

Original Research Article

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Effects of Dietary Chitosan and Neem Leaf Meal Supplementation on Digestive Enzyme Activities and Fat Deposition in Broiler Chickens

D. Sirsat Shraddha^{1*}, P. Visha² and K. Nanjappan²

¹Department of Veterinary Physiology, Bombay Veterinary College, Mumbai, India

²Department of Veterinary Physiology, Veterinary College and Research Institute, Namakkal, India

*Corresponding author

ABSTRACT

Keywords

Abdominal fat, broiler, chitosan, digestive enzymes, neem leaf meal.

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A study was conducted to determine the effects of dietary supplementation with chitosan, neem leaf meal and their combination at different levels in broiler on fat deposition and intestinal pH and digestive enzymes, amylase and lipase. Two hundred forty broilers (Vencobb) were randomly assigned to eight groups with three replicates of ten birds each and were fed for six weeks and slaughtered. Supplementation of antibiotic, chitosan, NLM and chitosan NLM combination had no influence on the activities of intestinal amylase and lipase except that the chitosan supplemented groups recorded lower lipase activity than other treatment groups. The birds supplemented with chitosan at 0.025% and 0.05% alone or in combination with NLM at 0.025% chitosan and 0.5% neem leaf meal and 0.05% chitosan and 1.0% neem leaf meal both the levels resulted in significant ($p < 0.05$) reduction in abdominal fat in comparison to all other treatment and control groups.

Introduction

An emphasis on nutrition and health, mainly diet, saturated fat, cholesterol and obesity by consumers has changed the demand for food products, especially meat. Health conscious consumers associate diet with the probability of experiencing health problems or diseases. The desire of the consumers to eat healthy foods which are free from antibiotic residues and other negative attributes lead to the search for natural or organic substitutes for synthetic antibiotics which is commonly used by poultry producers today. The growth and expansion of the poultry industry is confronted by high cost of feed and drugs and

their occasional shortages. However, there is the fear that the continuous subtherapeutic use of in-feed antibiotics could lead to the development of antibiotic resistant bacteria, which can be harmful to humans (Nasir and Grashorn, 2006). Thus, efforts have been made in different parts of the world to ban the inclusion of all types of antibiotic growth promoters in animal feeds.

Chitin is a cellulose-like biopolymer found in the arthropods (Austine *et al.*, 1981). Chitosan, a deacetylated product of chitin, is produced industrially from crab and shrimp

shell waste obtained from the sea. Chitosan derived from shell wastes has multifunctional activities such as immunopotentiating, antibacterial, antifungal, antioxidant, wound healing, antiulcer and antitumor effects. It has a unique combination of properties such as biocompatibility, biodegradability, biorenewable and biofunctional activity which makes it an ideal polymer for wide range of applications including agriculture, food science, pharmaceuticals and biomedical fields (Shahidi *et al.*, 1999; Yin *et al.*, 2009). Additionally, chitosan enhances the digestion and absorption of nutrients when incorporated at lower levels in diets. (Gopalakannan and Venkatesan, 2006) and it has synergistic action with antibiotics and neem against many bacteria (Tin *et al.*, 2009; Rajendran *et al.*, 2012).

Previously, it was reported that dietary chitosan inhibited the increases in plasma triglyceride concentration and abdominal fat weight induced by the feeding of high-fat diets in laying-type chicks (Kobayashi and Itoh, 1991). Dietary chitosan also reduced plasma cholesterol concentrations (Razdan and Pettersson, 1994; Razdan *et al.*, 1997), postprandial plasma triglyceride (Razdan and Pettersson, 1994) and ileal fat digestibility (Razdan and Pettersson, 1996) in broiler chickens. Accordingly, it was suggested that dietary chitosan may decrease the absorption of dietary fat and the deposition of fat in broiler chickens. Pancreatic lipase and amylase plays an important role in fat absorption.

Azadirachta indica, commonly known as neem, has attracted worldwide attention in recent years, owing to its wide range of medicinal properties. Neem is a tropical evergreen tree native to Indian sub-continent. Neem possesses limonoids, protolimonoids, tetranortriterpenoids, pentanortri-terpenoids, hexanortriterpenoids, and some nonterpenoid (Koul *et al.*, 2006). Most of the plant parts

such as fruits, seeds, leaves, bark, and roots contain compounds with proven antiseptic, antiviral, antipyretic, anti-inflammatory, antiulcer, and antifungal properties (Brahmachari, 2004). Neem leaves exhibit immunomodulatory, anti-inflammatory, antihyperglycaemic, antiulcer, antimalarial, antifungal, antibacterial, antiviral, antioxidant, antimutagenic and anticarcinogenic properties (Tiwari *et al.*, 2014). Owing to these properties, neem leaf meal has been tried as a feed additive in animals.

Thus present experiment was conducted to determine the effect of dietary chitosan and neem leaf meal and their combinations at different level on fat deposition and digestive enzyme activities in broiler chickens

Materials and Methods

Two hundred and forty numbers (240) of day-old broiler chicks (Vencobb 400) were wing banded, weighed and randomly distributed to eight groups with three replicates of ten chicks each based on the body weight to namely T1,T2,T3,T4,T5,T6,T7 and T8. The experiment was undertaken in the Department of Veterinary Physiology, Veterinary College and Research Institute, Namakkal (Tamilnadu). All the chicks were reared up to 42 days in cage system under standard managemental practices with *ad. lib.* feed and water supply. The experimental diets were formulated according to the standards prescribed in Bureau of Indian Standards (BIS, 1992) and fed to the birds as per the following schedule. At the end of the experiment (42nd day), six birds per treatment group were randomly selected and slaughtered by halal method. The intestinal contents were collected in separate sterile vials for the estimation intestinal enzyme activities. The abdominal fat was collected and weighed. The eight treatment groups are as follows:

- Group T1: Basal diet without antibiotic
- Group T2: Basal diet with 50 mg Oxytetracycline/kg
- Group T3: Basal diet without antibiotic + 0.025% chitosan
- Group T4: Basal diet without antibiotic + 0.05% chitosan
- Group T5: Basal diet without antibiotic + 0.5% neem leaf meal
- Group T6: Basal diet without antibiotic + 1.0% neem leaf meal
- Group T7: Basal diet without antibiotic + 0.025% chitosan and 0.5% neem leaf meal
- Group T8: Basal diet without antibiotic + 0.05% chitosan and 1.0% neem leaf meal

Estimation of amylase activity

Estimation of amylase activity of the intestinal contents was done as per the method of Coles (1986). Briefly, one gram of intestinal contents was homogenized with 5 ml of distilled water, centrifuged and the supernatant was diluted to 1:1000 with 0.9 percent saline. To 1 ml of 0.02 percent buffered starch substrate solution, 0.1 ml of diluted supernatant was added and incubated for 9 min at 37°C. In another test tube 1 ml of 0.02 percent buffered starch substrate solution was taken as control and incubated. After incubation, one ml of working iodine solution was added to both the tubes. The optical density (OD) was read at 660 nm in a UV spectrophotometer using distilled water as blank.

Amylase activity (Units/ml) =

$$\frac{\text{OD of control} - \text{OD of test}}{\text{OD of control supernatant}} \times \text{Volume of sample} \times \text{Dilution factor}$$

Estimation of lipase activity

The lipase activity of the digesta was determined as per the method of Boutwell, (1962). Briefly, one gram of intestinal contents was homogenized with 10 ml of

distilled water, centrifuged and the supernatant was used for lipase estimation. Six ml of substrate buffer mixture was taken in two separate test tubes. One tube served as blank and in another tube, 5 ml of the digesta supernatant was added, mixed thoroughly and both the tubes were incubated at 37°C for 24 h. At the end of incubation, 5 ml of the digesta supernatant was added to the blank. Then, five drops of phenolphthalein was added to the mixture and titrated against 0.05 N sodium hydroxide until the appearance of a distinct pink color.

Lipase activity (Units/g) =

$$\frac{\text{Titre value of test} - \text{Blank value}}{\text{Weight of the intestinal content (g)}} \times \text{Dilution factor}$$

Statistical analysis

The data collected were analysed using SPSS® 20.0 software package. The completely randomized design method was followed for the experiment (Snedecor and Cochran, 2007) and the data collected were analysed using SPSS® 20.0 software package. Post-hoc analysis was done by Tukey honestly significance difference test.

Results and Discussion

The mean enzyme activities and the abdominal fat are presented in the Table 1.

Amylase activity

The mean intestinal amylase activity (U/ml) in broiler chickens were 49.15, 54.98, 55.98, 50.54, 51.98, 58.99 and 59.19 in the treatment groups T₂ to T₈ as compared with 48.14 in the control group of broiler chickens.

Even though there was no significant difference in the mean amylase activity among any of the treatment groups and control group of broiler chickens, there was a

numerical increase in the activity of amylase in all the treatment groups supplemented with chitosan, neem and their combination as compared with the control group of birds. Similar findings were recorded by Chen and Zhou (2005) in *Silver crucian carp* and Rui *et al.*, (2013) in broiler chickens.

Lipase activity

The mean intestinal lipase activity (U/g) in broiler chickens were 41.84, 38.58, 37.27, 40.73, 39.28, 38.10 and 37.11 in the treatment groups T₂ to T₈ as compared with 42.07 in the control group of broiler chickens.

There was no significant difference in the mean lipase activity in any of the treatment groups as compared with the control. There was a numerical decrease in the lipase activity in the chitosan supplemented groups (T₃, T₄, T₇ and T₈) and these groups recorded lower lipase activity.

The results of the present study agree with the findings of Xu *et al.*, (2014) who observed that the lipase activity of proximal jejunum contents was not affected by increasing chitosan supplementation from 0-500mg/kg in weaned piglets on days 14 and 28. The results of the present study differs with those of Kobayashi *et al.*, (2002) who observed dietary chitosan (50gm/kg) in broiler chicken diet significantly ($p < 0.01$) decreased lipase activity per gm of small intestinal contents. The lipase activity in the treatment groups did not show any significant variation as the dose of chitosan (0.025 - 0.05%) supplemented in the present study was comparatively lesser.

Bile salts are essential for emulsification of dietary fat and activation of pancreatic lipase activity. Dietary chitosan binds with the bile salts and decreases bile acid concentration in the duodenum of chickens (Razdan and Pettersson, 1994) which might have lowered the intestinal lipase activity.

Hence, it could be inferred that the supplementation of antibiotic, chitosan, NLM and chitosan NLM combination had no influence on the activities of intestinal amylase and lipase except that the chitosan supplemented groups recorded lower lipase activity.

Abdominal Fat

The mean abdominal fat (%) in broiler chickens were 1.85, 1.39, 1.29, 1.82, 1.84, 1.37 and 1.26 in the treatment groups T₂ to T₈ as compared with 2.03 in the control group of broiler chickens.

The mean abdominal fat (%) was significantly ($p < 0.05$) higher in the control group, T₂, T₅ and T₆ groups of broiler chickens as compared with the treatment groups T₃, T₄, T₇ and T₈.

There was no significant difference in the mean abdominal fat in the NLM supplemented group and control birds. These findings concur with those of Landy *et al.*, (2011) who reported that the neem fruit powder supplementation at 7 or 12 g/kg diet had no significant influence on the abdominal fat percentage in broilers. Similar observations were made by Bonsu *et al.*, (2012) who found that fat isolated from the lower abdomen was significantly ($P < 0.05$) higher in the control birds when compared with that of birds fed the NLM at 2 per cent but did not differ significantly ($P > 0.05$) from the NLM at 1.5 and 2.5 per cent. Dietary chitosan inhibits the micelle formation during the lipid digestion in the digestive tract by forming ionic bond with the bile salts and acids, lipids and fatty acids directly thereby decreases the body fat deposition by reducing intestinal fat absorption and hepatic triglyceride synthesis and increasing the fecal lipid excretion (Zhou *et al.*, 2006).

Table.1 Mean (\pm S.E.) Intestinal digestive enzymes and abdominal fat of broiler chickens

Treatment groups	Amylase activity (U/ml)	Lipase activity (U/g)	Abdominal fat (%)
T ₁ - Basal diet (without antibiotic)	48.14 \pm 1.56	42.07 \pm 0.35	2.03 ^b \pm 0.23
T ₂ - Basal diet + 50 mg Oxytetracycline/kg	49.15 \pm 1.84	41.84 \pm 0.35	1.85 ^b \pm 0.24
T ₃ - Basal diet + 0.025% chitosan	54.98 \pm 1.67	38.58 \pm 0.64	1.39 ^a \pm 0.16
T ₄ - Basal diet + 0.05% chitosan	55.98 \pm 1.46	37.27 \pm 0.43	1.29 ^a \pm 0.20
T ₅ - Basal diet + 0.5% NLM	50.54 \pm 1.56	40.73 \pm 0.17	1.82 ^b \pm 0.14
T ₆ - Basal diet + 1.0 % NLM	51.98 \pm 1.58	39.28 \pm 0.27	1.84 ^b \pm 0.11
T ₇ - Basal diet 0.025% chitosan +0.5% NLM	58.99 \pm 1.65	38.10 \pm 0.38	1.37 ^a \pm 0.21
T ₈ - Basal diet + 0.05% chitosan +1.0% NLM	59.19 \pm 1.46	37.11 \pm 0.20	1.26 ^a \pm 0.19

Means within the same column bearing different superscripts differ significantly ($p < 0.05$)

Chitosan can therefore reduce the ectopic accumulation of lipids (triglycerides) both in the liver and muscle tissue. Chitosan counteracts triglyceride accumulation in the liver (decrease of triglyceride content by 39%) and in the muscle (decrease of triglyceride content by 66%) (Neyrinck *et al.*, 2009).

Supplementation of chitosan alone or in combination with NLM at both levels resulted in significant reduction in abdominal fat in comparison to all other treatment and control groups.

In conclusion, modern broiler strains have excessive abdominal fat deposit, has a negative impact on poultry production, as evidenced by the increase in feed cost during rearing, decrease in the final meat quality, and the significant economic loss to poultry-processing plants. Due to the economic concerns and consumer aversion to excess fat

deposition, excess fat control and improvement of meat quality are among the targets of broilers production. Chitosan, an abundantly available biopolymer in combination with neem leaf meal can be supplemented in the diet of broiler chickens to reduce the abdominal fat without having any adverse effect on the intestinal enzyme activities.

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