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Clinicopathology of gout in growing layers induced by high calcium and high protein diets

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Abstract 1. An experiment was conducted to test the independent and combined effects of high dietary calcium and protein concentrations on the induction of visceral gout in growing birds of a layer strain.

2. One hundred and sixty healthy birds were randomly divided into 4 groups at 35 d of age. The different groups were given 4 diets containing normal or high concentrations of dietary calcium or crude protein in a 2 × 2 factorial experiment for 30 d. The diets contained normal calcium (Ca) and crude protein (CP) (NCNP, 8.5 g Ca/kg and 175 g CP/kg), high calcium and normal protein (HC, 36.3 g Ca/kg and 175 g CP/kg), normal calcium and high protein (HP, 8.8 g Ca/kg and 245 g CP/kg) or high calcium and high protein (HCHP, 36.8 g Ca/kg and 242 g CP/kg), respectively.

3. Typical visceral gout was induced by the HCHP diet. The HCHP and HC diet caused severe kidney damage. The HP diet did not cause kidney damage, but significantly increased plasma uric acid and inorganic phosphorus concentrations.

4. The HC diet significantly increased plasma uric acid, calcium and sodium, but significantly decreased plasma inorganic phosphorus, potassium and magnesium concentrations. The HCHP diet significantly increased plasma uric acid, calcium and sodium.

5. Urine volumes were significantly higher on the HCHP and HC diets than on the control. The growers raised on HC and HCHP diets had significantly higher total quantity of 24 h urinary excretion of uric acid, calcium, magnesium, inorganic phosphorus and potassium and a significantly lower 24 h urinary excretion of sodium. The growers fed on the HP diet had a higher 24 h urinary excretion of uric acid and inorganic phosphorus than the control.

6. It is concluded that growing layer birds should not be fed on layer rations.

INTRODUCTION

Gout is a common metabolic disorder that results in abnormal accumulation of urates in domestic birds (Damodaran *et al.*, 1978). It occurs as two distinct forms, namely, visceral and articular gout. Visceral gout has been reported in various caged and aviary birds from different parts of the world. It is among the most commonly diagnosed causes of mortality in poultry (Riddell, 1987; Brown, 1996). Typical clinicopathology of visceral gout is hyperuricaemia. Early attempts to determine the cause of visceral gout were focused on conditions in the layer house. However, field reports have made it apparent that immature birds also experience outbreaks of visceral gout. Many factors, such as exposure of birds to nephrotrophic strains of infectious

bronchitis virus, influenza virus, cryptosporidium, high crude protein diets, diets containing oosporem, vitamin A deficiency, water deprivation or metabolic alkalosis induced by elevated dietary sodium plus potassium over chloride ratios have been reported to be associated with visceral gout (Pegram and Wyatt, 1981; Siller, 1981; Chong and Apostolv, 1982; Sharma and Kaushid, 1986; Hocking, 1989; Slomens *et al.*, 1990; Hocking and Bernard, 1997; Mubarak and Sharkawy, 1999; Trampel *et al.*, 2000).

Visceral gout in birds is an economically important disease in China. Case histories of affected bird flocks often, but not always, showed that excessive calcium and protein had been fed and that visceral gout could be induced by feeding growers on a commercial layer ration (containing 36 to 38 g Ca/kg compared with the

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normal range of 8 to 10 g Ca/kg). An experiment in adult feed-restricted broiler breeder males showed that plasma uric acid concentration increased linearly above 106 g crude protein (CP)/kg and gout was observed in birds fed on 400 g CP/kg (Hocking, 1989). The concentrations of CP in grower diets are in the range 160 to 180 g CP/kg, according to National Research Council (1994) recommendations. To date, little experimental research has been conducted on the relationship between the disease and independent and combined dietary calcium and protein concentrations and previous investigations of gout have little clinicopathological evaluation. The present study was designed to test the independent and combined effects of dietary calcium and protein levels on the incidence of visceral gout, plasma and urinary concentrations of calcium, magnesium, sodium, potassium, inorganic phosphorus and uric acid in growing layer chicks. At the end of the experiment, we determined clinical signs and pathological lesions of the affected birds.

MATERIALS AND METHODS

Experimental design

One hundred and eighty 1-d-old ISA Brown female chicks were placed in brooders and given a commercial chick starter diet (Table 1), prepared according to National Research Council (1994) recommendations. At 35 d of age, 160 healthy growers were chosen for the experiment. The design consisted of 4 dietary treatments with 4 replicate pens (16 pens, 4 blocks) of 10 birds. Over the entire experimental period (35 to 65 d), the growers were allowed *ad libitum* consumption of feed and water. The control diet contained normal calcium and normal crude protein

(NCNP, 8.5 g/kg calcium and 175 g CP/kg), meeting National Research Council (1994) recommendations. The high calcium (HC) diet contained 36.3 g Ca/kg and 169 g CP/kg. The high protein (HP) diet contained high CP and normal calcium (8.8 g Ca/kg and 245 g CP/kg). The high calcium and high protein (HCHP) diet contained 36.8 g Ca/kg and 243 g CP/kg. Compositions and nutritive values of the different diets are shown in Table 1.

Clinical observations

The birds were inspected daily. Pen mean feed intake was measured between 35 and 65 d and mean pen body weight was measured at 35 and 65 d of age. Necropsies were performed on all birds that died during the experiment. Five randomly selected growers from each replicate treatment pen were killed by cervical dislocation at 65 d of age. After death, necropsies were performed and post-mortem lesions were recorded.

Histopathology

Tissue samples from kidneys were taken from treated and control birds and fixed in 10% neutral buffered formalin. Tissue samples were processed routinely for paraffin embedding followed by sectioning and staining with haematoxylin and eosin (HE).

Blood collection and analysis

Plasma samples were obtained from 5 birds in each replicate treatment pen at 52 d of age by wing venipuncture prior to morning feeding at 08:30 h. Heparinised blood samples were centrifuged immediately after collection and the plasma was divided into aliquots and stored at -20°C for analysis. A Cobas Bio Spectrophotometric Auto-analyser was used to measure the concentrations of calcium, magnesium, sodium, potassium, inorganic phosphorus and uric acid in the plasma.

Urinary collection and analysis

To prevent the back-flow of urine from the cloaca to the caecum and to collect urine, one bird from each replicate treatment pen was colostomised as described by Belay *et al.* (1993) at 51 d of age. After one day, a urine collection bottle was attached to the anal collar of each colostomised bird for urine collection and 24 h urine volume was determined. Urine samples were divided into aliquots and stored at -20°C for analysis. The concentrations of calcium, magnesium, sodium, potassium,

Table 1. Composition of experimental diets (g/kg): normal (NCNP), high calcium (HC), high protein (HP) or high calcium and high protein (HCHP)

Ingredient	Starter	NCNP	HC	HP	HCHP
Maize	650.0	630.0	645.0	500.0	460.0
Soybean meal	240.0	220.0	235.0	410.0	430.0
Wheat bran	80.0	120.0	20.0	60.0	0
Bone meal	20.0	20.0	20.0	20.0	20.0
Limestone	0	0	80.0	0	80.0
Premix ¹	10.0	10.0	10.0	10.0	10.0
ME (MJ/kg)	12.30	12.05	11.80	11.92	11.25
Calcium	8.5	8.5	36.3	8.8	36.8
Available phosphorus	4.3	4.4	4.2	4.5	4.4
Crude protein	179.6	175.3	169.2	245.2	242.5

¹Premix provided per kg of diet: vitamin B₁ 0.5 mg, vitamin B₂ 4.0 mg, vitamin E 10 mg, choline chloride 400 mg, vitamin B₁₂ 0.01 mg, pantothenic acid 8 mg, niacin 30 mg, folic acid 0.5 mg, vitamin K₃ 2 mg, vitamin B₆ 2 mg, copper 6.0 mg, iron 40 mg, manganese 70 mg, zinc 50 mg, iodine 0.30 mg, selenium 0.10 mg.

inorganic phosphorus and uric acid in the urine were subsequently determined as described above.

Statistics

The concentrations of calcium, magnesium, sodium, potassium, inorganic phosphorus and uric acid in the plasma and in the urine were analysed on an individual bird basis. Body weight, weight gain and the ratio of feed to gain were analysed on a pen basis. All data were subjected to analysis of variance using the General Linear Models procedure of SAS software (SAS Institute, 2001) using a 2×2 factorial model. All data are shown as means \pm SEM, and values were considered to differ significantly at $P < 0.05$.

RESULTS

Performance

Live performance of growers is given in Table 2. At 65 d of age, body weight was significantly lower for HC and HCHP growers, compared to the control and HP growers. Through the whole experimental period, feed intake was significantly higher for the control and HP growers than for the HC and HCHP growers. The ratio of feed and gain was significantly lower for the control and HP growers than for the HC and HCHP growers.

Clinical signs and gross pathology

No clinical signs or mortalities were recorded for the control and HP growers. Some growers in the HC and HCHP groups began to show signs of depression, loss of appetite, gradual emaciation, diarrhoea, intense thirst, dehydration and body weight by d 7 of the experiment. They had a tendency to hide and appeared to be chilled. Almost all growers in the HC and HCHP groups showed typical clinical signs as described above by d 17 of the experiment (that is, 52 d of age). Nineteen birds died in the HC and 21 in the HCHP groups during the experiment. Post-mortem examination showed that the dead growers were emaciated and the

breast, leg and thigh muscles were atrophied and dehydrated. Enlarged kidneys were observed, but typical visceral gout was not found in growers raised on the HC diet. Conversely most of the growers in the HCHP group developed typical visceral gout. The ureters were enlarged with white urate deposits and the kidneys were swollen and had pale regions in more of the divisions. There were noticeable chalky white masses surrounding the heart, kidneys and other organs. The most common extra-renal lesions were urate accumulation in the pericardial sac and in the epicardium.

Histopathology

In the HC and HCHP growers, there were similar appreciable changes in kidney parenchyma and substantial deposits of urate in glomeruli. Kidney histological changes were more extensive with complete destruction of the renal tubules with swollen, degenerated and putrescence epithelial cells. The remaining structures of kidney parenchyma showed compensatory changes consisting of tubular dilation and hypertrophy and hyperplasia of tubular epithelium.

Blood chemistry

Plasma concentrations are shown in Table 3. Inorganic phosphorus, potassium and magnesium for HC growers were significantly lower whereas uric acid, calcium and sodium were significantly higher than the control growers. Uric acid and inorganic phosphorus were significantly higher for HP than for the control growers and potassium, magnesium, calcium and sodium were similar. Plasma concentrations of uric acid, calcium and sodium were significantly higher for HCHP than for the controls and potassium, magnesium and inorganic phosphorus were similar in these two treatments also.

Urinary volumes and urinary values

Urine volumes for 24 h in growers raised on normal, HC, HP and HCHP diets were 37 ± 8.0 , 124 ± 11.3 , 40 ± 7.2 and 116 ± 17.1 ml,

Table 2. Performance of birds fed on normal, high calcium (HC), high protein (HP) or high calcium and high protein (HCHP) diets from 35 to 65 d of age

Trait	Control	HC	HP	HCHP
Body weight, g (35 d)	225 \pm 1.7	227 \pm 1.7	223 \pm 1.7	228 \pm 1.7
Body weight, g (65 d)	707 \pm 1.3 ^a	560 \pm 2.4 ^b	703 \pm 3.1 ^a	572 \pm 2.6 ^b
Feed intake, g (35 to 65 d)	2358 \pm 3.1 ^a	1838 \pm 2.9 ^b	2372 \pm 2.9 ^c	1893 \pm 3.2 ^d
Feed: gain, g:g (35 to 65 d)	4.89 \pm 0.03 ^a	5.52 \pm 0.07 ^b	4.97 \pm 0.01 ^a	5.47 \pm 0.07 ^b

Values in the same row with no common superscripts differ significantly ($P < 0.05$).

Table 3. Plasma concentrations in growers raised on normal, high calcium (HC), high protein (HP) or high calcium and high protein (HCHP) diets

Trait (mmoles/l)	Control	HC	HP	HCHP
Uric acid	0.41 ± 0.02 ^a	1.06 ± 0.08 ^b	0.91 ± 0.04 ^b	0.90 ± 0.04 ^b
Calcium	2.20 ± 0.12 ^a	2.89 ± 0.10 ^b	2.06 ± 0.07 ^a	3.05 ± 0.10 ^b
Inorganic phosphorus	1.80 ± 0.06 ^a	0.77 ± 0.07 ^b	2.18 ± 0.07 ^c	1.61 ± 0.08 ^a
Sodium	113.4 ± 4.28 ^a	151.1 ± 2.04 ^b	119.0 ± 2.40 ^a	142.3 ± 5.60 ^b
Potassium	7.11 ± 0.47 ^a	4.73 ± 0.28 ^b	7.24 ± 0.59 ^a	7.14 ± 0.39 ^a
Magnesium	0.93 ± 0.05 ^a	0.68 ± 0.03 ^b	0.95 ± 0.57 ^a	0.97 ± 0.16 ^a

Values in the same row with no common superscripts differ significantly ($P < 0.05$).

Table 4. Urinary concentrations in growers raised on normal, high calcium (HC), high protein (HP) or high calcium and high protein (HCHP) diets

Trait (mmoles/l)	Control	HC	HP	HCHP
Uric acid	0.33 ± 0.05 ^a	0.37 ± 0.01 ^a	0.53 ± 0.03 ^b	0.50 ± 0.04 ^b
Calcium	5.47 ± 0.22 ^a	4.29 ± 0.13 ^b	5.35 ± 0.15 ^a	3.92 ± 0.36 ^b
Inorganic phosphorus	0.69 ± 0.13 ^a	0.96 ± 0.15 ^{ab}	1.13 ± 0.15 ^b	1.01 ± 0.33 ^{ab}
Sodium	11.20 ± 0.90 ^a	1.76 ± 0.28 ^b	9.11 ± 0.88 ^a	1.99 ± 0.24 ^b
Potassium	37.84 ± 5.34 ^a	16.76 ± 2.47 ^b	36.13 ± 3.20 ^a	16.34 ± 3.40 ^b
Magnesium	3.69 ± 0.07 ^a	2.92 ± 0.18 ^b	3.44 ± 0.12 ^{ab}	3.09 ± 0.13 ^b

Values in the same row with no common superscripts differ significantly ($P < 0.05$).

Table 5. Total quantity of urinary excretion in growers raised on normal, high calcium (HC), high protein (HP) or high calcium and high protein (HCHP) diets

Trait (μmoles)	Control	HC	HP	HCHP
Uric acid	12.5 ± 1.8 ^a	46.6 ± 1.7 ^b	21.1 ± 1.1 ^c	57.7 ± 4.5 ^d
Calcium	204.4 ± 7.7 ^a	534.9 ± 16.2 ^b	214.5 ± 6.0 ^a	452.6 ± 41.4 ^c
Inorganic phosphorus	25.8 ± 4.7 ^a	128.1 ± 14.2 ^b	48.2 ± 4.5 ^c	128.9 ± 29.7 ^{bc}
Sodium	418.0 ± 33.4 ^a	219.5 ± 34.3 ^b	365.2 ± 35.2 ^a	230.3 ± 27.4 ^b
Potassium	1412.3 ± 199.5 ^a	2089.5 ± 307.4 ^b	1448.6 ± 128.3 ^a	1887.7 ± 392.4 ^b
Magnesium	137.9 ± 2.2 ^a	363.7 ± 22.7 ^b	137.9 ± 4.7 ^a	357.1 ± 15.1 ^b

Values in the same row with no common superscripts differ significantly ($P < 0.05$).

respectively. Urine volumes in growers raised on HC and HCHP diets were significantly higher than the control and HP treatments. Urinary concentrations of inorganic elements are presented in Table 4. Calcium, sodium, potassium and magnesium were significantly lower for HC and HCHP compared with the control and HP diets. Inorganic phosphorus was significantly higher for HP than the control whereas uric acid was significantly increased in HP and HCHP treatments compared with the control and HC diets.

Total 24 h urinary excretion

Total 24 h urinary excretion was calculated from urinary values and 24 h urine volume for birds raised on the different diets (Table 5). The total 24 h urinary excretion of sodium was significantly lower for HC and HCHP than for control and HP birds. The total 24 h urinary excretions

of uric acid and inorganic phosphorus were significantly greater for HP birds than for the controls. The total 24 h urinary excretion of uric acid, calcium, inorganic phosphorus, potassium and magnesium were significantly greater for HC and HCHP birds than for the controls.

DISCUSSION

In the present study, growers fed on the HC diets had severe kidney damage, which further confirms a previous report (Wideman *et al.*, 1985) that rearing pullets on high calcium diets can cause kidney lesions. Moreover, growers fed on the HCHP diets in our experiment had not only severe kidney damage but also typical visceral gout. In the previous studies, several investigators have suggested that hyperuricaemia was preceded by the development of tophaceous deposits in parenchymatous organs (Pegram and Wyatt, 1981; Siller, 1981). The results in

the present experiment suggested that high dietary calcium could cause significant hyperuricaemia in growing layers. The experiment also indicated that plasma uric acid concentration significantly increased in growers fed on high dietary protein, which is in agreement with a previous report (Hocking, 1989). The combined effects of high dietary calcium and high dietary protein in the HCHP group resulted in the occurrence of typical visceral gout in our experiment. We believe that high dietary calcium for growers was the primary cause of visceral urate deposition and that the increase in urate excretion associated with the HP diet is a secondary cause of visceral urate deposition.

In previous studies, growers fed on HP diets developed classical signs of articular gout (Siller, 1981; Hocking, 1989). However, no signs of gout were observed in growers fed on HP diets in our experiment although plasma uric acid concentration significantly increased. We suggested that the different results might be associated with the concentration of protein in the diet and length of feeding.

The HP diet caused hyperphosphataemia and significantly higher urinary phosphorus excretion compared with the control, which suggested that growers reared on a high protein diet had higher rates of intestinal phosphorus absorption. Birds fed on the HC diet had hypercalcaemia and hypophosphataemia and significantly higher urinary calcium and phosphorus excretion, suggesting that growers raised on this diet had higher rates of intestinal calcium absorption and lower rates of intestinal phosphorus absorption. Hypercalcaemia was associated with the HCHP diet but it did not cause hyperphosphataemia, suggesting that growers raised on HCHP diet had higher rates of intestinal calcium absorption and normal rates of intestinal absorption.

Plasma uric acid concentrations were significantly higher in the HC and HCHP growers than in the controls. Urinary uric acid excretion in birds with severe kidney damage caused by the HC and HCHP diets was not significantly less than in the control, suggesting that increased plasma uric acid is not the result of decreased urinary uric acid excretion. Our results also supported a previous study showing that plasma uric acid concentrations are not directly related to calcium-induced kidney damage (Wideman *et al.*, 1993). Other factors such as the higher protein ingested in HCHP may have increased plasma uric acid.

Urine volumes were significantly greater in growers raised on HC and HCHP diets than the control diet, which caused severe dehydration in the birds in our experiment. We believe that the increased urine volumes and severe

dehydration were associated with kidney failure induced by high dietary calcium and were a primary cause of deaths.

Based on the results of the present study, it is concluded that both high dietary calcium and crude protein concentrations caused hyperuricaemia in growers, which resulted in the occurrence of typical visceral gout. Therefore, recommended dietary concentrations of calcium and crude protein should not be exceeded and, specifically, growing layers should not be fed on a commercial layer ration.

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