimental diet (%)

Ingredients	Starter	Finisher
Yellow corn	48.4	48.4
Milo	5.0	15.0
Wheat	3.0	—
Cassava meal	—	5.0
Defatted soybean meal	20.0	11.0
Corn gluten meal	5.0	—
Corn jam	—	1.0
Defatted sesame meal	3.0	—
Defatted rapeseed meal	2.0	3.0
Fish meal	5.0	5.0
Meat and bone meal	4.0	4.0
Feather meal	—	2.0
Tallow	1.0	3.0
Alfalfa meal	1.0	—
Calcium carbonate	1.4	1.4
Calcium phosphate tribasic	0.7	0.7
Sodium chloride	0.2	0.2
Vitamin mixture[1)]	0.25	0.25
Mineral mixture[2)]	0.05	0.05
Salinomycin	7.5×10^{-3}	5.0×10^{-3}
Enramycin	0.5×10^{-3}	0.3×10^{-3}
Chemical composition		
Moisture	11.17	10.38
Crude protein	23.63	19.25
Crude fat	5.59	7.64
Nitrogen-free extract	51.18	54.59
Crude fiber	2.83	2.52
Crude ash (Calcium)	5.60 (1.54)	5.62 (1.58)
ME kcal/kg	3,022	3,139

[1)] 10 g Vitamin A (200,000 IU/g), 7 g Vitamin D_3 (30,000 ICU/g), 1.6 g Thiamine HCl, 8 g Riboflavin, 1.6 g Pyridoxine, 96 g Choline HCl, 1.6 g Nicotinic acid, 3.2 g Ca Panthothenate and 8 g Folic acid per kg, respectively.

[2)] 8.0% Manganese, 5.0% Zinc, 0.6% Iron, 0.1% Iodine and 0.06% Copper.

commercial hatchery and kept in electrically heated battery cages. At 5 days old, 40 chicks showing almost similar body weight were selected from each sex group and divided into 4 groups. Basal diets were conventional starter mash from 5 to 31 days old and finisher mash from 32 to 54 days old. In mammals, calsium absorption rate was increased in the diets containing low calcium level (HENRY and KON, 1953 ; ZEMEL, 1985), but calcium retention was elevated in the diets containing high calcium and phosphate levels (ZEMEL, 1985). Therefore, basal diets were added with high percentage of $CaCO_3$ and $Ca(H_2PO_4)_2 \cdot H_2O$ as shown in Table 1, in which proportions of ingredients and chemical composition of the basal diets are also shown. Experimental diets were prepared by adding 0 (control), 1, 2, or 3% PA to the basal diets and placed in a polyethylene bag, sealed and stored in about 0°C room. The experimental diets and water were provided twice a day from 5 to 54 days old *ad libitum*. Food and water intakes were daily recorded and weekly for body weight. At 54 days old, TMT of each bird was immediately detached after sacrificing and skin, muscle and connective tissue were removed. Then, fresh weight, length, and long and short axes of the TMT were recorded. Bones were ovendried at 60°C for 6 hours, followed by 1 hour at 100°C, cooled and weighed to determine the dry weight. Bones were kept in a room temperature and subjected for measuring the elasticity, and fracture and crash strength using a Maruto testing machine (Maruto Co.). For the elasticity and fracture strength, the midpoint of TMT laid on a 4.7 cm iron ring was pressed with the axle tip of 1 mm diameter and crash strength was determined on the surface of an iron flat plate. From crashed TMT, samples of 2 to 2.5 g were ashed in a muffle furnace at 600°C for 6 hours, cooled and determined ash weight. Ashed samples were digested by boiling in 30 m*l* of 12% HCl solution for 30 minutes. After cooling, samples were added distilled water up to 250 m*l*, filtered and calcium content was analyzed using atomic absorption spectrophotometer (Hitachi 308).

Results and Discussion

Each size of TMT of chicks fed experimental diets is shown in Table 2. In males, fresh weight, length and dry weight of TMT were increased, although not statistically significant, in 2% PA-fed chicks, while long axis was slightly decreased by dietary PA. Thus, a consistent trend between dietary PA and TMT size was not observed except for a tendency of increased dry weight/fresh weight with elevating dietary PA percentage. In females, length of TMT showed no changes among diets but diameter, fresh and dry weights, and dry weight/fresh weight were increased, although not statistically significant, by addition of PA. However, generally, each size of bone has a possibility to be altered by body weight of measured birds. Birds used in this study were the same with the previous paper (IBARDOLAZA *et al.*, 1992) in which mean body weights of each group were as follows : in males, 2.439 kg in 0% PA, 2.411 kg in 1% PA, 2.407 kg in 2% PA and 2.324 kg in 3% PA ; in females, 2.092 kg in 0% PA, 2.200 kg in 1% PA, 2.122 kg in 2% PA and 2.096 kg in 3% PA. Weight of TMT in 3% PA-fed chicks showing the lighest body weight showed the lowest value. However, when this TMT weight was expressed as TMT weight per kg body weight, the obtained value

Table 2. Size of tarsometatarsal bone of broiler chickens fed dietary propionic acid

Sex	PA level (%)	Fresh TMT					Dry TMT (g)	Dry/Fresh (%)
		Weight (g)	Length (mm)	Short axis (mm)	Long axis (mm)	Dia meter* (mm)		
Male	0	16.6±0.5^{a}	99.6±1.0^{ab}	6.6±0.2	12.1±0.4	9.4	8.7±0.3^{ab}	52.4
	1	15.9±0.8^{ab}	96.3±1.5^{b}	6.7±0.1	11.3±0.8	9.0	8.4±0.4^{ab}	52.8
	2	17.8±0.6^{a}	100.6±1.1^{a}	6.6±0.2	12.0±0.2	9.3	9.4±0.4^{a}	52.8
	3	14.5±0.4^{b}	98.9±1.0^{ab}	6.1±0.3	11.7±0.5	8.9	8.0±0.3^{b}	54.2
Female	0	10.1±0.3	89.4±1.0	5.4±0.1	10.2±0.1	7.8	5.8±0.2	57.4
	1	10.6±0.4	90.4±1.2	5.4±0.1	10.4±0.3	7.9	6.2±0.2	58.5
	2	10.4±0.3	90.2±1.2	5.6±0.2	10.6±0.2	8.1	6.1±0.2	58.7
	3	10.6±0.3	89.4±1.3	5.8±0.2	10.6±0.2	8.2	6.2±0.2	58.5
Average	0	13.4±0.6	94.5±1.5	6.0±0.2	11.2±0.2	8.6	7.3±0.3	54.9
	1	13.3±0.6	93.4±0.9	6.0±0.2	10.9±0.4	8.5	7.3±0.3	55.7
	2	14.1±0.7	95.4±1.2	6.1±0.2	11.3±0.2	8.7	7.8±0.3	55.7
	3	12.6±0.4	94.2±1.0	6.0±0.1	11.2±0.2	8.6	7.1±0.2	56.8

Values are means±SE of 8 birds.
* (Short + long axis)/2.
PA, Propionic acid.
Means having different superscripts within a column are significantly different ($P<0.05$).

Table 3. Elasticity and strength of tarsometatarsal bone of broilers fed dietary propionic acid

Sex	PA level (%)	Elasticity		Fracture strength		Crash strength	
		(mm)	(mm/dia meter*)	(kg)	(kg/dia meter*)	(kg)	(kg/dia meter*)
Male	0	2.46±0.19	0.26	32.8±2.5	3.5	19.7±2.1^{b}	2.1
	1	2.46±0.19	0.27	32.6±2.5	3.6	21.7±2.0^{ab}	2.4
	2	2.50±0.17	0.27	33.4±2.2	3.6	22.9±2.4^{ab}	2.5
	3	2.59±0.10	0.29	34.5±1.3	3.9	25.7±2.0^{a}	2.9
Female	0	2.24±0.15	0.29	29.9±2.0	3.8	20.5±1.9^{b}	2.6
	1	2.44±0.23	0.31	32.5±3.4	4.1	20.7±1.6^{b}	2.6
	2	2.45±0.13	0.30	32.6±1.7	4.0	22.1±1.5^{ab}	2.7
	3	2.48±0.22	0.30	33.0±3.0	4.0	26.2±1.4^{a}	3.2
Average	0	2.35±0.12	0.27	31.4±1.6	3.7	20.1±1.4^{b}	2.4
	1	2.45±0.29	0.29	32.6±2.1	3.9	21.2±1.3^{a}	2.5
	2	2.48±0.11	0.29	33.0±1.4	3.8	22.5±1.4^{a}	2.6
	3	2.54±0.12	0.30	33.8±1.6	4.0	26.0±1.2^{a}	3.0

Values are means±SE of 8 birds.
* See Table 2 for diameter value.
PA, Propionic acid.
Means having different superscripts within a column are significantly different ($P<0.05$).

increased almost nearly to the level of the control group. Also in female, values of TMT/kg body weight increased with elevating PA levels except that length and diameter of TMT/kg body weight were lower in 1% PA group than those in 0%.

Elasticity of TMT in PA-fed birds tended to increase with elevating PA levels in both sexes. This suggests that bone elasticity was increased by supplementation of PA. Fracture strength of bone tended to be higher in 2% and 3% PA-fed males and in all PA-fed females. Crash strength of TMT was increased in both sexes with increasing PA levels in diets and the value of female chicks fed 3% PA was significantly higher than those in 0% and 1% PA-fed groups. Almost similar tendency was obtained in crash strength of TMT/mm diameter. The estimated linear regressions between weight for crash strength (Y) and dietary PA level (X) were followed : males, $Y = 17.62 + 1.92\ X$ ($r = 0.355$, $P < 0.05$) ; females, $Y = 19.61 + 1.85\ X$ ($r = 0.427$, $P < 0.05$). Average of crash strength in both sexes was significantly increased ($P < 0.05$) with the increasing dietary PA level and the estimated linear regression was $Y = 19.61 + 1.90\ X$ ($r = 0.379$, $P < 0.05$).

Crude ash and calcium contents of TMT in chicks fed dietary PA were significantly increased only in 3% PA-fed male group ($P < 0.05$) and in all PA-fed female groups. The estimated linear regressions between crude ash (Y) and dietary PA level (X) were $Y = 28.33 + 1.88\ X$ ($r = 0.483$) in males, $Y = 35.61 + 1.35\ X$ ($r = 0.426$) in females and $Y = 31.97 + 1.62\ X$ ($r = 0.417$) in average of both sexes. Coefficient correlation in each group was significant ($P < 0.05$). The estimated linear regressions between calcium (Y) and dietary PA level (X) were $Y = 12.45 + 0.80\ X$ ($r = 0.43$) in males, $Y = 13.58 + 1.57\ X$ ($r = 0.591$) in females and $Y = 13.16 + 1.26\ X$ ($r = 0.542$) in average of both sexes, suggesting positive correlations at $P < 0.05$.

In comparative anatomical studies on the tibia between White Leghorn and BR male chickens maintained under the same environment from 0 to 154 days old, length, weight and volume of the tibia showed higher values in BR than White Leghorn chickens but no differences were found in the density of the tibia (REILAND *et al.*, 1978). Besides, in microradiographical observations the tibia in BR showed slower calcification of the hypertrophic cartilage cell zone than White Leghorn, resulting in cartilage fragility. HENRY and KON (1953) reported a high content of moisture in the bone and a less calcium content in the dry matter in young animals. The present results showing the increased dry weight/fresh weight of TMT in males and the elevated diameter, dry weight and dry weight/fresh weight of TMT in females by feeding the dietary PA suggest that growth of TMT in BR chicks was improved by supplementation of PA, although histological observations on TMT was not carried out.

It is well known that bone strength in BR differs in strains, breeding methods and mineral contents such as calcium in diets. ANDERSON *et al.* (1979) described that content of crude ash in the tibia as well as breaking strength of the bone were higher in chickens raised in floor system than those in cage system. A significant difference in strains was observed in tibia strength but no apparent difference was found in tibia ash among strains (ROWLAND *et al.*, 1972). Besides, ANDERSON *et al.* (1979) reported

Table 4. Crude ash and calcium contents of tarsometatarsal bone of broiler chickens fed dietary propionic acid

Sex	PA level (%)	Crude ash (%)	Calcium (%)
Male	0	29.9±1.2^{b}	13.0±0.7^{b}
	1	29.7±1.4^{b}	13.2±0.5^{b}
	2	28.4±0.6^{b}	12.5±0.3^{b}
	3	36.6±1.1^{a}	15.9±0.8^{a}
Female	0	35.3±1.0^{b}	12.3±0.7^{b}
	1	36.9±1.0^{ab}	16.6±0.9^{a}
	2	39.3±1.4^{a}	17.6±0.9^{a}
	3	39.0±1.3^{a}	17.2±0.6^{a}
Average	0	32.6±0.9^{b}	12.7±0.5^{c}
	1	33.3±1.1^{b}	14.9±0.6^{b}
	2	33.9±1.2^{b}	16.1±0.6^{ab}
	3	37.8±0.7^{a}	16.5±0.5^{a}

Values are means±SE of 8 birds.
PA, Propionic acid.
Means having different superscripts within a column are significantly different ($P<0.05$).

that percentage of broken bone during picking through the pickers was reduced in birds raised in floor system. MAY *et al.* (1981) investigated that in chickens kept in pen cage, the smaller body sized birds showed the higher feed requirement, weaker bone strength and higher percentage of bone fracture during picking through the pickers. In turkeys fed high calcium diets, the percentage of bone fracture was lowered by brooding in high temperature (ANDERSON *et al.*, 1979). Generally, bone ash is known to be increased by elevating the calcium contents in diets and increase bone breaking strength (ROWLAND *et al.*, 1967 ; MEYER and SUNDE, 1974 ; ANDERSON *et al.*, 1979). WHITEHEAD *et al.* (1971) studied that calcium retention in chicks was reduced with increasing dietary energy levels by adding maize oil to diets. According to ANDERSON *et al.* (1979), the addition of maize oil to diets lowered the breaking strength of the bone in turkeys, resulting in an increase of percentage of bone fracture during picking through the pickers. PA in this study has high energy among the volatile fatty acids and supplementation of PA is also thought to elevate the dietary energy level. However, contents of crude ash and calcium in TMT as well as breaking strength of TMT were significantly increased in 3% PA-fed chicks. In addition to calcium, PA is also known to be absorbed in the upper part of the intestine (BOLTON and DEWAR, 1965). Therefore, as suggested by WATANABE and MUTAI (1975) the present results indicate a possibility that dietary PA accelerate the absorption of mineral such as calcium in the intestine.

The bone of pig was strengthened by an addition of calcium to diets but no differences were observed in bone strength between both sexes (CRENSHAW *et al.*, 1981). In BR, incidence of bone breaking during picking through the pickers is higher in

females than males. In this study, however, breaking strength of TMT per diameter was higher in females fed dietary PA than males. Taking this fact into consideration, the high incidence of bone breakage in females seems to be caused by the small diameter of the bone. The findings that diameter, calcium content, and fracture and crash strengths of TMT were improved by the supplementation of PA suggest that dietary PA is effective in the prevention of bone breakage during picking through the pickers rather than an improvement of feed efficiency (IBARDOLAZA *et al.*, 1992).

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ブロイラー飼料へのプロピオン酸添加が中足骨の強度に及ぼす影響

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慣用のブロイラー用配合飼料にプロピオン酸（PA）を1，2，および3%配合した飼料をブロイラー専用雛に5日齢から54日齢まで水と共に自由摂食させた。給与試験終了後，直ちに中足骨を採取し，乾燥後，その大きさ，強度および粗灰分とカルシウム成分を測定した。

1） PA給与鶏の雄では，骨の大きさには一定の傾向が認められなかったが，新鮮重当たりの乾物重の割合は増加した。雌では，中足骨の直径，乾物重および新鮮重当たりの乾物重比が増加した。

2） 雌雄とも，中足骨のたわみ，骨折および破骨はPA配合により改善され，3%区が最も顕著であった。

3） 中足骨の粗灰分カルシウムは雌雄ともPAの給与により増加し，いずれもPAの配合量との間に正の相関関係がみられた。

以上のことから，ブロイラー飼料へのPA配合はカルシウム吸収の促進により，骨中のカルシウム量が増加し，骨が強度になったことを示唆するものと思われる。

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