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Effects of Extra Dietary Calcium in Form of Fossil Shells Flour, or a Mixture of Fossil Shells Flour and Fermented Plant Concentrate on Growth Performance, Blood Cholesterol Levels, Abdominal Fat Content and Mechanical Bone Strength in Broilers

[1]F. Kanyinji, [1]T. Maeda and [2]N. Araki
[1]Graduate School of Biosphere Science, Hiroshima University, 1-4-4 Kagamiyama,
Higashi-Hiroshima 739-8528, Japan
[2]GaiaTec Co., Inc., 5-1 Nishimukouda, Satsumasendai, Kagoshima, 895-0027, Japan

Abstract: On the basis of experimental evidence showing that high Calcium (Ca) intake lowers blood Cholesterol (Ch) in rats, this study sought to evaluated the influence of extra dietary Ca in form of fossil shells (Aragonite) flour or its blended product (M-arago (a 1:1 mixture of Aragonite flour and fermented plant concentrate)) in broiler diets, on growth performance, blood Ch levels, Abdominal Fat Content (AFC) and bone mechanical strength in male broilers. Forty five 3 days old chunky male broiler chicks were selected by weight into 3 groups (15 chicks/treatment: 3 replications with 5 chicks/replicate)and fed *ad libitum,* diets containing 0 and 0.5% Aragonite flour, or 1% M-arago as extra Ca sources, until 70 days of age from hatching. Daily feed intakes, weight gains, weekly body weights and Feed Conversion Ratios (FCR) were assessed up to 63 days of age. Also, blood samples were collected at 3, 14, 28, 42, 56 and 70 days of age to measure Ch and Ca levels. At 70 days of age, all birds were sacrificedand the AFC and shanks were collected and weighed. Shank bones were tested for strength by measuring the fracture stress force required to break them. Daily feed intakes were similar for all the groups, but weight gains, FCR and body weights at 63 days of age were lower in treated birds than control. Blood Ch concentration was significantly lower in birds receiving extra Ca than control. In contrast, blood Ca levels in treated birds were higher than in control birds. AFC and bone mechanical strength were similar among groups. Thus, overall growth performance of treated birds was below that of control birds and decline in blood Ch and increase in blood Ca did not change their AFC or bone mechanical strength.

Key words: Fossil shells, fermented plant concentrate, extra calcium, weight gain, conversion ratio

INTRODUCTION

Over the last decades, occurrence of cardiovascular diseases has risen with increases in amounts and proportion of animal fat in the diets of humans (Katan, 2000; Ponte *et al*., 2004). Elevated intake of fats raise blood total Cholesterol (Ch) levels (Grundy and Denke, 1990; Sacks, 2002) and hypercholesterolemia is a risk factor for cardiovascular diseases. Although, diagnoses and treatments for cardiovascular diseases have advanced, the most important treatment is prevention and a major preventive measure is reducing intake of total fatty acids (Denke *et al*., 1993; Evans *et al*., 2002).

An alternative approach to reducing intake of total fatty acids would be to reduce Ch and fat contents in animals raised for meat. To this effect, attempts have previously been made to decrease Ch and fat contents in chicken meat by providing supplements such as garlic, copper or ω-3 fatty acids, to broilers (Pesti and Bakalli, 1996; Ayerza *et al*., 2002; Chowdhury *et al*., 2002). Despite these efforts, there is still a paucity of knowledge regarding alternative supplements that can achieve this important goal.

Recently, studies in humans and rats have shown that Calcium (Ca) intake above the usual dietary intake lowers blood serum total Ch concentrations (Ditscheid *et al*., 2005; Malekzadeh *et al*., 2007). Mechanisms proposed for this include saponification due to ionic binding of Ca^{2+} to saturated fatty acids, which interferes with their absorption from the small intestines into the blood stream (Grundy and Denke, 1990; Denke *et al*., 1993). Additionally, it is asserted that the negatively charged carboxyl group of the unconjugated bile acids binds to the positively charged Ca^{2+} to form precipitates that are excreted with feces, thereby withdrawing bile acids from enterohepatic circulation (Ditscheid *et al*., 2005). These two processes are supposed to result in a reduction in levels of total Ch in

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Corresponding Author: T. Maeda, Graduate School of Biosphere Science, Hiroshima University, 1-4-4 Kagamiyama, Higashi-Hiroshima 739-8528, Japan

blood. However, studies on the effects of high dietary Ca supplementation on Ch levels in poultry are scarce. Additionally, though blood ionized Ca is a highly regulated parameter under the strict control of parathyroid hormone, it is tempting to speculate that supplying extra dietary Ca to birds would increase mechanical strength of their bones since one primary function of Ca in animals is as a structural component of the bones.

Fossil shells (Aragonite) flour is a calcitic compound with 38% Ca (Finkelstein *et al*., 1993), which is similar to most Ca sources often used in animal feeding. In Japan, this compound is commercially available either as a pure product, or as a mixture (M-arago) of Aragonite flour and fermented plant concentrate mixed at 1:1 on w w^{-1} basis (GaiaTec Co., Inc., Kagoshima, Japan). The biological availability of Ca in Aragonite is similar to most common Ca sources, which ranges from 93-102% (Ross *et al*., 1984). Thus in theory, the hypocholesterolemic actions of conventional Ca sources observed in rats and humans should be consistent with Aragonite. However, published literature on whether or not high dietary Ca in form of Aragonite flour or M-arago in poultry diets can exert hypocholesterolemic effects in the birds is scarce. In addition, whether blending Aragonite flour with fermented plant concentrate would suppress growth performance of birds fed the mixture is not established.

Thus, the objective in this study was to evaluate the effects of feeding broilers with diets fortified with extra Ca in form of Aragonite flour or M-arago on their growth performance, concentrations of blood plasma total Ch and AFC. Additionally, because extra dietary Ca may impact on blood Ca levels, which may influence the degree of bone mineralization, the mechanical strength of their bones were also assessed by measuring the fracture stress force required to break the shank bones.

MATERIALS AND METHODS

Birds, management and diets: A total of 100 newly hatched male Chunky broiler chicks were purchased from a local hatchery (Fukuda, Okayama, Japan). Chunky broiler belongs to the Cornish line (Shen *et al*., 2002) and is the most commonly reared strain in Japan therefore, was the immediate available strain for the study. The chicks were reared according to guidelines stipulated by the Hiroshima University Animal Ethics Committee. Chicks were housed in an electrically heated battery brooder and fed a starter diet until 3 days of age. Then, 45 chicks with almost the same body weights (63.7-64.2 g) were selected and randomly assigned into 3 treatment groups so that there were 15 chicks/treatment (3 replications/treatment and 5 chicks/replicate). All the groups were put in one house. Two groups were randomly assigned a basal diet containing 0.5% Aragonite flour or 1% M-arago as sources of extra dietary Ca. The third group was the control and was fed a diet without any extra Ca source. Thus, the groups received (on dry weight basis): a basal diet without extra Ca (control); a basal diet fortified with extra Ca in form of Aragonite flour at 0.5% and a basal diet fortified with extra Ca in form of M-arago at 1%. All chicks had free access to drinking water and received 14 h of lighting each day until they were 70 days old. The basal diet, from a local feed mill (Nihonnousan Co. Ltd, Okayama, Japan), was a commercial starter (0-28 days) and finisher diets (28-70 days) formulated to meet requirements of the Japanese Feed Standards for Poultry (National Research Council of Agriculture, Forestry and Fishery, 2004).

The Ca and phosphorous contents of the experimental diets are shown in Table 1 and were measured using the inductively coupled argon plasma method (ICAP-575, Nippon Jarrel, Kyoto, Japan) after digesting a pre-weighed 1 mm ground sample with nitric acid and hydrogen peroxide. The other nutrients are as calculated. Both Aragonite flour and M-arago were supplied by a local mining company (GaiaTec Co., Inc., Kagoshima, Japan) and M-arago was a 1:1 (on w w^{-1} basis) mixture of Aragonite flour and fermented plant concentrate. Details of the process by which the fermented plant concentrate is made are not available due to copyright protection, but in short, it is the end product obtained when apples, oranges, rice, bananas, persimmons, soybean, pineapples, carrots, sea weeds, grapes, sesame, bee honey, garlic, peach and black sugar are mixed and fermented for more than 4 years in the presence of yeast, lactic acid forming bacteria and *Bacillus subtilis*.

Data collection: Body weights of the birds were recorded prior to commencement of the feeding trial and at 14, 21, 28, 35, 42, 49, 56, 63 and 70 days of age from hatching. Total feed leftover by each group was measured daily before putting in fresh feed to determine the day's intake. Daily weight gain and FCR were calculated up to 63 days of age.

Blood sample collection: A 1~2 mL blood sample from each bird in each group was collected prior to commencement of dietary treatment and at 14, 28, 42, 56 and 70 days of age into test tubes treated with heparin for measurement of concentrations of blood plasma total Ch and Ca. At 3 and 14 days of age, blood was collected from the chick's jugular vein in the neck area using sterilized 27 gauge needles and 1 mL syringes. Thereafter, samples were collected from the brachial wing vein using sterilized 23 gauge needles and 2.5 mL syringes. No mortalities due to bleeding were recorded at 3 and 14 days of age. Blood samples were centrifuged at 1, 000× g for 10 min at 5°C

Table 1: Nutrient composition of control and diets supplemented with Aragonite flour or M-arago

			Dietary supplement			
	Control		Aragonite flour		M-arago*	
Nutrient (%)[1]	Starter	Finisher	Starter	Finisher	Starter	Finisher
Crude protein	22	18	22	18	22	18
Crude fat	4.0	4.5	4.0	4.5	4.0	4.5
Crude fibre	5.0	5.0	5.1	5.0	5.1	5.2
Ash	8.0	8.0	8.0	8.0	8.0	8.0
Calcium[2]	0.80	0.70	0.99	0.89	0.98	0.90
Phosphorous[2]	0.50	0.55	0.51	0.56	0.51	0.56
Metabolizable energy (kcal)	3100	3200	3100	3200	3100	3200

[1]All nutrients (except calcium and phosphorous) were calculated. [2]Measured from feed leftovers using inductively coupled Argon plasma method (ICAP-575, Nippon Jarrel, Kyoto, Japan)

and the supernatant was used to determine total Ch and Ca concentrations. Total Ch and Ca were determined colorimetrically, using locally available commercial diagnostic kits (Wako Tcho 439-17501 and Wako Ca 272-21801) supplied by Wako Pure Chemical Industries Ltd., Tokyo, Japan.

AFC and Bone strength: At 70 days of age, all birds were sacrificed by decapitating and allowing them to bleed into a conical bucket. They were then immersed in water at 70°C for 1 min and defeathered before chilling the carcasses at 8°C overnight and removing the shanks for weighing and testing for mechanical strength. Also, AFC was measured by removing and weighing all the adipose tissues surrounding the gizzard, cloaca and adjacent muscles as described by Kubena *et al.* (1974).

To test for mechanical strength of the bones, muscles and connective tissues were first manually removed from the shank bones before drying them at 55°C in a forced air oven until a constant air dry weight was obtained. Then, the fracture stress force required to break the bones was measured using a three-point bending rheolometer (Autograph AGS-H, Shimadzu, Kyoto, Japan).

The principle of the breaking property test is shown in Fig. 1 and the illustrations were adopted from Omi and Ezawa (1993) and Takada *et al.* (1997). Breaking of diaphysis of the bone was conducted under the following measurement conditions: the sample space was 1.0 cm, the plunger speed was 60.0 cm sec^{-1}, the load range was 50.0 kg and the chart speed was 60.0 cm min^{-1}.

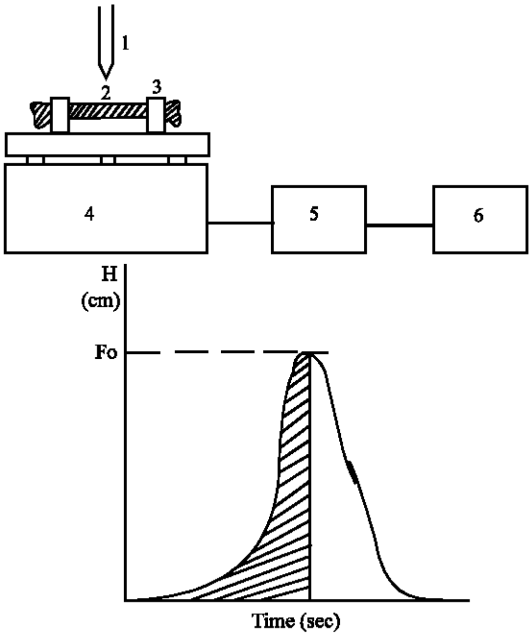

Fig. 1: Measurement of fracture stress force of shank bone by rheolometer (*Adopted from* Omi and Ezawa, 1993; Takada *et al*., 1997). 1: Plunger, 2: Bone sample (Shank bone), 3: Sample holder, 4: Load cell, 5: Amplifier, 6: Recorder, Fo: Full scale of load cell = 50 kg force, H: Chart full length = 2.5 cm

Statistical analysis: All data collected were analyzed using JMP® 8.0.1 statistical software program developed by SAS institute, Carry, NC. Growth parameters (i.e., daily feed intake, daily weight gain, weekly FCR and weekly body weights) and concentration of blood total Ch and Ca were analyzed using two-way ANOVA for multiple comparisons, with treatment group and age as factors based on the mathematical model:

$$Y_{ijkl} = \mu+T_j+P_j+TP_{ij}+A_k+e_{ijkl}$$

Where:

Y_{ijkl} = The observation
μ = The overall population mean
T_i = The effect of treatment
P_j = The effect of age
Tp_{ij} = The interaction effect of T_i and P_j
A_k = The effect of the bird
e_{ijkl} = Random error

Table 2: Daily feed intake, weight gains, FCR and weekly body weights at different ages of male broilers supplemented with Aragonite or M-arago

		Age from hatching (day)								
Parameters	Groups	14	21	28	35	42	49	56	63	Averaged over time
Average daily feed intake (g/bird/d)	Control	32.9 ± 3.7^{a}	82.2 ± 7.9^{b}	130.0 ± 5.8^{c}	168.0 ± 4.8^{de}	202.5 ± 4.5^{f}	198.1 ± 8.5^{fg}	189.9 ± 9.2^{fgh}	158.2 ± 6.2^{ei}	145.2 ± 21.4^{A}
	Aragonite	32.6 ± 3.6^{a}	80.0 ± 7.6^{b}	127.2 ± 6.7^{c}	165.2 ± 4.5^{de}	205.4 ± 4.4^{f}	200.4 ± 8.3^{fg}	171.8 ± 4.6^{deh}	140.2 ± 9.2^{ci}	140.4 ± 21.1^{A}
	M-arago	32.4 ± 3.5^{a}	73.3 ± 6.4^{b}	130.8 ± 6.3^{c}	162.4 ± 4.8^{e}	203.0 ± 3.8^{f}	201.5 ± 3.8^{fg}	181.9 ± 1.7^{fgh}	167.4 ± 8.3^{de}	144.1 ± 21.9^{A}
Average daily weight gain (g/bird/day)	Control	26.2 ± 0.9^{ab}	61.3 ± 1.8^{acde}	73.5 ± 1.3^{cde}	91.7 ± 2.0^{cd}	93.5 ± 2.1^{cd}	79.4 ± 4.5^{cd}	73.2 ± 7.6^{cd}	29.6 ± 22.0^{ab}	66.1 ± 9.1^{A}
	Aragonite	26.3 ± 0.9^{ab}	59.3 ± 2.0^{acde}	74.2 ± 2.2^{cde}	88.7 ± 2.5^{cd}	98.9 ± 3.6^{c}	84.2 ± 6.4^{acde}	62.5 ± 24.6^{a}	21.4 ± 17.2^{b}	64.4 ± 10.0^{A}
	M-arago	25.0 ± 1.6^{ab}	54.4 ± 2.3^{abde}	73.3 ± 4.1^{cde}	87.7 ± 4.3^{cd}	97.9 ± 3.6^{cd}	82.7 ± 8.9^{cd}	63.7 ± 15.8^{acde}	16.7 ± 30.5^{f}	62.7 ± 10.3^{B}
FCR	Control	0.80 ± 0.03^{a}	0.75 ± 0.02^{a}	0.57 ± 0.01^{b}	0.55 ± 0.01^{bc}	0.46 ± 0.01^{bcdef}	0.40 ± 0.02^{ef}	0.41 ± 0.04^{de}	0.21 ± 0.09^{g}	0.52 ± 0.1^{A}
	Aragonite	0.81 ± 0.03^{a}	0.74 ± 0.02^{a}	0.58 ± 0.02^{b}	0.54 ± 0.02^{bcd}	0.48 ± 0.02^{bcde}	0.42 ± 0.03^{cdef}	0.27 ± 0.13^{g}	0.12 ± 0.10^{h}	0.48 ± 0.1^{B}
	M-arago	0.77 ± 0.05^{a}	0.74 ± 0.03^{a}	0.58 ± 0.03^{b}	0.54 ± 0.03^{bcd}	0.48 ± 0.02^{bcde}	0.38 ± 0.05^{ef}	0.33 ± 0.08^{fg}	0.09 ± 0.16^{h}	0.49 ± 0.1^{B}
Average body weight (g/bird)	Control	325 ± 9^{a}	755 ± 21^{b}	1269 ± 26^{c}	1911 ± 30^{d}	2563 ± 32^{e}	3121 ± 42^{f}	3623 ± 83^{gh}	3872 ± 207^{g}	2180 ± 470^{A}
	Aragonite	327 ± 9^{a}	742 ± 22^{b}	1261 ± 34^{c}	1882 ± 48^{d}	2574 ± 66^{e}	3163 ± 56^{f}	3358 ± 175^{i}	3680 ± 202^{ghi}	2123 ± 447^{AB}
	M-arago	314 ± 16^{a}	695 ± 31^{b}	1207 ± 58^{c}	1821 ± 85^{d}	2506 ± 97^{e}	3052 ± 52^{f}	3447 ± 157^{i}	3593 ± 266^{hi}	2079 ± 446^{B}

Values are means±SE for 15 broilers per group. $^{a-i}$Means within same row and column for each parameter with different superscripts differ ($p<0.05$). $^{A, B}$Means within the same column for each parameter with different superscripts differ ($p<0.05$). FCR: Feed Conversion Ratio

Shank weight, AFC weight and bone fracture stress force were analyzed using one-way ANOVA based on the same mathematical model except that the interaction effect (TP_{ij}) was not included. When ANOVA indicated significant difference, the student's t-test was used to compare differences. Differences among means with $p<0.05$ were accepted as representing statistical differences. However, differences among means with $p<0.05$-0.10 were accepted as representing tendencies to differ.

RESULTS AND DISCUSSION

Growth parameters: Average daily feed intake increased with age in all treatment groups, attaining peak intakes at 42 days of age (Table 2). Thereafter, intakes did not change significantly until 56 days of age when they decreased in birds in control ($p = 0.02$) and Aragonite flour ($p = 0.002$) groups. Decline in intake in birds receiving M-arago supplement was not significant between 56 and 63 days of age. Between treatments, intakes were similar for all treatment groups at all ages, except at 63 days of age, at which control birds tended ($p = 0.07$) to exhibit lower intake than those receiving Aragonite flour. When compared to M-arago supplemented group, feed intake by control birds was significantly lower ($p = 0.02$). Overall, feed intakes were, however, similar for all groups.

The trend in daily weight gain in each group was similar to that of feed intake. Peak weight gains were attained at 42 days of age in all the groups. Thereafter, daily gains did not change in all groups until 56 days of age when control birds exhibited a tendency ($p = 0.08$) to gain less. Surprisingly, gains of birds treated with M-arago significantly ($p = 0.001$) decreased compared to control despite their feed intake being higher. No differences were however, observed between groups at all ages, except at 56 and 63 days of age. At 56 days of age, birds fed Aragonite flour or M-arago had similar weight gains that were significantly lower ($p = 0.03$) than control birds. Birds receiving M-arago had lower weight gain compared to either control ($p = 0.002$) or Aragonite flour ($p = 0.03$) group at 63 days of age. Overall weight gain was significantly lower in birds receiving M-arago compared to control ($p = 0.003$) or Aragonite flour ($p = 0.05$) fed birds.

There was significant difference ($p = 0.05$) in overall FCR between control birds and those that received M-arago or Aragonite flour. Also, FCR decreased with age ($p = 0.001$) in all treatment groups. FCR for control birds was lower than that of Aragonite flour ($p = 0.02$) or M-arago ($p = 0.004$) supplemented groups. Significant decreases were also observed between 21 and 28 days of age ($p = 0.001$) for all groups, 49 and 56 days of age ($p = 0.003$) for Aragonite flour group, as well as between 56 and 63 days of age for the control ($p = 0.004$) and M-arago ($p = 0.006$) groups. Only a tendency to decline was detected between 35 and 42 days of age ($p = 0.09$) and 42 and 49 days of age ($p = 0.06$) in all treatment groups. Between treatments, FCR among groups were similar at all ages, except at 56 days of age, where birds treated with Aragonite flour exhibited significantly lower FCR than control ($p = 0.001$) or M-arago ($p = 0.04$) groups. No differences were observed at this age between control birds and those that received M-arago.

Average body weights significantly increased with age ($p = 0.001$) from the initial weights and were similar for all groups at all ages, except at 56 and 63 days of age. At 56 days of age, body weights of birds in the control group were significantly higher than those fed Aragonite flour ($p = 0.01$) or M-arago ($p = 0.04$) and continued to be so at 63 days of age when compared to M-arago ($p = 0.03$) but was similar to Aragonite flour-fed birds. Overall, body weights of birds fed M-arago were significantly lower ($p = 0.01$) than those in the control group.

Levels of Ch and Ca in blood plasma: Mean total Ch and Ca concentrations at different ages are shown in Table 3.

Table 3: Concentrations of blood Ch and Ca at different ages of male broilers supplemented with Aragonite or M-arago

	Blood Ch and Ca concentration (mg dL^{-1})					
	Dietary supplement			Dietary supplement		
Age from hatching (days)	Control	Aragonite	M-arago	Control	Aragonite	M-arago
3	227.6±21.0^{a}	228.9±21.0^{a}	226.7±21.0^{a}	9.8±1.1^{ab}	9.6±1.1^{ab}	9.7±1.1^{ab}
14	124.7±5.4^{bc}	113.9±6.7^{bcd}	101.1±4.9^{cd}	2.8±0.8^{c}	3.3±1.0^{cd}	3.9±1.4^{cde}
28	124.4±6.9^{bc}	113.9±5.9^{bcd}	101.8±5.6^{cd}	3.7±1.0^{cde}	4.4±1.3^{de}	5.1±1.8^{e}
42	138.6± 12.4^{b}	117.8±4.2^{bcd}	114.1±4.1^{bcd}	7.8±2.1^{f}	9.3±2.7^{bf}	9.9±2.1^{ab}
56	109.4±6.3^{bcd}	96.2±6.7^{cd}	81.7±10.5^{d}	10.3±0.3^{ab}	11.1±0.2^{ag}	11.3±0.4^{g}
70	85.2±10.4^{bcd}	112.1±11.6^{bcd}	96.6±12.8^{cd}	9.0±0.8^{bf}	10.2±2.4^{abg}	11.7±0.3^{g}
Averaged over time	135.0±6.8^{A}	130.5±4.8^{AB}	120. ±5.1^{B}	7.2±0.4^{A}	8.0±0.6^{AB}	8.6±0.3^{B}

Values are means±SE for 15 broilers per group. $^{a,\,b}$Means within same row and column for each parameter with different superscripts differ ($p<0.05$). $^{A,\,B}$Means within the same row for each parameter with different superscripts differ ($p<0.05$). Ch: Cholesterol; Ca: Calcium

Table 4: Weights of shanks and AFC, as well as shank bone fracture stress force of male broilers supplemented with Aragonite or M-arago

		Dietary supplement	
Parameters	Control	Aragonite	M-arago
Shanks (g/bird)	163.9±10.0	163.3±6.3	163.2±13.6
AFC[1]	19.9±2.3	19.6±2.1	14.4±1.8
Bone fracture stress force (10^{7} N m^{-2})[2]	2.1±0.3	2.3±0.2	3.3±0.7

Values are means±SE for 15 broilers per group. [1]Expressed as g kg^{-1} body weight. [2]Amount of energy required to break the bone expressed as Newton m^{-2}. AFC: Abdominal Fat Content

Total Ch levels in all treatment groups declined (p = 0.001) between 3 and 14 days of age. However, Ch concentrations from 14-70 days of age were similar in each group. When averaged over time, Ch concentration in control birds tended to be high compared to those that received Aragonite flour (p = 0.09) and significantly higher (p = 0.03) than the M-arago fed birds.

Similarly, Ca concentration in all the groups significantly decreased (p = 0.001) between 3 and 14 days of age. Thereafter, increases in Ca levels to that recorded at 28 days of age in all groups were not significant, though birds receiving extra Ca in their diets showed a tendency (p = 0.09) to increase. From 28-56 days of age, Ca levels increased with age ($p<0.05$) in all groups and concentrations recorded at 56 days of age were similar to those measured at 70 days of age. No differences were detected between Aragonite flour-fed group and those that received M-arago between 14 and 70 days of age.

However, a comparison between control group and M-arago showed similarity in Ca concentrations at 14 days of age and a tendency (p = 0.06) to be low in control birds at 28 days of age. Beyond the age of 28 days, control birds had significantly lower blood Ca levels than birds supplemented with M-arago at 42 (p = 0.03), 56 (p = 0.04) and 70 days of age (p = 0.01). When averaged over time, birds fed Aragonite flour or M-arago exhibited higher (p = 0.001) blood Ca levels than control group. No differences were detected between Aragonite fed group and those that received M-arago.

AFC and bone strength: The effects of the supplemental Ca from the two sources on weights of fresh shanks, AFC and mechanical strength of shank bones are shown in Table 4. Dietary treatment with Aragonite flour or M-arago did not affect AFC or mechanical bone strength. Only a tendency to be low in birds supplemented with M-arago (p = 0.007) or Aragonite flour (p = 0.09) was detected. No differences were observed between control and Aragonite flour supplemented birds. Similarly, comparison of data on shank bone strength as measured by fracture stress force did not yield any significant differences or tendencies to differ among groups.

Though, the feed intake parameter indicates that extra dietary Ca in form of Aragonite flour or M-arago did not affect the growth performance of the birds, daily weight gain, FCR averaged over time, as well as body weights at 63 days of age suggest otherwise. In the absence of data on nutrient digestibility, it appears that the rate of passage of digesta may have been higher in birds fed Aragonite flour or M-arago than in control birds, particularly after 49 days of age. This allowed for both greater feed intake and less gut fill and increase in rate of passage would normally reduce digestibility, which may account for the lower daily weight gain, FCR and body weights observed in treated birds at 63 days of age. Therefore, it is likely that the supplements may have had laxative effects in the birds. Perhaps this phenomenon was more severe in birds supplemented with M-arago than those that received Aragonite flour because of the presence of plant materials in the fermented plant concentrate.

Shafey *et al.* (1990) had observed an improvement in weight gains when extra Ca was supplemented at 1%. We thus, also expected an improvement in growth performance in birds that received extra Ca. This, however, did not happen and probably, may not be surprising considering the level of supplementation administered. Additionally, the inconsistence of the results with those of Shafey *et al.* (1990) is not without precedence. In fact, not all studies on dietary Ca supplementation in broilers have produced consistent results. For example, in a study by Hurwitz *et al.* (1995) excess Ca suppressed growth in broilers, but Scheideler *et al.* (1995) and Smith *et al.* (2003) did not observe any negative effects of high dietary Ca on growth performance in their studies. In the present study, the highest level of Ca was 0.99%, which was below that of Shafey *et al.* (1990), therefore may not have been high enough to significantly change growth parameters, particularly during the first 49 days of the study. Additionally, Fleming (2008) suggested that current recommended Ca levels in broiler feeds may no longer be optimal because growth rates in broilers have improved considerably over the years without corresponding adjustments in Ca levels in their diets (Driver *et al.*, 2005). Thus, the 0.5% Aragonite and 1% M-arago added to the feed in the present study may have just contributed to the normal Ca requirements of the birds. Furthermore, the extra Ca was not part of the principal nutrients required for growth and it appears that the fermented plant concentrate did not alter feed palatability or biological availability of principal nutrients in the first 49 days. However, the laxative effects of the supplements after 49 days of age resulted into suboptimal overall growth performance of treated birds compared to control birds.

Initial blood Ch levels were higher than those recorded on day 14 in all the groups (Table 3). This was as expected because chicks emerge from eggs with large deposits of Ch in their blood (Tarugi *et al.*, 1989). Thus, decline in Ch levels during the first 14 days of the study were not attributed to the actions of extra Ca in the diets. Rather, it may have been due to accelerated conversion of blood Ch to bile acids, an increased secretion of Ch into the bile or into the circulation as a component of lipoproteins, or the utilization of Ch for the synthesis of membranes of proliferating hepatocytes (Tarugi *et al.*, 1989). Though, Ch concentration, when averaged over time, only tended to be higher in control birds than those receiving Aragonite flour, it was significantly higher than in birds fed M-arago. A statistical tendency to differ or an actual difference in this parameter between control and treated birds was attributed to the effects of extra Ca in the diets. According to Grundy and Denke (1990), Denke *et al.* (1993) and Ditscheid *et al.* (2005), extra dietary Ca lowers blood Ch by disrupting the absorption of lipids into the general circulation, as well as by preventing the recycling of bile acids into the blood stream. Moreover, these mechanisms may have been more intense in birds fed M-arago than in those that were on Aragonite flour treatment, probably because of the presence of certain ingredients in M-arago that were not in Aragonite flour. For example, sea weed and garlic in the plant concentrate used to make M-arago, which have been shown to exert hypocholesterolemic effects in animals (Sundaram *et al.*, 1997; Chowdhury *et al.*, 2002).

Similar to the trend of blood Ch, blood Ca also decreased during the first 14 days of this study. Williams *et al.* (1998) observed a rapid cortical bone formation and mineralization between 4 and 18 days of age in male broiler chickens. Accordingly, initial decline in blood Ca may have been due to increased demand for Ca for bone development. Overall, supplemented birds exhibited higher blood Ca concentrations than control group between 14 and 56 days of age, which was consistent with observations of Smith *et al.* (2003), Fujita *et al.* (1988), as well as Omi and Ezawa (1993). Increase in blood Ca was attributed to high biological availability of Ca in Aragonite flour (Ross *et al.*, 1984) and M-arago, coupled with its subsequent absorption in the intestines. Additionally, it appears that the fermented plant concentrate in M-arago did not alter Ca availability.

Another finding worth noting was that feeding extra dietary Ca to broilers did not change the strength of their shank bones or weight of their AFC (Table 4). When Larsen *et al.* (2000) investigated the effects of feeding 3 levels of dietary Ca (0.35, 0.94 and 1.64%) in growing pigs on their bone mass, it was concluded that 1.64% level resulted in a higher bone mass than 0.35 or 0.94%. Waldenstedt (2006) suggested that optimal Ca required for bone calcification, which affects bone strength, is much higher than that for growth. In the study, the level of total Ca in the diets (0.99% as the highest) were much lower than the 1.64% that increased bone mass in the study of Larsen *et al.* (2000). Therefore, it was not high enough to significantly improve bone strength.

About 80-85% of the fat that accumulates in the adipose tissue in broilers approaching market weight is derived from plasma lipids (Griffin *et al.*, 1992). Despite observing a tendency to be low in the weight of fat pad in treated birds, we could not attribute this directly to the effect of the supplements. This was because feed intake had drastically reduced two weeks prior to assessing AFC at 70 days of age as incidences of leg problems increased. This implied that the tendency to be low in AFC in treated birds may have been due to fat loss as a result of starvation caused by their failure to eat.

CONCLUSION

Extra dietary Ca in form Aragonite flour or M-arago did not affect growth performance of the birds during the first 49 days of the study, but had laxative effects during the last few weeks of the study, which resulted into below par overall growth performance of treated birds.

Additionally, though the extra dietary Ca exerted hypocholesterolemic actions in the birds especially in those that were fed M-arago, this was not effective enough to reduce AFC and perhaps, the level was not high enough to improve bone mechanical strength.

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