

Effects of High Dietary Calcium and Fat Levels on the Performance, Intestinal pH, Body Composition and Size and Weight of Organs in Growing Chickens

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ABSTRACT : The effects of fat supplementation of high calcium (Ca) diets on the performance, intestinal pH, body composition and size and weight of organs in growing chickens were investigated in two experiments.

Growing chickens tolerated a high dietary level of Ca (22.5 vs. 12.1 g/kg) in the presence of 6.3 g/kg of available phosphorus without any significant effect on performance. Intestinal pH was significantly increased by the addition of excess Ca and fat which probably created the right pH for the formation of insoluble Ca soaps. Excess dietary Ca increased carcass linoleic acid concentration at the expense of palmitic and stearic acid contents, whilst the addition of sunflower oil (80 g/kg diet) to the diet increased carcass linoleic acid concentration at the expense of palmitic acid content of the carcass.

Intestinal and visceral organ size and weight were not influenced by excess Ca or fat. However, there was a non significant increase in the intestinal dry weight per unit of length caused by excess dietary Ca.

It was concluded that excess dietary Ca of 22.5 g/kg did not significantly influence the performance of meat chickens. However, excess Ca increased intestinal pH and altered carcass fatty acid composition. Fat supplementation did not alter intestinal pH with high Ca diets. Excess dietary fat altered carcass fatty acid composition and reduced protein content. Intestinal and visceral organ size and weights were not influenced by excess dietary levels of Ca or fat.

(Key Words: Meat Chickens, Calcium, Fat, Body Composition, Intestinal pH, Intestinal Thickness)

INTRODUCTION

Dietary composition can affect growth performance and body composition. The level of Ca in growing chicken diets may vary greatly due to economic pressure to use meat and bone meal in the diet. Moderately excessive Ca intake in relation to recommendation is not uncommon when a substantial part of the diet is composed of meat and bone meal. Ca reacts with fat in the digestive tract resulting in the formation of Ca soaps (Fedde et al., 1960; Waibal and Mraz, 1964; Whitehead et al., 1971; Shafey, 1998). This reaction may alter the availability of some metallic ions and fatty acids (Shafey et al., 1991; Shafey, 1998). Bartov (1979) has reviewed studies on the effect of dietary composition on fat deposition but the effect of dietary calcium (Ca) has not been studied. The objectives of this study were to investigate the effects of dietary Ca and fat contents on the performance, intestinal pH, body composition and size and weight of a number of organs in growing chickens.

MATERIALS AND METHODS

Experiment 1

The effects of fat supplementation of high Ca diets on the performance, pH of intestinal contents (pHI) and body and fatty acids composition of growing chickens were investigated in this experiment. The experiment was a 2 × 2 factorial, with factors of: level of Ca (12.1 and 22.5 g/kg) and level of fat (31 and 111 g/kg). A total of 96, unsexed Ingham meat chickens, were fed a commercial diet until eight days old. At eight days of age, the chickens were weighed and individually caged with a separate feeding trough in an electrically heated battery brooder. Each of the experimental diets was fed to twenty-four chickens. The compositions of the experimental diets are shown in table 1. Increased dietary levels of Ca and fat were achieved by the addition of limestone and sunflower oil, respectively. The birds were weighed again at nineteen days of age when the experiment concluded. Food intake was measured over the eleven-day experimental period.

At the beginning of the experiment, eight chickens of

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Table 1. The composition of the experimental diets (g/kg)

Ingredient	Control fat- Control Ca	Control fat- High Ca	High fat- Control Ca	High fat- High Ca
Wheat	314	326.55	191.5	204
Sorghum	314	326.5	191.5	204
Cottonseed meal	40	40	40	40
Soy bean meal	70	90	80	100
Bran	115	30	270	185
Meat meal (53% protein)	100	100	100	100
Fish meal (65% protein)	40	40	40	40
Sunflower oil	—	—	80	80
Limestone	—	40	—	40
Sodium chloride	3	3	3	3
Celite (Insoluble ash)	2	2	2	2
Premix ¹	2	2	2	2
Analysis				
Crude protein (N% × 6.25)	20.6	20.9	22.6	22.4
Fat (%)	3.1	3.2	11.1	11.3
Ca (%)	1.21	2.25	1.2	2.3
Total phosphorus (%)	0.98	0.96	0.98	0.91
Calculated available phosphorus (%)	0.63	0.64	0.62	0.62
Calculated metabolizable energy (kJ/g)	11.6	11.5	12.5	12.3
Protein : energy ratio	1.78	1.81	1.8	1.82

¹ The composition of vitamins and minerals in the premix (per tonne of diet): retinol, 12g; cholecalciferol, 4g; D-L tocopheryl acetate, 10g; riboflavin, 3g; biotin, 0.5g; pyridoxine HCl, 0.5 g; menadione sodium bisulphate, 1.0g; zinc bacitracin (10%), 2.0 g; manganese sulphite, 60 g; zinc oxide, 50 g; ethoxyquin (33%), 150 g; DL-methionine, 500 g; L-lysine hydrochloride, 1,000 g; furazolidone, 100 g; carrier (pollard), to 5 kg.

average initial body weight were killed for initial body composition.

At the end of the experiment, three chickens from each of the dietary treatments were killed by cervical dislocation, and the intestinal tract from the base of the gizzard to the caecal junction was immediately removed. The intestinal contents were obtained by injecting 2 ml distilled water into the base of the gizzard, and then the contents were gently squeezed out into a beaker. The contents were further mixed with 5 ml of distilled water and pH read immediately to minimize changes in the digesta pH (Farner, 1942). Another eight chickens/diet were randomly selected, starved for three hours then killed without loss of blood by cervical dislocation and frozen immediately. Each carcass was chopped repeatedly into small pieces then minced in a meat grinder and then sampled for moisture analysis. Moisture was determined by drying the minced samples in a forced draught oven at 90°C for 48 hours. Dried samples were then reground prior to analysis of fat, protein, Ca and magnesium. Carcass total lipid was extracted with chloroform-

methanol by homogenizing in a high-speed motor-driven mixer (Folch et al., 1957) and fatty acid concentrations were determined by gas-liquid chromatography (Nugara and Edwards, 1970). Carcass protein was determined by the Kjeldahl procedure (AOAC, 1980). Ca and magnesium were determined by atomic absorption spectrophotometry after ashing carcass samples at 600°C overnight prior to digestion according to AOAC methods (1980). Calculations of gain were based on the formula: (nutrient gain/dry weight gain) *100.

Experiment 2

The effects of increasing dietary level of Ca on the size and weight of chicken organs were investigated in this experiment. A total of 40, Ingham unsexed chickens were fed on a commercial meat chicken diet until 9 days old. At 9 days of age, the chickens were weighed and allocated to two groups of twenty and housed in electrically heated battery brooders. The basal and high Ca diets (12.1 and 22.5 g of Ca/kg) of experiment 1 were used. The experiment lasted 14 days. At the end of the

experiment, eight chickens per treatment were randomly selected, then killed by cervical dislocation. The gastro-intestinal tract, pancreas, heart and liver were removed and all except the gastro-intestinal tract were immediately weighed. The gastro-intestinal tract was divided into proventriculus, gizzard, duodenum, jejunum, ileum, caecum and colon. The duodenum was that portion from the gizzard to the distal attachment of the pancreas, the jejunum was from duodenum to the yolk stalk, and the ileum was that portion from the yolk stalk to the ileocecal junction. The length of each segment was measured under a constant tension of a 10 grams weight. The segment was cut length wise, washed with saline solution (0.15 mol NaCl) and weighed. Intestinal weight per unit of length was also calculated. After weighing, the liver and the small intestines were dried at 55°C for 48 hours in a forced draught oven and dry weight was determined.

Data collected were subjected to analysis of variance (Steel and Torrie, 1980). Where significant variance ratios were detected, differences between treatment means were tested using the least significant difference (LSD) procedures.

RESULTS

Experiment 1

The addition of either Ca or fat to the diet did not significantly influence body weight gain or feed conversion ratio (table 2). However, increasing either dietary Ca or fat significantly increased pH, ($p < 0.01$ and $p < 0.05$ respectively). There was a significant interaction ($p < 0.01$) between Ca and fat levels, the addition of Ca or fat significantly increasing pH by approximately the same amount when added on their own, but no additive effect occurred when they were added together (table 3).

The effects of increasing dietary levels of Ca and fat on carcass composition of weight gain and on fatty acids are shown in tables 4 and 5, respectively. Variations in dietary levels of Ca and fat did not significantly affect either carcass dry matter or H₂O/N ratio. Increasing the dietary level of Ca significantly ($p < 0.01$) increased carcass concentration of linoleic acid and Ca ($p < 0.05$) and reduced palmitic and stearic acids concentrations ($p < 0.01$), whilst increasing dietary level of fat significantly ($p < 0.05$) increased carcass fat and linoleic acid concentrations ($p < 0.01$) and reduced carcass protein and palmitic acid concentrations ($p < 0.01$). However, the addition of fat and Ca together significantly increased carcass Ca and reduced protein contents ($p < 0.01$).

Table 2. The effect of dietary Ca and fat on body weight gain, FCR and intestinal pH of 19-day old meat chickens (Experiment 1)

Treatment	Body weight gain (g)	Feed conversion ratio (feed/gain)	Intestinal pH
Dietary Ca ¹			
Control Ca	291.9	1.85	5.91
High Ca	295.0	1.95	6.18**
Dietary fat ²			
Control fat	292.6	1.88	5.96
High fat	294.2	1.91	6.14*
SEM ³	7.2	0.07	0.06
Probability (P):			
Ca	0.1309	0.1354	0.0001
Fat	0.2369	0.3138	0.0289
Ca × Fat	0.3514	0.7954	0.0405

¹ Control Ca = 12.1 g Ca/kg diet; high Ca = 22.5 g Ca/kg diet;

² Control fat = 31 g fat/kg diet; high Fat = 111 g fat/kg diet;

³ Standard error of means;

Means within columns followed by different superscripts are significantly different. * = ($p < 0.05$); ** = ($p < 0.01$).

Table 3. The combined effects of dietary levels of Ca and fat on intestinal pH of 19-day old meat chickens (Experiment 1)

Diet	pH
Control Ca- Control fat	5.68 ^a
High Ca- Control fat	6.24 ^b
Control Ca- high fat	6.15 ^b
High Ca- high fat	6.12 ^a
LSD ¹ ($p < 0.05$)	0.24

^{a,b} Means followed by different superscripts are significantly different ($p < 0.05$).

¹ Least significant difference.

Experiment 2

The effects of Ca level on intestine length (cm), weight (g) and weight per unit of length are given in table 6 and on pancreas, heart and liver weights in table 7. The weight per unit of intestine length is highly correlated with thickness (Gordon and Bruckner-Kardoss, 1961 and Stutz et al., 1983). Neither of intestinal length and weight nor weights of the pancreas, heart or liver were significantly affected by dietary Ca level.

Table 4. The combined effect of dietary levels of Ca and fat on the composition of carcass weight gain of 19-day old meat chickens (Experiment 1)

Treatment	Weight Gain (g)	Dry weight (g) ¹	Fat (%) ⁴	Protein (%) ⁴	H ₂ O/N ratio	Ca (%) ⁴	Magnesium (%) ⁴
Dietary Ca ¹							
Control Ca	294.4	99.6	33.1	50.9	24.4	1.5	0.13
High Ca	284.5	93.9	32.7	48.4*	26.3	2.1*	0.11
Dietary fat ²							
Control fat	285.8	95.9	31.6	51.2	24.5	1.7	0.12
High fat	293.2	97.6	34.2*	48.1**	26.5	1.8	0.12
SEM ³	12.3	4.5	0.7	0.7	0.7	0.1	0.04
Probability (P):							
Ca	0.2083	0.2301	0.4845	0.0130	0.3518	0.0001	0.1003
Fat	0.3834	0.4034	0.0213	0.0001	0.1707	0.0727	0.4848
Ca × Fat	0.7811	0.3985	0.1577	0.0807	0.5862	0.2389	0.3801

¹ Control Ca=12.1 g Ca/kg diet; high Ca=22.5 g Ca/kg diet;² Control fat=31 g fat/kg diet; high Fat=111 g fat/kg diet;³ Standard error of means;⁴ Weight as % of dry weight;

Means within columns followed by different superscripts are significantly different, * = (p < 0.05); ** = (p < 0.01).

Table 5. The effects of dietary Ca and fat on carcass fatty acids composition (Experiment 1)

Treatment	Fatty acids (% of total methyl esters of carcass fat)				
	Palmitic 16:0	Stearic 18:0	Oleic 18:1	Linoleic 18:2	Linolenic 18:3
Dietary Ca ¹					
Control Ca	22.81	7.78	30.74	28.66	1.25
High Ca	19.93**	6.68**	31.27	31.29**	1.32
Dietary fat ²					
Control fat	23.57	6.42	26.65	32.60	1.33
High fat	17.73**	6.67	26.37	39.03**	1.16
SEM ³	0.35	0.16	0.46	0.44	0.78
Probability (P):					
Ca	0.0001	0.0001	0.1974	0.0001	0.5814
Fat	0.0001	0.4328	0.7053	0.0001	0.1467
Ca × Fat	0.4372	0.2118	0.3180	0.3731	0.1896

¹ Control Ca=12.1 g Ca/kg diet; high Ca=22.5 g Ca/kg diet;² Control fat=31 g fat/kg diet; high Fat=111 g fat/kg diet;³ Standard error of means;

Means within columns followed by different superscripts are significantly different, ** = (p < 0.01).

Table 6. The effect of dietary level of Ca on the weight and length and on the dry weight per unit of length of parts of the alimentary tract in 23-day old meat chickens¹ (Experiment 2)

Organ	Level of Ca (g/kg)	Weight (g)	Length ² (cm)	Dry weight/unit of length (g/cm)
Proventriculus	12.1	3.60 ± 0.23	—	—
	22.5	3.52 ± 0.19	—	—
Gizzard	12.1	24.12 ± 1.46	—	—
	22.5	23.61 ± 1.71	—	—
Duodenum	12.1	6.44 ± 0.51	24.94 ± 1.36	6.17 ± 0.29
	22.5	6.65 ± 0.68	27.75 ± 1.27	6.54 ± 0.28
Jejunum + Ileum	12.1	119.04 ± 1.07	121.18 ± 1.60	3.68 ± 0.07
	22.5	121.37 ± 6.63	126.01 ± 1.50	3.86 ± 0.28
Caeca	12.1	3.95 ± 0.29	30.81 ± 1.51	—
	22.5	4.27 ± 0.19	29.44 ± 0.73	—
Colon	12.1	1.07 ± 0.09	5.41 ± 0.30	—
	22.5	1.10 ± 0.08	5.75 ± 0.41	—

¹ Mean of eight chickens ± SE.² The length was measured under a constant tension of a 10 grams weight.³ Dry weight (g)/unit of length (cm) × 100 (mean of eight chickens ± SE).**Table 7.** The effect of dietary level of Ca on pancreas, heart and liver weights in 23-day old meat chickens¹ (Experiment 2)

Organ	Ca level (g/kg)	Fresh weight (g)	Dry weight (g)
Pancreas	12.1	2.23 ± 0.13	—
	22.5	2.48 ± 0.19	—
Heart	12.1	3.62 ± 0.09	—
	22.5	4.04 ± 0.21	—
Liver	12.1	17.74 ± 0.92	5.19 ± 0.27
	22.5	20.15 ± 1.12	5.87 ± 0.4

¹ Mean of eight chickens ± SE.

McDonald (1990) and Shafey (1993) who concluded that growing chickens could tolerate higher dietary levels of Ca without any significant effect on performance, providing the corresponding level of available phosphorus is also high. Whilst, the non-significant effects of dietary level of fat on body weight gain and FCR of chickens were in agreement with Whitehead et al. (1971) who reported that increasing dietary fat from 0 to 15% as maize oil did not significantly ($p < 0.05$) influence weight gain or FCR.

Intestinal pH was positively related to dietary levels of Ca and fat. McDonald and Solvyns (1964) using a similar technique reported that intestinal pH was significantly higher at 13 g/kg than at 9 g/kg dietary Ca, but that higher levels of Ca did not influence pH any further. This finding was not in agreement with Hurwitz and Bar (1968) and Mussehl et al. (1933) who concluded that chickens are able to adapt to a wide variety of dietary conditions without any change in digestive tract pH. However, March et al. (1958) showed that the intestinal pH could be altered by the addition of fat to the diet. Increasing pH of the gut content would alter the availability of some nutrients to the bird by increasing microbial activity in the gut (Coates et al., 1952) and reducing solubility and decreasing the absorption of some metallic ions (Shafey et

DISCUSSION

Results from these experiments demonstrated that excess dietary level of Ca from 12.1 to 22.5 g/kg did not adversely affect chicken performance, when available phosphorus was kept constant at 6.3 g/kg diet. These were in agreement with the results of Shafey and

al., 1991) and fatty acids (Shafey, 1998). Lev et al. (1957) found that an increase in intestinal microorganisms resulted in an increase in intestinal wall thickness and a reduction in its permeability to amino acids. Despite the non-significant increase in the intestinal weight per unit of length with high dietary level of Ca (22.5 vs. 12.1 g/kg), the trends in this study suggested that higher dietary levels of Ca than 22.5 g/kg may have a more pronounced effect on nutrient utilization and chickens performance.

The present study showed that the composition of the chicken body fat is highly susceptible to change by increasing dietary level of Ca or fat, whilst carcass fat content was not significantly altered by dietary treatments. Ca reacts with fat in the digestive tract resulting in the formation of Ca soaps that are excreted (Whitehead et al., 1971). The formation of insoluble soaps from divalent cations and fatty acids influences the metabolism of fatty acids and availability of Ca (Shafey, 1998). Excess dietary Ca caused a slight reduction in carcass fat content, whereas excess dietary fat increased carcass fat content by 2.6%. Excess dietary Ca reduces fat digestibility (Cheng et al., 1949) and increases faecal soap without any effect on total fat excretion (Shafey, 1998) and consequently alters the availability of fatty acids for absorption and deposition. Excess dietary Ca increased the deposition of linoleic acid in the carcass at the expense of palmitic and stearic acids, whilst the addition of vegetable fat to the diet increased carcass linoleic acid at the expense of palmitic acid deposition. As a result, the changes in C16 and C18 fatty acid concentration in the carcass will lead to a reduction in melting point of lipid and firmness of fat tissues.

Increasing dietary level of Ca and fat led to a reduction in carcass protein content by 2.5% and 3.1% and a slight increase in carcass water content by 1.9% and 2% respectively. Recently, Shafey (1998) showed that excess dietary Ca and fat reduced nitrogen utilization in growing chickens. Consequently, excess dietary levels of Ca and fat increased carcass water/nitrogen ratio by 7.5% and 8.2% respectively. These results were in agreement with those of Henry and Tohill (1962) and Delpech and Guillaume (unpublished, cited by Calet, 1967); they are at variance with the findings of Bender and Miller (1953) and Bender and Doell (1957) who reported that water/nitrogen ratio was constant, even in the presence of appreciable variation in body lipid content.

It was concluded that growing chickens tolerated a high dietary level of Ca (22.5 vs. 12.1 g/kg) in the presence of 6.3 g/kg of available phosphorus without any significant effect on performance. Intestinal pH was significantly increased by the addition of excess Ca and

fat that probably created the right pH for the formation of insoluble Ca soaps. Excess dietary Ca increased linoleic acid concentration in the carcass at the expense of palmitic and stearic acids, whilst the addition of vegetable fat to the diet increased linoleic acid concentration in the carcass at the expense of palmitic acid. Carcass content of fat was increased, while carcass protein content was reduced, by the addition of fat. Excess Ca or fat did not influence intestinal and visceral organ size and weight.

REFERENCES

- AOAC. 1980. Official Methods of Analysis. (W. Hurwitz ed.) 13th Ed., Association of Official Analytical Chemists, Washington, D.C.
- Bartov, I. 1979. Nutritional factors affecting quantity and quality of carcass fat in chickens. *Fed. Proc.* 38:2627-2630.
- Bender, A. E. and B. H. Doell. 1957. Biological evaluation of proteins: a new aspect. *Br. J. Nutr.* 11:140-148.
- Bender, A. E. and D. S. Miller. 1953. Constancy of the N/H₂O ratio of the rat and its use in the determination of the net protein value. *Biochem. J.* 53:VII-VIII.
- Calet, C. 1967. Methods for measuring Protein Quality With Chicks. In: "Protein Utilization by Poultry". pp. 16-47. (R. A. Morton and E. C. Amoroso eds.) Oliver and Boyd, Edinburgh and London.
- Cheng, A. L. S., M. G. Morehouse and H. G. Deuel. 1949. The effect of the level of dietary calcium and magnesium on the digestibility of fatty acids, simple triglycerides and some natural and hydrogenated fats. *J. Nutr.* 37:237-250.
- Coates, M. E., C. D. Dickinson, G. F. Harrison, S. K. Kon, J. W. G. Porter, S. H. Cummins and W. F. Cuthbertson. 1952. A mode of action of antibiotics in chick nutrition. *J. Food Sci. Agri.* 3:43-48.
- Farner, D. S. 1942. The hydrogen ion concentration in avian digestive tracts. *Poult. Sci.* 21:445-450.
- Fedde, M. R., P. E. Waibel and R. E. Burger. 1960. Factors affecting the absorbability of certain dietary fats in the chick. *J. Nutr.* 70:447-452.
- Folch, J., M. Lees and G. H. Sloane Stanley. 1957. A simple method for isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* 226:497-509.
- Gordon, H. A. and E. Bruckner-Kardoss. 1961. Effects of the normal microbial flora on various tissue elements of the small intestine. *Acta Anatomica*, 44:210-225.
- Henry, K. M. and J. Tohill. 1962. A comparison of the body-water and nitrogen balance-sheet methods for determining the nutritive value of proteins. *Br. J. Nutr.* 16:125-133.
- Hurwitz, S. and A. Bar. 1968. Activity, concentration, and lumen-blood electrochemical potential difference of calcium in the intestine of the laying hen. *J. Nutr.* 95:647-654.
- Lev, M., C. A. E. Briggs and M. E. Coates. 1957. The effects of bacteria on the mucosa of the intestine. *Br. J. Nutr.* 11:364-366.
- McDonald, M. W. and A. Solvyns. 1964. Dietary calcium levels and chickens growth. Proceedings, 1964 Australian Poultry Science Convention, Surfers Paradise, pp. 112-116.
- March, B., R. Tuckey and Y. J. Biely. 1958. The effect of diet

- on the intestinal tract of chicks. *Poult. Sci.* 37:405-410.
- Mussehl, F., M. J. Blish and C. W. Ackerson. 1933. Effect of dietary and environmental factors on the pH of the intestinal tract. *Poult. Sci.* 12:120-123.
- Nugara, D. and H. M. Edwards. 1970. Changes in fatty acid composition of cockerels testes due to age and fat deficiency. *J. Nutr.* 100:156-160.
- Shafey, T. M. 1993. Calcium tolerance of growing chickens: effect of ratio of dietary calcium to available phosphorus. *World's Poult. Sci. J.* 49:5-18.
- Shafey, T. M. 1998. Effects of dietary calcium, phosphorus, biotin and fat on the performance and nutrient utilization of meat chickens. *J. King Saud Univ.* in press.
- Shafey, T. M. and M. W. McDonald. 1990. Effects of dietary calcium: available phosphorus on calcium tolerance of broiler chickens. *Aust. J. Exp. Agri. Anim. Husb.* 30:483-490.
- Shafey, T. M., M. W. McDonald and J. Dingle. 1991. Effects of dietary calcium and available phosphorus concentrations on digesta pH and on the availability of calcium, iron, magnesium and zinc from the intestinal contents of meat chickens. *Br. Poult. Sci.* 32:185-194.
- Steel, R. D. G. and J. H. Torrie. 1980. *Principles and Procedures of Statistics* (McGraw-Hill Book Company, Inc., New York).
- Stutz, M. W., S. L. Johnson and F. R. Judith. 1983. Effect of diet, bacitracin and body weight restrictions on the intestine of broiler chicks. *Poult. Sci.* 62:1626-1632.
- Waibel, P. E. and F. R. Mraz. 1964. Calcium, strontium and phosphorus utilization by chicks as influenced by nutritional and endocrine variations. *J. Nutr.* 84:58-64.
- Whitehead, C. C., W. A. Dewar and J. N. Downie. 1971. Effect of dietary fat on mineral retention in the chick. *Br. Poult. Sci.* 12:249-254.