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Mineral Utilization in Layers as Influenced by Dietary Oligofructose and Inulin

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Abstract: Sixty White Leghorn hens (57 wk of age) were divided randomly into three groups with two birds per cage. One group received the basal diet, the second group received the basal diet supplemented with 1% (w/w) oligofructose and the third group received 1% (w/w) inulin in the form of 1.3% of a partially purified chicory root extract(Raftifeed®IPE).Birds were allowed free access to feed and water during the 4 wk observation period. Supplementing oligofructose and inulin increased ($P<0.05$) the layer's serum calcium levels at the end of experiment. Oligofructose and inulin dietary supplementation increased ($P<0.05$) eggshell weight percentage from the second week as compared with the control group; meanwhile overall eggshell weight showed 3.64 and 4.44% increases ($P<0.05$), respectively. Similarly, after 1 wk of supplementing diets with both prebiotics, eggshell strengths were increased ($P<0.05$) significantly. Supplementing with oligofructose or inulin increased ($P<0.05$) total ash, calcium, and phosphorus levels in the tibia. No effect ($P>0.05$) on the level of magnesium, potassium, copper, zinc or iron in the tibiae was observed among those treatments. In conclusion, dietary oligofructose and inulin can promote bird's health and improve eggshell quality.

Key words: Oligofructose, inulin, mineral, eggshell quality

Introduction

About 10% of all eggs produced are cracked or broken between oviposition and retail sale (Zeidler, 2001). Eggshell quality, especially shell strength, decreases with age of hens (Rodriguez-Navarro *et al*., 2002). Egg producers realize the seriousness of their egg breakage problem, especially as it affects total profit. Therefore, increasing eggshell quality should be helpful in the marketing of shell eggs.

Prebiotics are carbohydrates that are not digested by vertebrates and that interact selectively with intestinal fermentation. The particular modification of the composition of the intestinal flora, and the stimulation of the metabolic activity of a flora that contains relatively less pathogenic (proteolytic) micro-organisms exerts biological effects on the host. (Tomasik and Tomasik, 2003; Gibson *et al*., 2003). The non-digestible carbohydrates ("dietary fiber") have been reported to improve the intestinal absorption of minerals, presumably because of their binding or sequestering action (Coudray *et al*., 2003; Roberfroid *et al*., 2002). Roberfroid (2000) indicated that a higher concentration of short-chain carboxylic acids resulted from the colonic fermentation of non-digestible carbohydrates, accelerating the colonic absorption of minerals, particularly calcium (Ca^{2+}) and magnesium (Mg^{2+}). Increased levels of calcium and magnesium have been observed when feeding rats with oligosaccharides (Delzenne *et al*., 1995; Scholz-Ahrens *et al*., 2001; Kruger *et al*., 2003). An increased absorption of iron was obtained when iron-deficient, anemic rats were fed fructooligosaccharides (FOS) (Ohta *et al*., 1995). As stated by Ohta *et al*. (1994) and Takahara *et al*. (2000), no increased absorption of phosphorus was observed when feeding rats with prebiotic inulin-type fructans.

Gibson and Roberfroid (1995) indicated that FOS are the only products presently recognized and used as food ingredients that meet all the criteria allowing classification as prebiotics among the natural non-digestible oligosaccharides that fulfil the criteria of colonic food. They indicated also that short chains of fructooligosaccharides are known as oligofructose and medium chains as inulin. Calcium plays an important role in the formation of eggshell and bone. Increasing calcium absorption might be responsible for increased eggshell strength or decreased occurrence of bone fracture. Therefore, this study was designed to assess the effect on mineral utilization and eggshell strength of adding oligofructose and inulin into a basal diet.

Materials and Methods

Birds and diets: White Leghorn layers with similar weight, egg-laying performance and health condition were selected 2 wk prior to the feeding trial,. After selection, 60 White Leghorn hens of the same age (57 wk old) were obtained and divided randomly into three groups of 20 birds. Each group was assigned, with two birds per cage, to one of the following diets:

1) Basal diet (antibiotic and wheat-free regular diet) (Table 1).
2) Basal diet with 1.0% (w/w) oligofructose (Raftifeed®OPS, Orafti, Belgium). Chain length [degree of polymerization (DP)] varies between of 2 and 8, with an average of 4.

3) Basal diet with 1% (w/w) inulin administered as 1.3% (w/w) of a semipurified chicory inulin extract (Raftifeed®IPE, Orafti, Belgium).

Chain length (DP) varies between 2 and 65, with an average of 10.

The prebiotics were mixed into the basal diet manually and stored at room temperature for less than 3 d. The birds were fed their assigned diets *ad libitum* and had free access to water during the 4 wk feeding period. A regimen of 16 h light:8 h dark was provided throughout the experiment study, which last for 4 wk.

Table 1: Major ingredients of the basal layer diet (g/kg)

Ingredients	Composition (g/kg)
Corn	676
Soybean meal	190
Dicalcium phosphate	22
Limestone	90
dl-Methionine	0.5
Vitamin mixture	3.5
Salt	5
Fat	13
Calculated nutrient content	
Crude protein (g/kg)	150
Metabolizable energy (MJ/kg)	11.99

Serum calcium content: At the end of the 4 wk trial, one layer per cage for each treatment was sacrificed for blood collection. About 5 ml of blood from each bird were collected in VACUTAINER® tubes (non-anticoagulant inclusion) (Becton Dickinson VACUTAINER Systems, Franklin Lakes, NJ) and left for 1 h. The tubes were centrifuged (Lourdes Clinical Centrifuge, Serial #3394, model CHT, Lourdes Instrument Corp, Brooklyn, NY) at 27,000 g for 30 min to collect serum. Serum calcium levels were measured with a Kodak, Ektachem DT60 Analyzer (Estman Kodak Company, Rochester, NY) with Ca DT slides (Ortho-Clinical Diagnostics, INC., Rochester, NY).

Eggshell weight percentage/eggshell strength: Eggs produced every 7th day were collected and selected randomly for measurements of eggshell weight and eggshell breaking strength. Egg-white and yolk were removed, and the eggshell was cleaned gently with warm water to avoid crushing. All eggshells were dried for 24 h at room temperature (20°C) (Doyon *et al.*, 1985). Eggshell breaking strength was measured by a method reported by Rodriguez-Navarro *et al.* (2002). An Instron (model 1130, Intreon Corporation, Canton, MA) was used for the measurement.

Tibia collection and tibia mineral analyses: At the end of the 28 d feeding trial, 10 birds from each treatment were sacrificed for tibia collection. The collected tibiae were frozen and sent with solid CO_2 by courier to Orafti, Belgium. An induced coupled plasma method (ICP-AES) was used for mineral analyses.

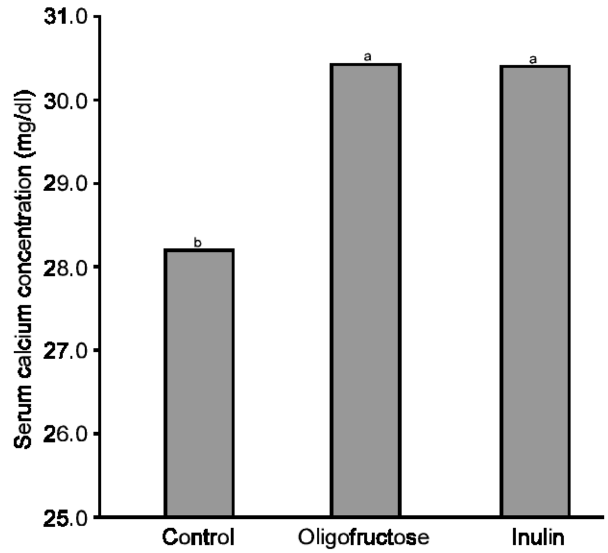

Fig. 1: Serum calcium concentration of layers as affected by dietary oligofructose and inulin. a-b, Columns with unlike letters differ significantly (P<0.05)

Statistical analyses: The experiment was conducted using a completely random design (CRD) (Steel and Torrie, 1980). Data were analyzed using analysis of variance (ANOVA) (SAS Institute, Inc., 1993). A significant difference was used at the 0.05 probability level. The least significant difference (LSD) was used to separate different mean values (Freud and Wilson, 1997).

Results and Discussion

Serum calcium levels and tibia mineral analyses: Supplementing layer's diets with oligofructose and inulin for 4 wk increased (P<0.05) serum calcium levels as compared with the controls (Fig. 1). The increases of serum calcium levels were 8.02 and 7.84% for oligofructose and inulin, respectively. Layers fed oligofructose and inulin prebiotic showed higher (P<0.05) total ash, calcium, and phosphorus levels in tibiae than the controls. An increase (P<0.05) of sodium level in the tibiae was noted only in layers fed inulin (Table 2). No effect (P>0.05) on the levels of magnesium, potassium, copper, zinc, or iron in the tibiae of either prebiotic product was observed. The higher (P<0.05) calcium levels in tibiae might be due to the higher (P<0.05) serum calcium levels from birds with prebiotic supplementation (Fig. 1 and Table 2). Apparently, prebiotic supplementation improved calcium and phosphorus absorption.

An improvement of mineral absorption by prebiotics in rats and humans has been reported (Delzenne *et al.*,

Table 2: Total ash and mineral contents in the tibia affected by dietary oligofructose and inulin

Parameter	Treatment		
	Control	Oligofructose	Inulin
Total ash (g/kg tibia)	560.07b	584.18a	587.94a
Calcium (g/kg tibia)	213.44b	221.53a	225.31a
Magnesium (g/kg tibia)	3.24a	3.36a	3.37a
Phosphorus (g/kg tibia)	100.71b	104.74a	106.30a
Sodium (g/kg tibia)	4.46b	4.67ab	4.83a
Potassium (g/kg tibia)	0.73a	0.75a	0.79a
Copper (mg/kg tibia)	< 4.56	< 5.13	<4.49
Zinc (mg/kg tibia)	267.48a	257.87a	253.10a
Iron (mg/kg tibia)	405.45a	345.03a	367.62a

Each reading is a mean of 10 replications.
a-b, Means within a row followed by unlike letters differ significantly (P<0.05).

Table 3: Mean eggshell weight percentage and eggshell breaking strength as affected by dietary oligofructose and inulin

Parameter	Treatment		
	Control	Oligofructose	Inulin
Day 7	8.66a	8.77a	8.90a
Day 14	9.03b	9.31a	9.30a
Day 21	8.68b	9.29a	9.34a
Day 28	8.81b	9.09a	9.19a
Overall	8.79b	9.11a	9.18a
Eggshell breaking strength (kg)			
Day 7	2.02a	2.04a	2.01a
Day 14	2.00b	2.09a	2.13a
Day 21	2.05b	2.24a	2.19a
Day 28	2.01b	2.21a	2.23a
Overall	2.02b	2.15a	2.12a

Each reading is a mean of 10 replications.
a-b, Means within a row of each parameter followed by unlike letters differ significantly (P<0.05).

1995; Scholz-Ahrens *et al*., 2001; Kruger *et al*., 2003). Roberfroid (2000) and Coudray *et al*. (2003) reported that a higher concentration of short-chain carboxylic acids resulting from the colonic fermentation of non-digestible carbohydrates accelerates the colonic absorption of minerals, particularly calcium and magnesium. Kruger *et al*. (2003) indicated that improved calcium absorption could decrease the occurrences of bone fracture and osteoporosis.

Eggshell weight percentage and eggshell strength: Supplementing layers with oligofructose and inulin did not (P>0.05) affect eggshell weight percentage during the first week of feeding (Table 3). However, the increasing effects (P<0.05) of both prebiotics were observed after 1 wk of feeding when compared with the controls. Overall, oligofructose and inulin increased (P<0.05) 3.64 and 4.44% eggshell weight percentages, respectively. Similarly, oligofructose and inulin increased (P<0.05) eggshell strength after 1 wk of feeding (Table 3).

Approximately 10% of all eggs produced are cracked or broken between oviposition and retail sale (Zeidler, 2001), and eggshell quality, especially shell strength, decreases with age of hens (Rodriguez-Navarro *et al*., 2002). Increasing calcium absorption is thought to result in a higher eggshell weight percentages and eggshell strength. The results of this study show an increased calcium content in both the bones and the eggshells of the birds as compared to control (Table 2 and 3; and Fig. 1). It is observed that the eggshell strength has improved, which is a consequence of the increased mineral absorption. The phenomenon of increased mineral absorption and increased deposit of calcium and magnesium in bone tissue of animals has been described in experimental models (Roberfroid *et al*., 2002; Scholz-Ahrens *et al*., 2002). The present study confirmed this property of inulin-type fructans in laying hens. Inulin can contribute to improved eggshell quality, which may result in reduced breaking of the shells and improved productivity of the laying hen batteries, especially with birds of relatively high age. This study should be beneficial to the egg industry and to the marketing of shell eggs.

Conclusion: Oligofructose and inulin increased serum calcium levels after 4 wk of feeding. Meanwhile, both prebiotics increased ($P<0.05$) total ash, calcium and phosphorus levels in the tibiae but an increase of sodium level in tibiae was observed only in the inulin group. There was no effect on the level of magnesium, potassium, copper, zinc, or iron levels in tibiae when layers were fed with prebiotic supplementation as compared with the controls. There was no difference between inulin and oligofructose-fed birds. Oligofructose and inulin increased ($P<0.05$) 3.64 and 4.44% eggshell weight, respectively. Eggshell quality decreases upon ages of layers. Increasing calcium absorption may result in a higher eggshell weight percentage and eggshell strength, and a decrease in the occurrence of bone fracture and osteoporosis. Therefore, this study should not only be of beneficial to the egg industry but also an onset of research about prebiotics on chicken health.

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Acronyms: FOS, fructooligosaccharide(s); ICP-AES, induced coupled plasma method; CRD, completely random design; ANOVA, analysis of variance; LSD, least significant difference