Journal of Animal and Veterinary Advances 10 (11): 1394-1400, 2011
ISSN: 1680-5593

Individual Plasma Lipoprotein Cholesterol Levels in Breeding Barred Plymouth Rock Male Chickens are Influenced by Additional Calcium in the Diet

[1]F. Kanyinji and [2]T. Maeda
[1]Department of Animal Sciences, Natural Resources Development College,
P.O. Box 310099, Chelstone, Lusaka, Zambia
[2]Japanese Avian Bioresource Project Research Center, Hiroshima University,
Higashi-Hiroshima, Japan

Abstract: Low Density Lipoprotein Cholesterol (LDL-Ch) is the main risk factor for atherogenesis. The efficacy of cardio-protective supplements may therefore be judged based on their ability to reduce LDL-Ch concentration in the blood. Additional dietary Calcium (Ca) decreases blood Total Ch (TCh) concentration. Thus researchers evaluated the influence of additional dietary Ca in form of fossil shells (Aragonite) on total Ch (TCh), LDL-Ch, High Density Lipoprotein Ch (HDL-Ch), Tiglycerols (TG) and Non-esterified Fatty Acids (NEFA) concentrations in chickens. Thirty 21 weeks old Barred Plymouth Rock roosters were fed diets containing 0, 2 or 4% Aragonite flour from 21-53 weeks of age. At 21, 25, 29, 34, 37, 42, 46, 51 and 53 weeks of age, birds were individually weighed and TCh, LDL-Ch, HDL-Ch, TG and NEFA levels in blood were measured. Body weights and NEFA levels were similar among treatments. Treated males showed significantly lower TCh, LDL-Ch and TG levels but higher HDL-Ch concentration than control males. However, these parameters were similar between treated males. Thus, additional dietary Ca in form of Aragonite flour did not affect the nutritional status of the birds when fed at 2 or 4% but beneficially altered levels of TCh, LDL-Ch, HDL-Ch and TG in blood.

Key words: Additional calcium, high density lipoproteins, low density lipoproteins, total cholesterol, triglycerols, nutritional status

INTRODUCTION

The role of blood plasma Total Cholesterol (TCh) as a factor in stimulating atherogenesis is now widely recognized by health experts. Concentration of TCh in the blood is strongly influenced by the quantity and quality of fat in the diet. For this reason, most previous studies focused on the effects of diet on blood TCh concentrations. However, recently attention has shifted to individual lipoprotein Ch fractions, i.e., low density lipoprotein Ch (LDL-Ch) and high density lipoprotein Ch (HDL-Ch). This has been prompted by the discovery that most of the Tch in the blood is contained in the LDL-Ch fraction and that it is this component that has the most undesirable effects on health. Though LDL-Ch provides cells with their Ch requirements, its excessive supply causes atherosclerosis (Castelli *et al*., 1986; Ohlsen and Rogers, 2004a; Stanford, 2005) whereas high HDL-Ch levels are cardio-protective, independent of other blood lipid measures (Castelli *et al*., 1977; Ohlsen and Rogers, 2004b). On this basis, LDL-Ch levels or ratio of LDL-Ch to HDL-Ch have been used as summary measures of risks of atherosclerosis.

Although, diagnoses and treatment for cardiovascular diseases have advanced the most important treatment is prevention and a major preventive measure is averting hypercholesterolemia. Daily dietary supplementation with Calcium (Ca) from conventional Ca carbonates has been reported to modify blood lipid profiles towards low TCh and LDL-Ch concentrations in humans (Denke *et al*., 1993; Reid, 2004; Ditscheid *et al*., 2005), rats (Vaskonen *et al*., 2002; Sun *et al*., 2004), rabbits (Renaud *et al*., 1983; Hsu and Culley, 2006) and goats (Hines *et al*., 1985). The mechanisms by which it achieves this are attributed to the mineral's propensity to bind with fatty acids (Grundy and Denke, 1990) and bile acids (Lupton *et al*., 1994; Ditscheid *et al*., 2005) in the gut which interferes with their absorption into the blood circulatory system. Thus, a reduction in the absorption of fats, especially saturated fatty acids,

Corresponding Author: Francisco Kanyinji, Department of Animal Sciences, Natural Resources Development College (NRDC), P.O. Box 310099 Chelstone, Lusaka, Zambia

decreases not only blood Tch but also LDL-Ch concentrations (Grundy and Dunke, 1990; Vaskonen, 2003) whereas a decrease in the re-absorption of bile acids induces an increase in the removal of Ch from the circulatory system by the liver for conversion to bile acid (Vaskonen *et al*., 2002). These physiological processes cause an overall reduction in TCh and LDL-Ch levels in the blood.

Fossil shells (Aragonite) flour is a Ca carbonate of marine origin with 38% Ca (Finkelstein *et al*., 1993). The biological availability of its Ca is similar to most conventional Ca carbonates (Ross *et al*., 1984). This compound is commercially available in Japan (GaiaTec Co., Inc., Kagoshima, Japan) and may be used as a possible therapeutic agent for cardio-protection. However, because its origin (i.e., marine animal/plant fossils vs mineral deposits) and chemical structure are different from most conventional Ca sources (Ajakaiye *et al*., 1996), its physiological effects on individual plasma lipoprotein Ch profiles may also be different but scientific evidence for this conclusion is not readily available in the literature.

Recently, the laboratory evaluated the hypocholesterolemic potential of Aragonite flour in chickens. The results from these studies showed that additional dietary Ca in form of Aragonite flour had indeed Tch-lowering actions in both male broilers (Kanyinji *et al*., 2010) and breeding Barred Plymouth Rock males (Kanyinji and Maeda, 2010). However, in these studies, no attempts were made to measure levels of individual lipoprotein Ch fractions of TCh (i.e., LDL-Ch and HDL-Ch) in the blood. Since LDL-Ch fraction is the main atherogenic agent (Kannel *et al*., 1986; Isles and Paterson, 2000; Ohlsen and Rogers, 2004a) whereas HDL-Ch is cardioprotective (Miller and Miller, 1977; Castelli *et al*., 1986; Ohlsen and Rogers, 2004b), the health benefits gained from the hypocholesterolemic characteristics of Aragonite can therefore, best be judged based on its influence on levels of LDL-Ch and HDL-Ch. Moreover, Ohlsen and Rogers (2004a) argued that the risks of coronary heart diseases cannot be evaluated based on levels of TCh alone but also on levels of individual lipoprotein Ch fractions.

Therefore, the present research sought to evaluate the influence of feeding 2 or 4% additional Ca in form of Aragonite flour to breeding Barred Plymouth Rock males on levels of TCh and its individual lipoprotein fractions in the blood. Additionally because high dietary Ca in the diet precipitates lipids in the gut thereby inhibiting their digestion and absorption despite lipids being essential for normal growth and development (Hulan *et al*., 1984), researchers endeavored to establish the effects of additional dietary Ca on the nutritional status of the birds fed additional dietary Ca by measuring body weights and levels of Non-Esterified Fatty Acids (NEFA).

MATERIALS AND METHODS

Animals, diets and experimental design: The birds used in this study were managed according to the guidelines outlined by the Hiroshima University Animal Ethics Committee. The chickens, diets and details of the design of the experiment are as described in Kanyinji and Maeda (2010). Briefly, thirty 21 weeks old Barred Plymouth Rock roosters were divided into three groups based on their body weights and put in individual cages under one house. The birds were fed *ad libitum* breeders diet (Nishinihon Feed Co. Ltd., Okayama, Japan) fortified with 0, 2 or 4% Aragonite flour (GaiaTec Co., Inc., Kagoshima, Japan) as source of additional dietary Ca until 53 weeks of age. At 21, 25, 29, 34, 37, 42, 46, 51 and 53 weeks of age, body weights of individual males were recorded and a 1~2 mL blood sample from each bird was also collected into 15×85 mm Borosilicate disposable culture test tubes (CAT No. 14-961-28 Fisher brand, Thermo Fisher Scientific Inc., MA, USA) treated with heparin (Novo-Heparin, Mochida Pharmaceutical Co., Ltd., Tokyo, Japan). The samples were then centrifuged at 1,000×g for 15 min at 5°C and the plasma obtained was stored frozen at -20°C until analysis.

Laboratory analysis: Frozen plasma samples of all males were thawed at room temperature (25°C) for determination of levels of TCh, LDL-Ch, HDL-Ch, Triglycerols (TG) and NEFA. These parameters were measured from a 20 μmL plasma sample of each bird at each sampling age using an automated Beckman Coulter AU480 instrument (Beckman Coulter Inc., Fullerton, CA, USA).

Data analysis: The data were analyzed as repeated measure data using PROC MIXED of SAS (2003) based on the mathematical model:

$$Y_{ijk} = \mu+L_i+T_j+(LT)_{ij}+A_{ik}+\varepsilon_{ijk}$$

Where:

Y_{ijk} = The observation
μ = The overall mean
L_i = The fixed effect of Ca level (treatment group)
$(LT)_{ij}$ = The fixed interaction effect of L_j and T_j
A_{ik} = The random effect of the individual bird within L_i
ε_{ijk} = The random error

Age of the birds at which blood was sampled was used as repeated measure. Means were compared using Tukey test (SAS, 2003). Differences among means with

$p<0.05$ were accepted as representing statistically significant differences. However, differences among means with $0.05<p<0.10$ were accepted as representing statistical tendency to differ.

RESULTS

The mean body weights recorded at 21, 25, 29, 34, 37, 42, 46, 51 and 53 weeks of age are shown in Fig. 1a. Initially, there was a tendency ($p = 0.08$) to decline in body weights in all treatment groups but from 25 weeks of age onwards, they increased with time ($p = 0.02$). No differences ($p = 0.98$) in this parameter were observed between treated males and those in the control group nor between 2 and 4% groups ($p = 0.73$).

Concentration of NEFA in treated birds and those fed control diet are shown in Fig. 1b. Initially, levels of NEFA in all treatment groups were high but declined significantly with time ($p = 0.05$) until 25 weeks of age after which they stabilized. However, no differences ($p = 0.62$) were observed between treated birds and those fed control diets at all ages. Likewise, NEFA levels between birds fed diets with 2 or 4% Aragonite flour were similar ($p = 0.91$).

Blood TCh levels in Barred Plymouth Rock roosters fed diets with or without additional Ca source between 21 and 53 weeks of age are shown in Fig. 2a. From 25 weeks onwards, males receiving additional Ca (i.e., 2 or 4% additional Ca groups) exhibited lower blood total Ch levels than birds fed control diet ($p = 0.01$) but no differences ($p = 0.80$) were observed between 2 and 4% additional Ca-fed groups during this period. TCh levels were not affected ($p = 0.97$) by the age of the birds at which sampling was done.

Figure 2b shows the LDL-Ch levels in the birds that received diets with or without additional Ca source. In all the treatment groups, mean LDL-Ch levels for the whole trial period averaged 87.9, 78.8 and 78.3 mg dL^{-1} for the control, 2 and 4% additional Ca-fed groups, respectively. This implies that LDL-Ch levels were at least 10% lower in the birds that received additional Ca source in the diet than those fed control diet. The LDL-Ch levels for treated birds (i.e., 2 or 4% additional Ca-fed groups) were different ($p = 0.001$) from those of males in the control group but no differences ($p = 0.89$) were observed between the 2 and 4% additional Ca source-fed males. Moreover, the age at which blood sampling was conducted did not significantly affect ($p = 0.99$) LDL-Ch levels.

Figure 2c shows the HDL-Ch levels in males that received diets with additional Ca source and those fed control diet. In all the treatment groups, mean HDL-Ch levels between 21 and 53 weeks of age averaged 26.0, 28.7 and 28.9 mg dL^{-1} for the control, 2 and 4% additional Ca

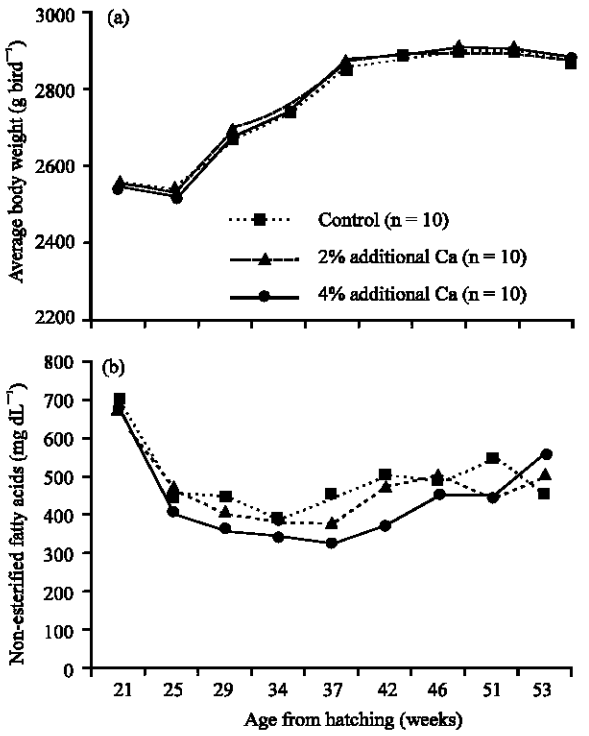

Fig. 1: a) average body weight and b) Non-Esterified Total Fatty Acids (NEFA) levels in blood of Barred Plymouth Rock males fed diets containing 0 (control), 2 and 4% additional Ca in form of Aragonite flour sfrom 21-53 weeks of age

source-fed males, respectively, implying that HDL-Ch levels were also at least 10% higher in the birds that received diets with additional Ca compared to those fed control diet. The HDL-Ch values for treated birds were significantly different from those of the control group ($p = 0.001$, control vs. 2 or 4% additional Ca fed groups). A decline in LDL-Ch and increase in HDL-Ch consequently changed the ratio of LDL-Ch to HDL-Ch (LDL-Ch:HDL-Ch) in treated birds to 2.8:1 and 2.7:1 for 2 and 4% additional Ca-fed groups, respectively from 3.4:1 for males fed control diets. The LDL-Ch:HDL-Ch ratios for both 2 and 4% additional Ca-treated males were significantly different ($p = 0.001$) from that of control group.

However, no differences in HDL-Ch levels were observed between 2 and 4% additional Ca-fed birds ($p = 0.67$) or LDL-Ch to HDL-Ch ratios ($p = 0.93$). Additionally, the age at which blood sampling was done did not influence HDL-Ch levels ($p = 0.60$).

Levels of TG in males that received diets with or without additional Ca source are shown in Fig. 2d. Mean TG levels between 21 and 53 weeks of age were 34.1, 32.2 and 32.0 mg dL^{-1} for males fed control, 2 and 4%

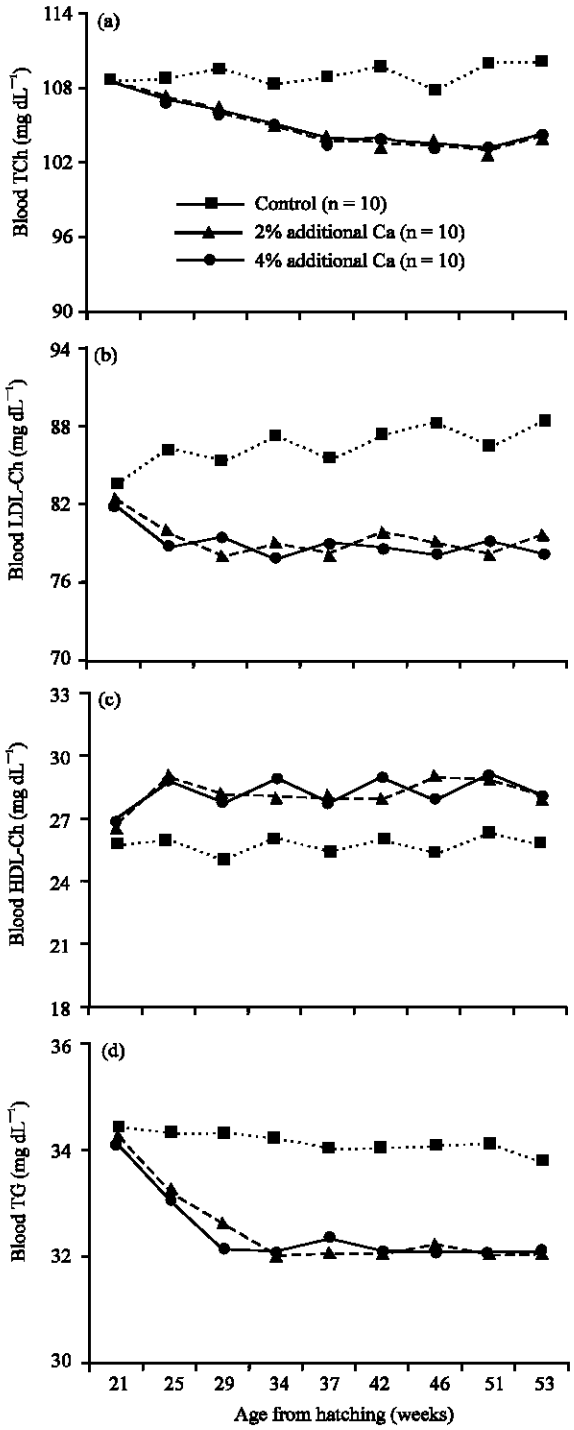

Fig. 2: a) Total Cholesterol (TCh); b) Low density Lipoprotein Cholesterol (LDL-Ch); c) high density cholesterol (HDL-Ch) and d) Triglycerols (TG) levels in blood of Barred Plymouth Rock males fed diets containing 0 (control), 2 and 4% additional Ca in form of Aragonite flour from 21-53 weeks of age

additional Ca-fortified diets, respectively. Statistical analysis showed that additional Ca in the diets reduced TG levels ($p = 0.001$) in treated birds (2 or 4% additional Ca-fed birds vs control) when compared to the control. However, no differences ($p = 0.79$) were observed when TG levels for 2% additional Ca-fed males were compared to those that received feed with 4% Aragonite flour. As was the case with TCh, LDL-Ch and HDL-Ch levels, the age at which blood samples were collected did not influence ($p = 0.99$) TG levels in the birds.

DISCUSSION

The results described in this study agreed with the suggestion by Grundy and Denke (1990) that dietary induced changes in plasma TCh levels yield essentially reciprocal alterations in individual lipoproteins. They have also demonstrated that the hypocholesterolemic characteristics of additional dietary Ca in form of Aragonite flour not only reduced levels of TCh in Barred Plymouth Rock males but also modified the lipoprotein Ch fractions towards lower LDL-Ch and higher HDL-Ch levels which improved the LDH-Ch to HDL-Ch ratio. Furthermore, this study permitted the validation of the relationship between the level of Ca in the diet and level of lipoprotein Ch fractions. For example, in spite of similarities in macronutrient compositions between control diet and diets with 2 or 4% additional Ca tabulated by Kanyinji and Maeda (2010), plasma LDL-Ch and TG levels significantly reduced while HDL-Ch levels increased in birds fed diets with additional Ca source compared to those in the control group. This suggested that macronutrients could not be responsible for the observed differences in lipoprotein Ch fractions.

The control-treated groups differences in lipoprotein Ch fractions in the present study concurred with observations of Denke *et al.* (1993) and Reid *et al.* (2002) in humans with regard to additional dietary Ca-plasma LDL-Ch and HDL-Ch changes. Thus, these results suggested a beneficial influence of additional Ca in the form of Aragonite flour not only on the TCh levels but also on LDL-Ch and HDL-Ch levels. The decrease in TCh and LDL-Ch levels in this study could be due to several effects attributed to high Ca intake by the chickens such as a reduction in fatty acid absorption, resulting most likely from the formation of insoluble Ca-fatty soaps in the gut (Denke *et al.*, 1993; Reid, 2004; Ditscheid *et al.*, 2005). According to Grundy and Denke (1990) and Vaskonen (2003), decreased absorption of fat especially of saturated fatty acids, reduces both TCh and LDL-Ch concentrations.

Another suggested Ch-lowering mechanism of additional dietary Ca in the present study is with regards to the mineral's propensity to bind and precipitate bile acids in the gut (Lupton *et al*., 1994; Welberg *et al*., 1994; Shahkhalili *et al*., 2001). Though there did not measure fecal bile acids, precipitation of bile acids by additional Ca would reduce the intestinal concentrations of bile acids available for fat digestion as well as that which is supposed to return to the liver via the entero-hepatic circulation. Thus, additional dietary Ca changed the fat/Ch uptake and metabolism as well as bile acid feedback mechanism. Decrease in the bile acids returning to the liver induced an increase in the conversion of Ch to bile acids by the liver (Fuchs, 2003). This mechanism is known at least from the lipid-lowering effects of cholestyramine and other bile acid binding resins (Witztum, 1996; Ohlsen and Rogers, 2004b). Thus, the interruption of the re-absorption of bile acids into the entero-hepatic circulation by additional Ca modified the hepatic metabolism of Ch. A high bile acid biosynthesis induced by treatment with additional Ca may have caused an increase in demand for Ch by the liver and the liver responds to this by enhancing the synthesis of Ch and uptake of LDL-Ch via the LDL receptors with the overall effect of reduced plasma TCh and LDL-Ch levels.

Furthermore, the lipid-lowering mechanism attributed to additional Ca could also have been due to the mineral's capability to influence adipocyte activity. About 99% of Ca in the body is stored in the extracellular spaces and intracellular cytosolic soluble Ca mediates many metabolic pathways including platelet aggregation and insulin resistance. Calcitropic hormones such as parathyroid hormone and 1, 25-hydroxy vitamin D3, regulate intracellular Ca concentration (Ca^{2+}). Low dietary Ca intake for example, stimulates high levels of parathyroid hormone and 1, 25-hydroxy vitamin D3 which in turn stimulate high levels of intracellular (Ca2+) in adipocytes that promote lipogenesis while inhibiting lipolysis (Kelly and Gimble, 1998; Zemel *et al*., 2000). In contrast, high dietary Ca intake depresses levels of parathyroid hormone and 1, 25-hydroxy vitamin D3 which results in low levels of intracellular [Ca^{2+}] leading to inhibition of lipogenesis and stimulation of lipolysis (Zemel, 2002, 2003). Thus, the Ca level in the diet may determine whether adipocytes store or break down fats. In the present study, it is possible that increased lipolysis usually resulting from Ca-rich diets favored lipid mobilization in birds that received additional Ca and may explain the observed reduction in TG levels. Additionally, it has been noted that an increase in intracellular (Ca^{2+}) in hepatocytes stimulates microsomal triacylglycerol transfer protein that has been implicated in the formation and secretion of very low density lipoproteins (Cho *et al*., 2005). On this understanding, it is likely that ingested additional Ca in treated birds suppressed increase in hepatocellular (Ca^{2+}) thereby inhibiting the formation and secretion of very low density lipoproteins that elevate TG and LDL-Ch levels.

The levels of some of the blood metabolites indicate the nutritional status of an animal (Russell and Wright, 1983). For example, in ruminants, plasma concentrations of NEFA have been related to their growth (Ellenberger *et al*., 1989) and growth depends on the nutritional plane of the animal. The effects of feed intake on plasma NEFA levels (Grummer, 1995; O'Doherty and Crosby, 1998) have been investigated and results have shown that this parameter increases during periods of energy restriction. In the present study, no differences in NEFA concentrations were detected between treated birds and those fed control diet. Though feed intake was not measured, lack of differences in NEFA levels may at least suggest that the energy status of birds in all treatment groups was similar. Since body weight is also another good indicator of the nutritional status of animals (Sheehan *et al*., 1977; Juarez-Reyes *et al*., 2004), this supposition is supported by lack of significant differences in body weights between treated birds and those fed control diets. It also implies that additional dietary Ca source did not significantly change the availability of macronutrients required for growth.

In all treatment groups, NEFA levels were high at 21 weeks of age but declined with time before stabilizing at lower levels from 25 weeks of age onwards. Hershock and Vogel (1989) stated that stress as well as dietary influences may affect TG, NEFA and TCh levels in humans and animals. Interestingly, the occurrence of high NEFA levels in the present study corresponded with the time the males had just been transferred into their individual cages which led to fights between neighboring males. It was also at the time during which the males were being prepared for another study on sperm parameters which entailed clipping their tail feathers and feathers around the cloaca and training them for semen collection by massage. Thus, in the absence of data on feed intake in the present experiment, it can only be speculated that high initial NEFA levels were due to stress induced by the described activities and as these activities ceased, NEFA levels also declined.

CONCLUSION

Additional dietary Ca in diets fed to Barred Plymouth Rock males at 2 or 4% as Aragonite flour had no adverse effects on the nutritional status of the birds compared to those fed control diet but beneficially decreased not only

blood TCh but also LDL-Ch and TG levels. A reduction in TCh and LDL-Ch levels resulted in an increase in the HDL-Ch fraction in treated birds which improved the LDL-Ch to HDL-Ch ratio. Since chickens are recognized as suitable animal models for studies on the comparative biochemistry of Ch metabolism and transport in humans (Chandler *et al*., 1979; Castillo *et al*., 1996), these results provide a reason to encourage trials of Aragonite flour in humans for possible health benefits.

ACKNOWLEDGEMENTS

The first researcher was supported by the Government of Japan through MEXT: Ministry of Education, Cultural, Sports, Science and Technology (Monbukagakusho). Both researchers are grateful to Dr Sugino for his help in the analysis of samples, Prof. Lawrence. M. Liao for proof reading this manuscript and GaiaTec Co., Inc., Kagoshima, Japan for kindly providing the Aragonite flour.

REFERENCES

Ajakaiye, A., J.O. Atteh and S. Leeson, 1996. Effects of calcium source, particle size and time on *in vitro* calcium solubility of some indigenous Nigerian mineral ingredients for poultry diets. Anim. Feed Sci. Tech., 65: 293-298.

Castelli, W.P., J.T. Doyle, T. Gordon, C.G. Hames and M.C. Hjortland *et al*., 1977. HDL Cholesterol and other lipids in coronary heart disease. The co-operative lipoprotein in phenotyping study. Circulation, 55: 767-772.

Castelli, W.P., R.J. Garrison, P.W. Wilson, R.D. Abbott, S. Kalousdian and W.B. Kannel, 1986. Incidence of coronary heart disease and lipoprotein cholesterol levels. The framingham study. J. Am. Med. Assoc., 256: 2835-2838.

Castillo, M., J.H. Hortal, E. Garcla-Fuentes, M.F. Zafra and E. Garcfa-Peregrfn, 1996. Coconut oil affects lipoprotein composition and structure of neonatal chicks. J. Biochem., 119: 610-616.

Chandler, R.F., S.N. Hooper and H.A. Ismail, 1979. Antihypercholesterolemic studies with sterols: Comparison of rats and chicks as animal model. Can. J. Pharm. Sci., 14: 15-20.

Cho, H.J., H.C. Kang, S.A. Choi, Y.C. Ju, H.S. Lee and H.J. Park, 2005. The possible role of Ca^{2+} on the activation of microsomal triglyceride transfer protein in rat hepatocytes. Biol. Pharm. Bull., 28: 1418-1423.

Denke, M.A., M.M. Fox and M.C. Schulte, 1993. Short term dietary calcium fortification increases fecal saturated fat content and reduces serum lipids in men. J. Nutr., 123: 1047-1053.

Ditscheid, B., S. Keller and G. Jahreis, 2005. Cholesterol metabolism is affected by calcium phosphate supplementation in humans. J. Nutr., 135: 1678-1682.

Ellenberger, M.A., D.E. Johnson, G.E. Carstens, K.L. Hossner, M.D. Holland, T.M. Nett and C.F. Nockels, 1989. Endocrine and metabolic changes during altered growth rates in beef cattle. J. Anim. Sci., 67: 1446-1454.

Finkelstein, A.D., J.E. Wohlt and S.M. Emanuele, 1993. Composition and nutritive value of ground sea clam shells as calcium supplements for lactating Holstein cows. J. Dairy Sci., 76: 582-589.

Fuchs, M., 2003. Bile acid regulation of hepatic physiology III. Regulation of bile acid synthesis: Past progress and future challenges. Am. J. Physiol. Gastrointest. Liver Physiol., 284: 551-557.

Grummer, R.R., 1995. Impact of changes in organic nutrient metabolism on feeding the transition dairy cow. Anim. Sci., 73: 2820-2833.

Grundy, S.M. and M.A. Denke, 1990. Review-Dietary influences on serum lipids and lipoproteins. J. Lipid Res., 31: 1149-1172.

Hershock, D. and W.H. Vogel, 1989. The effects of immobilization stress on serum triglycerides, nonesterified fatty acids and total cholesterol in male rats after dietary modifications. Life Sci., 45: 157-165.

Hines, T.G., N.L. Jacobson, D.C. Beitz and D.K. Littledike, 1985. Dietary calcium and vitamin D: Risk factors in the development of atherosclerosis in young goats. J. Nutr., 115: 167-168.

Hsu, H.H. and N.C. Culley, 2006. Effects of dietary calcium on atherosclerosis, aortic calcification and icterus in rabbits fed a supplemental cholesterol diet. Lipid Health Dis., 5: 1-9.

Hulan, H.W., F.G. Proudfoot and D.M. Nash, 1984. The effects of different fat sources on general performances and carcass fatty acid composition of broiler chickens. Poult. Sci., 63: 324-332.

Isles, C.G. and J.R. Paterson, 2000. Identifying patients at risk for coronary heart diseases: Implications from trials of lipid-lowering drug therapy. Quart. J. Med., 93: 567-574.

Juarez-Reyes, A.S., M.A. Cerrillo-Soto, C.A. Meza-Herrera and G. Nevarez-Carrasco, 2004. Diet composition, intake, plasma metabolites, reproductive and metabolic hormones during pregnancy in goats under semi-arid grazing conditions. J. Agric. Sci., 142: 697-704.

Kannel, W.B., J.D. Neaton, D. Wentworth, H.E. Thomas, J. Stamler, S.B. Hulley and M.O. Kjelsberg, 1986. Overall and coronary heart disease mortality rates in relation to major risk factors in 325,348 men screened for the MRFIT. Am. Heart J., 112: 825-836.

Kanyinji, F. and T. Maeda, 2010. Additional dietary calcium fed to Barred Plymouth Rock roosters reduces blood cholesterol, elevates seminal calcium, and enhances sperm motility, thermo-tolerance and cryosurvivability. Anim. Reprod. Sci., 120: 158-165.

Kanyinji, F., N. Araki and T. Maeda, 2010. 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. Res. J. Poult. Sci., 31: 12-18.

Kelly, K.A. and J.M. Gimble, 1998. 1,25-dihydroxy vitamin D3 inhibits adipocyte differentiation and gene expression in murine bone marrow stromal cell clones and primary cultures. Endocrinology, 139: 2622-2628.

Lupton, J.R., X.Q. Chen, W. Frolich, G.L. Schoeffler and M.L. Peterson, 1994. Rats fed high fat diets with increased calcium levels have fecal bile acid concentrations similar to those of rats fed a low fat diet. J. Nutr., 124: 188-195.

Miller, G.J. and N.E. Miller, 1977. Plasma high density lipoprotein concentration and development of ischaemic heart disease. Lancet, 1: 16-18.

Ohlsen, S. and D. Rogers, 2004a. Reducing hyperlipidaemia and CHD. Pharm. J., 273: 116-118.

Ohlsen, S. and D. Rogers, 2004b. Significance of lipid measurements. Pharm. J., 273: 57-58.

O'Doherty, J.V. and T.F. Crosby, 1998. Blood metabolite concentrations in late pregnant ewes as indicators of nutritional status. J. Anim. Sci., 66: 675-683.

Reid, I.R., 2004. Effects of calcium supplementation on circulating lipids: Potential pharmacoeconomic implications. Drug Age, 21: 7-17.

Reid, I.R., B. Mason, A. Horne, R. Ames and J. Clearwater *et al*., 2002. Effects of calcium supplementation on serum lipid concentrations in normal older women: A randomized controlled trial. Am. J. Med., 112: 343-347.

Renaud, S., Ciavatti, M., Thevenon, C. and J.P. Ripoll, 1983. Protective effects of dietary calcium and magnesium on platelet function and atherosclerosis in rabbits fed saturated fat. Atherosclerosis, 4: 187-198.

Ross, R.D., G.L. Cromwell and T.S. Stahly, 1984. Effect of source and particle size on the biological availability of calcium in calcium supplements for growing pigs. J. Anim. Sci., 59: 125-134.

Russell, A.J.F. and I.A. Wright, 1983. The use of blood metabolites in the determination of energy status in beef cows. J. Anim. Prod., 34: 335-343.

SAS, 2003. SAS/STAT® Users Guide: Statistics, Version 9.1. SAS Institute Inc., Cary, North Carolina, USA.

Shahkhalili, Y., C. Murset, I. Meirim, E. Duruz, S. Guinchard, C. Cavadini and K. Acheson, 2001. Calcium supplementation of chocolate: Effect on cocoa butter digestibility and blood lipids in humans. Am. J. Clin. Nutr., 73: 246-252.

Sheehan, W., M.J. Lawlor and I.H. Bath, 1977. Energy requirements of the pregnant ewe. Irish J. Agric. Res., 16: 233-242.

Stanford, M., 2005. Significance of cholesterol assays in the investigation of hepatic lipidosis and atherosclerosis in psittacine birds. Exotic DVM., 7: 28-34.

Sun, C., X. Yu, Y. Li and R. Liu, 2004. Effects of dietary calcium on the blood glucose, blood lipid and hormone of rat fed a high fat diet. Wei Sheng Yan Jiu, 33: 164-166.

Vaskonen, T., 2003. Dietary minerals and modification of cardiovascular risk factors. J. Nutr. Biochem., 14: 492-506.

Vaskonen, T., E. Mervaala, V. Sumuvuori, T. Seppanen-Laakso and H. Karppanen, 2002. Effects of calcium and plant sterols on serum lipids in obese Zucker rats on a low fat diet. Br. J. Nutr., 87: 239-245.

Welberg, J.W.M., J.F. Monkelbaana, E.G.E. de Vriesb, F.A.J. Muskietc and A. Catsa *et al*., 1994. Effects of supplemental dietary calcium on quantitative and qualitative fecal fat excretion in man. Ann. Nutr. Met., 38: 185-191.

Witztum, J.L., 1996. Drugs Used in the Treatement of Hyperlipoproteinemias. In: Goodman and Gilman's The Pharmacological Basis of Therapeutics, Hardman, J.G., E. Limbird, P.B. Molinoff and R.W. Ruddon (Eds.). McGraw-Hill, New York, USA., pp: 885-889.

Zemel, M.B., 2002. Mechanisms of dairy modulation of adiposity. J. Nutr., 133: 252-256.

Zemel, M.B., 2003. Role of dietary calcium and dairy products in modulating adiposity. Lipids, 38: 139-146.

Zemel, M.B., H. Shi, B. Greer, D. Di Rienzo and P.C. Zemel, 2000. Regulation of adiposity by dietary calcium. FASEB J., 14: 1132-1138.