UNIVERSITI PUTRA MALAYSIA

EFFECTS OF PHYTASE SUPPLEMENTATION ON PERFORMANCE, BONE CHARA CTERISTICS, BLOOD MINERALS AND CALCIUM AND PHOSPHORUS RETENTION IN BROILER QUAILS

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By

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Chairman: **Associate Professor Azhar B. Kasim, PhD**

Faculty: **Agriculture**

Two experiments were conducted to determine the appropriate ratio of calcium (Ca) and total phosphorus (tP) (to be used for the second experiment) capable to elicit good response on the addition of dietary phytase to broiler quail's diet, and to study the effect of varying levels of this enzyme on the growth performance, Ca and P retention, plasma's Ca and P, crude protein (CP) and phytate P (pP) retention and bone characteristics in broiler quails. In the first experiment 600 quails were subjected randomly to 5 treatments consisting ratios of Ca and tP: 1.39, 1.53, 1.66 (normal ratio), 1.81, and 1.96. Each treatment was replicated four times, consisting of 30 birds per replicate. The experimental period was 21 days. The second experiment used 480 quails, birds were randomly distributed into five treatment groups consisting of different levels of microbial phytase supplementation of 0, 250, 500, 750, 1000 FTU (phytase units). Each treatment was replicated four times, consisting of 24 birds per replicate, and

experimental period was also 21 days. Basal diet was based on corn and soybean meal. In Experiment 1, feed intake of quails across treatments did not show any significant difference. However, body weight gain (BWG) and feed conversion ratio (FCR) increased significantly ($P < 0.05$) by normal ratio (1.66) and low ratios (1.53 and 1.39) of these minerals. Lower ratios of Ca and tP (1.53 and 1.39) increased tibia ash content significantly ($P<0.05$). Ratios of Ca and tP (1.66, 1.53 and 1.39) resulted in significant ($P<0.05$) enlargement of proliferative zone of the tibial proximal end. P concentration in the plasma was not significantly affected. Decreasing ratio of these two minerals from 1.96 to 1.39 numerically increased the concentration of P in plasma. However, Ca level in plasma was significantly ($P < 0.05$) decreased. Retention of P in these ratios of Ca and tP (1.81, 1.66, 1.53 and 1.39) compared to ratio of 1.96 was significantly ($P < 0.05$) low and Ca retention in the ratios of 1.66, 1.53 and 1.39 was high. Hence, Ca and tP ratios of 1.81 and 1.96 caused negative effect on parameters measured. Between these two ratios (1.81 and 1.96), ratio of 1.81 selected to be used in the second experiment due to avoid adverse effect of high ratio of Ca and tP (1.96) on microbial phytase activity.

In the second experiment, BWG of broiler quails fed diets with different levels of phytase did not show any significant differences. However, feed intake (FI) and FCR were affected significantly by this enzyme ($P < 0.05$). Phytase significantly ($P < 0.05$) enhanced percentage of tibia ash. The proliferative zone was not significantly different ($P > 0.05$) by addition of dietary phytase even though it caused numerical increments in width of this zone. Dietary phytase had significant ($P<0.05$) impact on plasma P and Ca concentration. When diets were supplemented with incremental amounts of microbial

phytase, plasma P was enhanced significantly ($P<0.05$) but *vice versa* for plasma Ca concentration. The highest phytate P retention values ($P<0.05$) was obtained from quails fed with diet containing 1,000 FTU phytase. At this level of phytase supplementation, retention of crude protein (CP) was also significantly ($P < 0.05$) increased. Retention of P and Ca was increased ($P < 0.05$) by supplementation of diet with dietary phytase. It is concluded that supplemental phytase improved retention of Ca, P, phytate P, CP in broiler quails and the level of 1,000 FTU of this enzyme had optimum effect on the parameters measured in present study. Therefore, level of Ca and tP in quail diet can be reduced when dietary phytase is supplemented in the diet.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagi memenuhi keperluan untuk ijazah Master Pertanian Sains

Kesan-kesan phytase terhadap prestasi, ciri-ciri tulang, mineral-mineral darah dan retensi kalsium dan fosforus dalam burung puyuh pedaging

Oleh

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Dua eksperimen telah dijalankan untuk menentukan nisbah optimum Ca dan jumlah fosforus (untuk digunakan pada eksperimen kedua) mampu untuk meningkatkan tindak balas yang baik pada penambahan diet phytase kepada burung puyuh pedaging, dan untuk mengkaji kesan aras enzim yang berbeza pada prestasi pertumbuhan, retensi Ca dan P, plasma kalsium , protein kasar, dan retensi pP serta ciri-ciri tulang pada burung puyuh. Burung puyuh telah digunakan pada eksperimen pertama ialah sebanyak 600 ekor. Burung-burung tersebut diasingkan secara rawak kepada 5 perlakuan yang mengandungi nisbah Ca dan P seperti 1.39, 1.53, 1.66 (nisbah normal), 1.81 , dan 1.96. Setiap rawatan telah direplikasikan sebanyak 4 kali, iaitu sebanyak 30 ekor bagi setiap replikat. Tempoh eksperimen ialah selama 21 hari. Eksperimen kedua terdiri daripada

pemberian phytase dalam makanan pada aras yang berbeza iaitu 0, 250, 500, 750, 1000 FTU (unit phytase). Setiap perlakuan direplikat sebanyak empat kali, mengandungi 24 burung pada setiap replikat dan eksperimen juga selama 21 hari. Diet asas ialah berasaskan jagung dan mil kacang soya. Nisbah terpilih bagi Ca dan jumlah fosforus (tP) ialah 1.81, yang telah digunakan dalam eksperimen kedua. Dalam eksperimen pertama, pengambilan makanan burung puyuh antara pertakuan tidak menunjukkan sebarang perbezaan yang bererti ($P<0.05$). Walaubagaimanapun, penambahan berat badan (BWG), dan nisbah pertukaran makanan (FCR) bertambah baik ($P<0.05$) normal (1.66) dan nisbah rendah bagi mineral-mineral tersebut. BWG dan FCR meningkat . Nisbah-nisbah rendah Ca dan tP (1.53 dan 1.39) meningkatkan kandungan abu tibia dengan beerti ($P<0.05$). Penurunan nisbah Ca dan jumlah tP (1.66, 1.53 dan 1.39) mengakibatkan pembesaran zon proliferatif bagi hujung tibia proksimal ($P<0.05$). Kepekatan fosforus di dalam plasma tidak menunjukkan kesan signifikan. Penurunan nisbah bagi kedua-dua mineral (dari 1.96 ke 1.39) numerik, meningkatkan kepekatan P dalam plasma. Walaubagaimanapun, aras Ca dalam plasma menurun dengan bererti ($P < 0.05$). Retensi P pada nisbah yang rendah Ca:tP (1.81, 1.66, 1.53 dan 1.39) adalah kurang bererti($P< 0.05$) tetapi retensi Ca (dalam nisbah of 1.66, 1.53 dan 1.39) adalah tinggi. Di antra dua nisbah ini (1.81 dan 1.96), nisbah 1.81 dipilih untuk digunakan pada eksperimen kedua. Ini disebabkan kesan sampingan nisbah yang tinggi Ca dan tP 1.96 terhadap aktiviti phytas.

Pada eksperimen kedua, BWG bagi burung puyuh pedaging yang diberi makan dengan aras phytase yang berbeza tidak menunjukkan sebarang perbezaan bererti. Walaubgaimanapun, FI dan FCR telah dipengaruhi secara signifikan melalui enzim (P

<0.05). Phytase telah meningkatkan peratus abu tibia dengan bererti (P <0.05). Zon proliferatif tidak menunjukkan perbezan bererti (P <0.05) apabila phytase ditambah lagi. Walaubagaimanapun ia menunjukan pertambahan numerik pada kelebaran zon ini. Diet phytase mempunyai kesan bererti pada plasma P dan kepekatan Ca. Apabila diet diberikan jumlah phytase yang tinggi, plasma fosforus meningkat secara bererti (P <0.05). Namun, kepekatan plasma kalsium menunjukkan keputusan sebaliknya.

Nilai retensi phytate P yang tertinggi (P <0.05) didapati daripada burung puyuh yang diberi makan diet mengandungi 1,000 FTU phytase. Pada pemberian aras phytase ini, retensi bagi protein kering (CP) juga meningkat dengan bererti (P < 0.05). Retensi bagi P dan Ca juga bereti (P < 0.05) dengan penambahan phytase ke dalam diet. Ini dapat disimpulkan pemberian suplemen phytase dapat meningkatkan retensi Ca, P, phytate P,CP pada burung puyuh dan enzim pada aras 1,000 FTU ini mempunyai kesan optimum pada parameter yang diukur pada kajian yang lepas. Oleh sebab itu, aras Ca dan tP dalam diet burung puyuh dapat dikurangkan apabila phytase dimasukan ke dalam diet.

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ABBREVIATIONS

AA	amino acids
ALKP	alkaline phosphatase
AME	apparent metabolizable energy
aP	available phosphorus
BWG	body weight gain
Ca	calcium
CCD	couple charged device
CP	crude protein
Cu	copper
DCP	dicalcium phosphate
DM	dry matter
FCR	feed conversion ratio
FI	feed intake
FTU	phytase unit
g	gram
GE	gross energy
GIT	gastro intestinal tract
h	hour
kcal	kilo calorie
Kg	kilogram
LWG	live weight gain

MCP	mono calcium phosphate
ME	metabolize energy
Mg	magnesium
mg	milligram
ml	milliliter
mmol	milimol
Mn	manganese
N	nitrogen
NRC	National Research Council
npP	non phytate phosphorus
P	phosphoros
Pi	inorganic phosphorus
pP	phytate phosphorus
PU	phytase unit
SD	standard deviation
tP	total phosphorus
U	unit
WG	weight gain
wk	week
Zn	zinc

CHAPTER 1

INTRODUCTION

The requirement of calcium (Ca) and phosphorus (P) of poultry has been the subject of research for more than four decades. Both Ca and P co-exist in many biological functions (Hurwitz *et al.*, 1995; Underwood and Suttle, 2001) and the dietary requirement for these minerals is interdependent. Feed is the only source of these nutrients. However, it is recognized that some nutrients in feed ingredients are not entirely available to monogastric animals such as poultry and are chelated by phytate (the salt of acid phytic). Phytate has the property to bind to cations consisting of Ca, P, Co, Cu, Fe, Zn, Mn, Mg and Pb (Oberleas and Harland, 1996). This strong anti-nutritional characteristic of phytate limits the absorption of other important nutrients as well, such as protein (Okubo *et al.*, 1976) and carbohydrate (Thompson and Yoon, 1984).

Hence, phytate-nutrients complex remains undigested by poultry and are excreted in the feces. Among the bound minerals, P is most disruptive in polluting water. Animal droppings added to soil, increases P capacity of the soil, and the extra P leaches into rivers, lakes, and reservoirs causing eutrophication (Correll, 1999; Sharpley, 1999), odors, sedimentation and other problems that impose various health risks, not only to humans, but also to livestock and aquatic life.

Impact of environmental pollution due to the disposed of unutilized compounds such as phytate phosphorus (pP) from intensive poultry farming is of great concern. To overcome this problem, all feeds for monogastric animals need to supplement with phytase or other additives that reduce fecal phosphorus (Harter-Dennis and Sterling, 1999).

The beneficial effects of phytase on the availability of pP and other nutrients have been known since the 1960s (Nelson, 1967) with considerable research being undertaken. This environmental friendly enzyme is able to improve the usage of P and decreases the required dietary level of minerals, proteins and amino acids without affecting the requirements of poultry. This consequently contributes to environmental benefits and economical advantages.

Phytase breaks down phytate into inorganic P that can be directly used by chickens (Denbow *et al.*, 1995; Qian *et al.*, 1997; Ravindran *et al.*, 2000). This enzyme is found in the digestive tract of monogastric animals. However the amount of intestinal phytase is not enough to hydrolyze phytate to a considerable amount (Bitar and Reinhold 1972; Sugiura *et al.*, 1999). Thus, with no supplementation of dietary phytase non-ruminants require supplemental phosphorus in their ration.

To date, extensive research had carried out to determine the optimum level of supplemental phytase for fast growing commercial broiler chicken. However, not many studies on the effect of this enzyme on broiler quails fed corn soybean meal diet with

low levels of Ca and P were conducted. Also, in those studies the effect of phytase on limited numbers of parameters such as growth performance and tibia ash content (Sacakli *et al.*, 2006; Denek *et al.*, 2007; Osman *et al.*, 2009) had been investigated. Therefore, the present study has conducted on broiler quails to assay the effect of graded levels of this enzyme on several parameters at 3 wk of age.

Positive effect of microbial phytase on the reduction of inorganic phosphorus utilization in the diet seems not to be restricted to this age, as 3 to 5 week old birds also responded in a manner similar to the yaung quails and chickens (Sacakli *et al.*, 2006; Osman *et al.*, 2009; Mitchell and Edward, 1996). Hence, present study conducted until 3 week of age to assay the effect of this enzyme only for this age period.

Thus, the objectives of present study were to determine optimum ratio of Ca and tP capable to obtain good result on the addition of dietary phytase to broiler quail's diet, and to investigate the effect of different levels of this enzyme on growth performance, retention of Ca, P, pP and crude protein (CP), blood parameters and bone characteristics of broiler quails at three weeks of age.

CHAPTER 2

LITERATURE REVIEW

2.1 Japanese Quails

Origin and Characteristics

Quails along with chickens, pheasants and partridges belong to the Family Phasianoidea of Order Galliformes of the Class Aves of the Animal Kingdom. Species or subspecies of the genus *Coturnix* are native to all continents except the Americas. One of them *Coturnix coturnix* or common quail are migratory birds of Asia, Africa and Europe. Several interbreeding subspecies are recognized, the more important being the European quail, *Coturnix coturnix coturnix*, and the Asiatic or Japanese quail, *Coturnix coturnix japonica*. The quail used in this study belongs to the later quails (Howes, 1964).

Young *coturnix* are yellowish in appearance with stripes of brown and somewhat resemble turkey poults except for size. The newly hatched chicks weigh about 6 to 8 grams, but grow rapidly during the first few days. After three days flight feathers begin to appear and the birds are fully feathered about four weeks of age. Partial sexing is possible by three weeks of age by the cinnamon-colored feathers on the breast of the male bird, but there are some birds that defy definite sexing by this method, even when adults.

The adult male *coturnix* weighs about 140 grams. The male birds can be identified readily by the rusty brown colored feathers on the upper throat and lower breast region.

Males also have a cloacal gland, a bulbous structure located at the upper edge of the vent which secretes a white, foamy material. This unique gland can be used to assess the reproductive fitness of the males (Cheng *et al.*,1985).

The adult female *coturnix* is slightly heavier than the male, weighs about 160 grams. The body coloration of the female bird is similar to the male except that the feathers on the throat and upper breast are long, pointed, and much lighter cinnamon. Also, the light tan breast feathers are characteristically black-stippled.

Coturnix eggs are characterized by a variety of color patterns. The average egg from mature female weighs about 10 grams, about 8 percent of the body weight of the quail hen as compared to 3 percent for chicken eggs. The egg of Japanese quail contains 158 Cal. of energy, 74.6% water, 13.1% protein, 11.2% fat, and 1.1% total ash. The mineral content includes 0.59 mg calcium, 220 mg phosphorus and 3.8 mg iron. The vitamin content is 300 i.u. of vitamin A, 0.12 mg of vitamin B l, 0.85 mg of vitamin B2 and 0.10 mg nicotinic acid (Cheng *et al.*,1985).