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≪**Research Note**≫

The Effects of Dietary Chitosan or Glucosamine HCl on Liver Lipid Concentrations and Fat Deposition in Broiler Chickens

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Dietary chitosan, a polymer of glucosamine, decrease the absorption of dietary fat and then reduce the abdominal fat deposition in broiler chickens. Chitosan is also digested and absorbed in the form of glucosamine by hens and broilers. Thus, in broiler chickens, dietary chitosan may decrease lipogenesis and triglyceride (TG) synthesis in the liver in addition to decrease fat absorption, consequently resulting in a reduction of body fat deposition. However, little research has been done to determine whether dietary glucosamine decreases hepatic TG synthesis and body fat deposition in broiler chickens. The present experiment was conducted to determine the effects of dietary chitosan or glucosamine HCl on fat absorption, hepatic TG contents and body fat deposition in broiler chickens. Broiler chickens at 14 d old were fed on a control diet based on corn and soybean meal or diets containing 5% chitosan or glucosamine HCl for 3 weeks. Dietary chitosan and glucosamine HCl did not affect feed intake, body weight gain, feed efficiency and breast muscle weight. Dietary chitosan significantly ($P < 0.05$) decreased fat digestibility, the contents of total lipid and triglyceride in the liver and abdominal fat weight. The concentration of plasma very low density lipoproteins (VLDL) tended to decrease in broilers fed on the chitosan diet. Dietary glucosamine HCl significantly ($P < 0.05$) reduced hepatic total lipid and triglyceride contents, plasma VLDL concentration and abdominal fat deposition with no influence on fat digestibility. These results suggest that dietary chitosan may decrease the body fat deposition by reducing intestinal fat absorption and hepatic TG synthesis and dietary glucosamine may decrease the body fat deposition by reducing hepatic TG synthesis in broiler chickens.

Key words : broiler chickens, chitosan, fat deposition, glucosamine HCl, liver lipid concentration

Introduction

Excessive body fatness in broiler chickens is widely recognized as a problem in the poultry industry. In chickens, lipogenic activity in the liver is much greater than that in the adipose tissue, and most of the fats accumulated in the adipose tissues result from incorporation of triglyceride (TG) from plasma lipoproteins (in particular very low density lipoproteins (VLDL)) those are either synthesized in the liver or provided from dietary fats (Hermier, 1997). It has been reported that dietary chitosan, a polymer of glucosamine, decreased the absorption of dietary fat (Razdan and Pettersson, 1996) and then reduced the abdominal fat deposition in broiler chickens (Kobayashi and Itoh, 1991 ; Kobayashi *et al.,* 2002). Chitosan has been also reported to be digested by hens and broilers (Hirano *et al.,* 1990). Thus, in broiler chickens, chitosan may be hydrolyzed by chitosanase secreted from intestinal bacteria (Hirano *et al.,* 1990 ; Kurakake *et al.,* 2000 ; Omumasaba *et al.,* 2000 ; Kim *et al.,* 2004 ; Barbosa *et al.,* 2005) and absorbed in the form of glucosamine. On the other hand, glucosamine is known to inhibit glucose uptake into the liver (Virkamaki *et al.,* 1997) in rats. Accordingly, it is suggested that a part of diet-

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ary chitosan decrease the fat absorption and other part may be absorbed in the form of glucosamine and may decrease hepatic lipogenesis and TG synthesis, consequently resulting in a reduction of body fat deposition in broiler chickens. However, little research has been done to determine whether dietary glucosamine decreases hepatic TG synthesis and body fat deposition in broiler chickens.

The present experiment was conducted to determine the effects of dietary chitosan or glucosamine on fat absorption, hepatic TG contents and body fat deposition in broiler chickens.

Materials and Methods

One-d-old male broiler chicks (Chunky strain) were housed in an electrically heated battery brooder until 14 d of age and subsequently kept under continuous light with a commercial diet (CP 21%, ME 3100 kcal/kg, Chubu shiryo Co. Ltd., Chita, Japan). At 14 d of age, the birds were assigned to 3 groups with 6 chicks each according to body weight to equalize the mean body weight in each group. They were housed individually in wire cages and given the experimental diets for 3 weeks. Table 1 shows the composition of the experimental diets. The basal diet was formulated to contain all nutrients to meet the nutritional requirements of broiler chickens (NRC, 1994). Chitosan, which was processed industrially from marine crab shells, was purchased from Kyowatecnos Co. (Tokyo, Japan). Glucosamine HCl was purchased from Kantoh Chem. Co. (Tokyo, Japan). The chitosan and glucosamine HCl were added to the basal diet at 5% at the expense of cellulose. Because, the abdominal fat deposition in broiler chickens was markedly reduced by the addition of 5% chitosan to the diet in a previous study (Kobayashi *et al.,* 2002). The diets contained chromic oxide as an indigestible feces collection marker. During the last three days of the experimental period, the excreta in each bird were collected daily and stored at −20℃. The excreta were pooled per bird, dried for 48 h in a forced-air draft oven at 65℃. All samples of feed and excreta were ground prior to assay for chromium oxide and crude fat. Chromic oxide in samples of diets and excreta was analyzed by the method of Yoshida *et al.,* (1967). Crude fat contents of feed and excreta were determined by extraction of samples with ether using a Soxhlet apparatus.

Table 1. **Composition of experimental diets (%)**

Ingredients	Diets		
	Control	Chitosan	Glucosamine HCl
Corn	51.00	51.00	51.00
Soybean meal	18.00	18.00	18.00
Soybean protein	10.00	10.00	10.00
Soybean oil	5.50	5.50	5.50
Corn starch	6.37	6.37	4.05
Cellulose	5.00	—	—
Chitosan	—	5.00	—
Glucosamine HCl	—	—	5.00
Potassium bicarbonate	0.00	0.00	2.32
Dicalcium phosphate	1.93	1.93	1.93
Calcium carbonate	0.95	0.95	0.95
Vitamin and mineral premix[1)]	0.50	0.50	0.50
Sodium chloride	0.30	0.30	0.30
L-Methionine	0.29	0.29	0.29
Trace vitamin and mineral premix[2)]	0.04	0.04	0.04
Lysine-HCl	0.03	0.03	0.03
Threonine	0.01	0.01	0.01
Chromic oxide	0.08	0.08	0.08
Calculated analysis			
ME (kcal/g)	3.17	3.17	3.10
CP (%)	21.00	21.00	21.00

[1)] Kobayashi and Itoh (1989).

[2)] Provided the following per kg of diet : choline chloride 367 mg, nicotinic acid 10 mg, biotin 60 μg, folic acid 0.27 mg, vitamin B_{12} 10 μg, KI 0.5 mg.

The fat digestibility was calculated using the following formula :

$$\text{Fat digestibility}(\%) = 100 - [100 \times (\text{diet } Cr_2O_3 / \text{excreta } Cr_2O_3) \times (\text{excreta fat} / \text{diet fat})]$$

Experimental diets and water were provided *ad libitum*. Chickens were maintained on a 24-h constant light schedule in a temperature controlled room at 26±3℃. During the experimental period, feed consumption was measured daily. At the end of the experimental period, blood samples were collected into tubes containing heparin sodium after a 6-h fasting period. Plasma was prepared by centrifugation for 15 min at 1500×g. After the collection of blood, the chickens were weighed and killed by intravenous injection of sodium pentobarbital. The abdominal fat pads and the liver were rapidly removed and weighed. Plasma VLDL was measured using the turbidimetric method with analytical kit for VLDL (BLF EIKEN Ⅱ ; Eiken Chemical Co, Ltd, Tokyo). The liver lipids were extracted by the method of Folch *et al.,* (1957) and determined gravimetrically. Liver triglyceride contents were determined by the method of Fletcher (1968).

The data were analyzed statistically by one-way analysis of variance, followed by Tukey's multiple range test (Yoshida, 1978).

Results

Table 2 shows the effects of dietary chitosan and glucosamine HCl on feed intake, body weight gain, feed efficiency (gain/feed intake), breast muscle and abdominal fat weights of broiler chickens fed experimental diets for 21 days. Feed intake, body weight gain and feed efficiency of broiler chickens fed experimental diets for 21 days were not affected by either dietary chitosan or glucosamine HCl treatment. There were no significant differences in breast muscle weight among the dietary treatments. Absolute and relative weights of abdominal fat were significantly ($P<0.05$) reduced in birds fed the chitosan diet or the glucosamine HCl diet compared with control diet.

Table 3 shows the effects of dietary chitosan and

Table 2. **Effects of dietary chitosan and glucosamine HCl on feed intake, body weight gain, feed efficiency (gain/feed intake), breast muscle weight and abdominal fat weight of broiler chickens fed experimental diets for 3 weeks**

	Diets		
	Control	Chitosan	Glucosamine HCl
Feed intake (g/21 d)	2064±72	1989±46	1983±32
Body weight gain (g/21 d)	1392±33	1344±46	1315±23
Feed efficiency (gain/feed intake)	0.68±0.01	0.68±0.01	0.66±0.01
Breast muscle weight (g)	208±4	200±6	190±6
Abdominal fat weight (g)	22.3±1.8[a]	14.7±1.8[b]	11.8±1.6[b]
Abdominal fat weight (%/BW)	1.23±0.09[a]	0.85±0.11[b]	0.69±0.09[b]

Mean±SE with 6 observations.
[a b] Means within a row with different superscripts are significantly different ($P<0.05$).

Table 3. **Effects of dietary chitosan and glucosamine HCl on contents of liver weight, total lipid and triglyceride in the liver and plasma VLDL concentrations, and fat digestibility in broiler chickens fed the experimental diets for 3 weeks and fat digestibility during the last 3 days**

	Diets		
	Control	Chitosan	Glucosamine HCl
Liver weight (g)	36.7±1.2	38.8±0.9	35.9±1.3
Liver total lipid (%)	4.34±0.08[a]	3.90±0.10[b]	3.73±0.11[b]
Liver triglyceride (mg/g)	25.0±1.4[a]	20.8±1.4[b]	18.6±1.2[b]
Plasma VLDL(mg/dl)	48.9±2.6[a]	41.5±3.2[ab]	36.7±2.7[b]
Fat digestibility (%)	85.72±0.76[a]	75.60±2.38[b]	80.68±1.99[ab]

Mean±SE with 6 observations.
[a b] Means within a row with different superscripts are significantly different ($P<0.05$).

glucosamine HCl on contents of liver weight, total lipid and TG in the liver and plasma VLDL concentrations, and fat digestibility in broiler chickens fed the experimental diets for 21 days and fat digestibility during the last 3 days in the experimental period. The liver weight was not influenced by either dietary chitosan or glucosamine HCl treatment. The concentrations of total lipid and TG in the liver were significantly ($P < 0.05$) decreased by dietary chitosan or glucosamine HCl treatment. The plasma VLDL concentration tended to decrease in broilers fed the chitosan diet. Dietary glucosamine HCl decreased significantly ($P < 0.05$) the plasma VLDL concentration. Fat digestibility during the last 3 days in the experimental period was reduced in broilers fed on chitosan diet but not reduced in birds fed on glucosamine HCl diet.

Discussion

Feeding of glucosamine HCl to broiler chickens reduced hepatic total lipid and triglyceride contents, plasma VLDL concentration and abdominal fat weight with no influence on fat digestibility. Therefore, glucosamine HCl exhibits postabsorptive effects that could involve lipogenesis in the liver. In chickens, the liver is the main site of de novo lipogenesis, synthesis of fatty acids, which is very limited in the adipose tissue (Saadoun and Leclercq, 1987 ; Griffin *et al.,* 1992). Thus, most of the fat that accumulates in avian adipose tissue is either derived from the diet or synthesized in the liver (Hermier, 1997). The de novo synthesized fatty acids are incorporated into TG. The newly synthesized TG is incorporated into hepatic lipoproteins, mainly VLDL which are secreted into the blood and transported into adipose tissues. However, when the intensity of lipogenesis is higher than the hepatic capacity for VLDL synthesis and secretion, the de novo synthesized TG is stored into the hepatocytes (Hermir, 1997). Therefore, liver TG content reflects long-term endogenous TG synthesis in the liver. Reduced hepatic TG concentration, plasma VLDL concentration and abdominal fat weight indicate a suppression of hepatic lipogenesis and TG synthesis due to dietary glucosamine HCl. Therefore, in broiler chickens, dietary glucosamine HCl may decrease de novo lipogenesis and TG synthesis in the liver, consequently resulting in a reduction of fat deposition in the abdominal fat tissue. In avian species, glucose is used extensively and is a major substrate for hepatic lipogenesis (Riesenfeld *et al.,* 1981 ; Bedu *et al.,* 2002). It is reported that glucosamine decreases glucose uptake into the liver (Virkamaki *et al.,* 1997), hepatic glucokinase activity and glucose-6-phosphate formation (Barzilai *et al.,* 1996 ; Cadefau *et al.,* 1997) in rats. Thus, the suppression of hepatic de novo fatty acid synthesis by the feeding of glucosamine HCl may be due to reduced supply of glucose to the liver. On the other hand, it is known that TG synthesis and secretion by chicken hepatocytes is stimulated by insulin (Legrand *et al.,* 1996) and that a significant activation of insulin signaling in liver of fat chickens may account for their increased liver lipogenesis and ultimately their fattening (Dupont *et al.,* 1999). It is also reported that glucosamine impairs glucose-induced insulin secretion in rat (Balkan and Dunning, 1994 ; Giaccari *et al.,* 1995 ; Shankar *et al.,* 1998). Therefore, hepatic lipogenesis may also be reduced by lower insulin secretion in broiler chickens fed on glucosamine HCl diets.

Dietary chitosan decreased fat digestibility and abdominal fat weight. Therefore, it is suggested that dietary chitosan decreases the absorption of dietary fat and results in a reduction of fat deposition in the abdominal fat tissue of broiler chickens. The results of giving a chitosan diet in the present experiment confirmed the suggestion that dietary chitosan decreases the lipase activity (Kobayashi *et al.,* 2002) and fat absorption in the small intestine by reducing the concentration of bile acids in the duodenum (Razdan *et al.,* 1997), consequently resulting in a reduction of fat deposition in the abdominal fat tissue of broiler chickens (Kobayashi and Itoh, 1991 ; Kobayashi *et al.,* 2002). Dietary chitosan also decreased the concentration of total lipid and TG in the liver. This result suggests that a part of dietary chitosan is absorbed (Hirano, 1997) in the form of glucosamine and may decrease hepatic TG synthesis by reducing glucose uptake into the liver or insulin secretion, resulting in a reduction of body fat deposition.

The results of our study confirm that both dietary chitosan and glucosamine HCl have potent body fat-lowering effects in broiler chickens, but suggest that they do so through different mechanisms. In the case of chitosan, body fat lowering appears to be mediated through a reduction of dietary fat absorption and hepatic TG synthesis. Dietary glucosamine

HCl lowers the TG synthesis in the liver and results in a reduction of body fat deposition.

In conclusion, both dietary chitosan and glucosamine reduce body fat deposition in broiler chickens. The mechanism of body fat lowering appears to differ between the two. Dietary chitosan may decrease the body fat deposition by reducing intestinal fat absorption and TG synthesis in the liver. Dietary glucosamine HCl may decrease the body fat deposition through a reduction of TG synthesis in the liver.

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