



## Effect of different molecular weight of chitosans on performance and lipid metabolism in chicken

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### ABSTRACT

The effect of different molecular weight (MW) chitosans on lipid metabolism, body fat deposition, growth performance and mechanism of action of chitosans in broilers was investigated. A total of 192, 50-day-old local feather-frizzled female broilers with similar initial body weight ( $583 \pm 4$  g) were randomly divided into four groups with eight replicates of six broilers each and fed a corn–soybean meal based diet containing 0 (control), 2 (LMWC), 5 (MMWC) and 50 (HMWC) kDa MW chitosan for 42 days. Treatments did not affect the growth performance. The LMWC had no influence on fecal lipids, apparent fat metabolisability and serum concentrations of total cholesterol (TC) and triglyceride (TG), and decreased ( $P < 0.05$ ) the fat accumulation in both liver and muscle and abdominal fat yield. The MMWC and HMWC feeding increased fecal lipid excretion, decreased the fat accumulation in both liver and muscle and abdominal fat yield and also the serum concentrations of TC and TG ( $P < 0.05$ ). Both LMWC and MMWC reduced liver fatty acid synthase (FAS) activity ( $P < 0.01$ ), and increased liver lipoprotein lipase (LPL) and hepatic lipase activities ( $P < 0.05$ ). However, the HMWC feeding did not affect activities of tissue lipases except for a reduction in liver FAS activity. All three forms of chitosans improved the antioxidant status as seen by increased serum superoxide dismutase activity and decreased malondialdehyde content ( $P < 0.05$ ). The liver FAS mRNA did not differ among the treatment groups. There was a trend ( $P = 0.077$ ) for liver LPL mRNA to decrease as the chitosan MW increased. In conclusion, the three chitosans regardless of MW inhibited body fat deposition in broilers. The lipid-lowering effect of 50 kDa chitosan is probably mediated by reduced dietary fat absorption. The 2 kDa chitosan was useful in inducing fat catabolism, and the 5 kDa chitosan appeared to act via both these mechanisms.

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**Abbreviations:** DM, dry matter; FAS, fatty acid synthase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HDL-C, high-density-lipoprotein cholesterol; HL, hepatic lipase; HMWC, high molecular weight chitosan; HSL, hormone sensitive lipase; LDL-C, low-density-lipoprotein cholesterol; LMWC, low molecular weight chitosan; LPL, lipoprotein lipase; MDA, malondialdehyde; MMWC, medium molecular weight chitosan; MW, molecular weight; N, nitrogen; P, phosphorus; RQ, relative quantity; TC, total cholesterol; TG, triglycerides; SOD, superoxide dismutase.

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## 1. Introduction

Chitosan, a derivative of chitin is a natural polymer of glucosamine and N-acetylglucosamine extracted from the shells of crustaceans such as crab, lobster and shrimp (Kim and Rajapakse, 2005; Xia et al., 2011). Dietary chitosans exhibit potent hypocholesterolemic and hypolipidemic effects in both animals and humans (Liao et al., 2007; Zhang et al., 2008, 2012; Xia et al., 2011; Rizzo et al., 2013).

Molecular weight (MW) of chitosan and deacetylation biotransformation pathway are two important characteristics that affect its chemical and physiological properties. Although the effect of MW on hypolipidemic activity of chitosan has been extensively studied, the results are not consistent (Deuchi et al., 1995; Chiang et al., 2000; Yao et al., 2008; Liu et al., 2008; Czechowska-Biskup et al., 2005; Sumiyoshi and Kimura, 2006; Zhang et al., 2012, 2013). A major reason for diverse results appears to be because MW and the degree of deacetylation of the chitosans used in these studies were different. In most studies, only the chitosans with a relatively high MW of more than 50 kDa were tested. But the absorption of chitosan varies with MW (Chae et al., 2005). It is assumed that lower MW chitosans are more readily absorbed through the intestine, enter into the blood and exert biological effects on lipid metabolisms in the organism. However, there is only limited research on the effects of low MW chitosans (oligochitosan, <10 kDa chitosans) with the same degree of deacetylation to alter tissue lipid metabolism and prevent body fat deposition in animals.

The emphasis of our research in recent years has been to decrease body fat deposition and alter tissue fat constituents to improve the carcass characteristics and meat quality in broilers. In this study, the effect of different MW chitosans with the same degree of deacetylation, on lipid metabolism, body fat deposition and potential mechanism of action in local feather-frizzled broilers was investigated.

## 2. Materials and methods

### 2.1. Chitosan preparation

The 2, 5, and 50 kDa MW chitosans in powder form, prepared from chitin deacetylated to 90%, were purchased from Jinan Haidebei Marine Bioengineering Co. Ltd., China. All three preparations contained  $\leq 78$  g/kg water and  $\leq 10$  g/kg ash. The water solubility of chitosan supplements were  $\geq 990$  g/L.

### 2.2. Experimental design and diets

One-day-old feather-frizzled broilers were obtained from a local hatchery and fed a commercial starter diet containing 20.12 g/kg crude protein and 11.69 MJ/kg apparent metabolizable energy to 49 days of age. On day 50, a total of 192 broilers with similar initial body weight ( $583 \pm 4$  g) were assigned to 1 of 4 treatments (8 cage replicates of 6 birds each per treatment) using a completely randomized design and were fed a corn–soybean meal based diet containing 0 (control), 2 (LMWC), 5 (MMWC) and 50 HMWC) kDa MW chitosan for 42 days (until day 92). The diet (Table 1) was formulated to meet the nutrient requirements of Chinese color-feathered chicken (NY/T 33–2004) during the period of more than 8 weeks of age. Feed samples were ground to pass through a 1-mm screen, after which they were analyzed for dry matter (DM; method 934.01; AOAC, 2000), nitrogen (N; method 968.06; AOAC, 2000), calcium (method 984.01; AOAC, 1995), and total phosphorus (P; method 965.17; AOAC, 1995). Dry matter was determined by drying in an oven at 103 °C for 8 h. Nitrogen was determined (Kjectec 2300 Nitrogen Analyzer; Foss Tecator AB, Hoeganaes, Sweden), and crude protein was calculated as  $N \times 6.25$ . The other values including metabolizable energy, available P, lysine, methionine and methionine + cysteine of the experiment diet were calculated from chemical composition (NRC, 1994). Chitosan in the treatments was set 7.5 g/kg of the diet by replacing an equal part of zeolite powder in the corn–soybean meal based diet.

### 2.3. Birds, housing and management

The study was approved by the Animal Welfare Committee of Guangdong Ocean University. Chickens were housed in an electrically heated, thermostatically controlled room equipped with fiberglass feeders, waterers and stainless-steel cages (100 cm  $\times$  50 cm  $\times$  50 cm). They were maintained at a temperature of  $24 \pm 2$  °C and on a 24 h constant-light schedule. The lighting regimen and ventilation were monitored continuously. All chickens had ad libitum access to the diets and water. Twice a week, the birds were weighed and the feed intake recorded.

### 2.4. Sampling and sample processing procedure

Eight birds from each group were randomly selected at the end of the feeding period and allotted to individual metabolism cages to study the effects of different MW chitosans on fat utilization. After a four-day adaptation period, total excreta were collected for three days from each cage, pooled per cage, weighed, subsampled and dried at 60 °C for 72 h. Feed intakes were recorded daily. The feed and dried excreta samples were ground to pass through a 40-mesh screen and mixed thoroughly before analysis. At the end of the experiment, 2 chickens from each cage were selected according to the average body weight of birds within the cage after a 10 h fast. Blood samples were collected via jugular venipuncture into vacutainer tubes, and

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