

Original Research Article

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## Effects of Dietary Chitosan and Neem Leaf Meal Supplementation on Intestinal Bacterial Count in Broiler Chickens

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### ABSTRACT

#### Keywords

Neem leaf, Meal supplementation, Bacterial count

#### Article Info

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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 intestinal bacterial count and total Coliform and Clostridium count). 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 caused a significant reduction in the total gut bacterial counts and total *Clostridial* and *Coliform* counts of broiler chickens. 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 intestinal bacterial count and total Coliform and Clostridium count in comparison to all other treatment and control groups.

### Introduction

Chitosan is a biocompatible polymer derived by deacetylation of chitin from shellfish, and its use in industry, agriculture, food science, pharmaceuticals and biomedical fields is well described (Rabea *et al.*, 2003; Senel and McClure, 2004; Yin *et al.*, 2009; Friedman and Juneja, 2010). The interest in chitosan as a biological sanitizer arises from several studies reporting its antimicrobial and anti-oxidative effects in foods (No *et al.*, 2002; Friedman and Juneja, 2010). The antimicrobial activities of

chitosan against Food borne pathogens have been extensively investigated in the food industry (Singla and Chawla, 2001; No *et al.*, 2002; Senel and McClure, 2004; Petrovich *et al.*, 2008; El Hadrami *et al.*, 2010; Kong *et al.*, 2010; Vargas and Gonzalez-Martinez, 2010).

*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, proto-limonoids,

tetranortri-terpenoids, pentanortri-terpenoids, hexanortri-terpenoids, and some non-terpenoid (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 immune-modulatory, anti-inflammatory, anti-hyperglycaemic, antiulcer, antimalarial, antifungal, antibacterial, antiviral, antioxidant, anti-mutagenic and anti-carcinogenic properties (Tiwari *et al.*, 2014). Also chitosan has synergetic effect with Neem leaf meal 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 (42<sup>nd</sup> 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 intestinal bacterial count

The microbial count was done as per the method of Smits *et al.*, (1998). Briefly, immediately after slaughter, one gram of intestinal content was transferred to sterile tube and diluted with 9 ml of sterile normal saline. All the samples were subjected to six serial dilutions.

One ml of the last dilution was inoculated into sterile Mac Conkey agar, Perfringens agar, MRS agar plates for isolation and enumeration of *Escherichia coli*, *Clostridium spp* and *Lactobacillus spp* respectively. The agar plates were incubated for 36 h and the colonies were counted. The result was expressed as log<sub>10</sub> colony forming unit/ gram of digesta.

### 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 effect of supplementation of chitosan, neem leaf meal and their combination on the intestinal bacterial count in broiler chickens is presented in Table 1, Plate 1, Plate 2 and Plate 3.

The mean total intestinal bacterial count ( $\log_{10}$  cfu/g) in broiler chickens were 5.37, 5.88, 5.23, 5.81, 5.07, 4.15 and 4.12 in the treatment groups T<sub>2</sub> to T<sub>8</sub> as compared with 6.58 in the control group of broiler chickens.

The mean total intestinal bacterial count was significantly ( $p < 0.05$ ) higher in the control group, T<sub>3</sub> and T<sub>5</sub> as compared with T<sub>7</sub> and T<sub>8</sub> groups of broiler chickens. The mean total intestinal bacterial count was significantly

( $p < 0.05$ ) lowest in the T<sub>7</sub> and T<sub>8</sub> groups of broiler chickens.

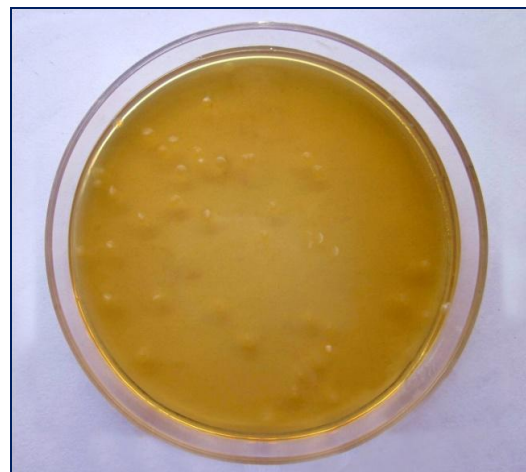
The mean total Coliform count ( $\log_{10}$  cfu/g) in broiler chickens were 4.30, 4.59, 4.34, 4.32, 3.79, 3.58 and 3.28 in the treatment groups T<sub>2</sub> to T<sub>8</sub> as compared with 4.86 in the control group of broiler chickens. The mean total Coliform count was significantly ( $p < 0.05$ ) higher in the control group as compared with T<sub>7</sub> and T<sub>8</sub> treatment groups. The T<sub>8</sub> group of broiler chicken had a significantly ( $p < 0.05$ ) lowest mean total Coliform count. The treatment groups T<sub>2</sub>, T<sub>4</sub>, T<sub>5</sub> and T<sub>6</sub> did not differ significantly ( $p < 0.05$ ) in their mean Coliform count.

The mean total Clostridial count ( $\log_{10}$  cfu/g) in broiler chickens were 2.68, 2.82, 2.20, 2.39, 1.92, 1.65 and 1.39 in the treatment groups T<sub>2</sub> to T<sub>8</sub> as compared with 3.34 in the control group of broiler chickens. The mean total Clostridial count was significantly ( $p < 0.05$ ) higher in the control group as compared with T<sub>4</sub>, T<sub>6</sub>, T<sub>7</sub> and T<sub>8</sub> treatment groups. The T<sub>7</sub> and T<sub>8</sub> groups of broiler chickens had significantly ( $p < 0.05$ ) lowest mean total Clostridial count. The treatment groups T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub>, T<sub>5</sub> and T<sub>6</sub> did not differ significantly ( $p < 0.05$ ) in their mean Clostridial count.

**Plate.1** Intestinal total bacterial count in broilers



**Control**



**0.05% Chitosan and 1% NLM Group**

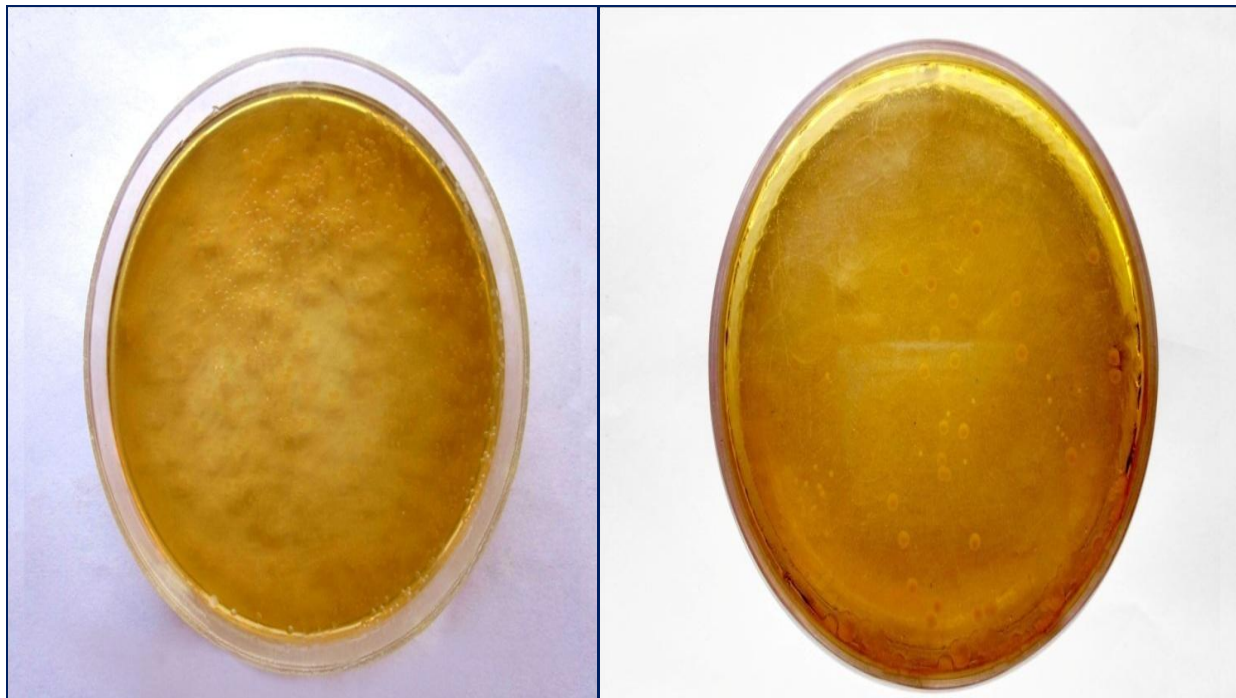
**Plate.2** Intestinal *Escherichia coli* Count in broiler chickens



**Control**

**0.05% Chitosan and 1% NLM Group**

**Plate.3** Intestinal *Clostridium* Count in broiler chickens



**Control**

**0.05% Chitosan and 1% NLM Group**



**Table.1** Mean ( $\pm$  S.E.) intestinal microbial count ( $\log_{10}$  cfu/g) of broiler chickens as influenced by dietary inclusion of chitosan and neem leaf meal at 6<sup>th</sup> week of age

Treatment groups	Total bacterial count	Coliform count	Clostridium count
T <sub>1</sub> - Basal diet (without antibiotic)	6.58 <sup>b</sup> $\pm$ 0.04	4.86 <sup>c</sup> $\pm$ 0.05	3.34 <sup>c</sup> $\pm$ 0.03
T <sub>2</sub> - Basal diet + 50 mg Oxytetracycline/kg	5.37 <sup>ab</sup> $\pm$ 0.02	4.30 <sup>abc</sup> $\pm$ 0.04	2.68 <sup>bc</sup> $\pm$ 0.02
T <sub>3</sub> - Basal diet + 0.025% Chitosan	5.88 <sup>b</sup> $\pm$ 0.02	4.59 <sup>bc</sup> $\pm$ 0.02	2.82 <sup>bc</sup> $\pm$ 0.03
T <sub>4</sub> - Basal diet + 0.05% Chitosan	5.23 <sup>ab</sup> $\pm$ 0.03	4.34 <sup>abc</sup> $\pm$ 0.04	2.20 <sup>ab</sup> $\pm$ 0.03
T <sub>5</sub> - Basal diet + 0.5% NLM	5.81 <sup>b</sup> $\pm$ 0.01	4.32 <sup>abc</sup> $\pm$ 0.03	2.39 <sup>abc</sup> $\pm$ 0.02
T <sub>6</sub> - Basal diet + 1.0 % NLM	5.07 <sup>ab</sup> $\pm$ 0.02	3.79 <sup>abc</sup> $\pm$ 0.03	1.92 <sup>ab</sup> $\pm$ 0.02
T <sub>7</sub> - Basal diet 0.025% chitosan +0.5% NLM	4.15 <sup>a</sup> $\pm$ 0.02	3.58 <sup>ab</sup> $\pm$ 0.02	1.65 <sup>a</sup> $\pm$ 0.03
T <sub>8</sub> - Basal diet + 0.05% chitosan +1.0% NLM	4.12 <sup>a</sup> $\pm$ 0.04	3.28 <sup>a</sup> $\pm$ 0.04	1.39 <sup>a</sup> $\pm$ 0.02

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

The results of the microbial count is in agreement those of Menconi *et al.*, (2014) in broiler chickens and Liu *et al.*, (2010) in Landrace pigs for chitosan supplementation and Bonsu *et al.*, (2012) in broiler chickens for neem supplementation.

The reduction in the mean total intestinal bacterial count in the chitosan supplemented groups of broiler chickens could be due to the fact that the chitosan acts as an effective antimicrobial agent. Two main mechanisms have been suggested as the cause of the inhibition of microbial cells by chitosan. One mechanism is that the polycationic nature of chitosan interferes with bacterial metabolism by electrostatic stacking at the cell surface of bacteria (Chung *et al.*, 2004; Je and Kim, 2006). The other is blocking of transcription of RNA from DNA by adsorption of penetrated chitosan to DNA molecules. Immune response by the cooperation of cytokines to activate the non-specific immune system of animals (Lambrecht *et al.*, 1999).

During this process, the activated macrophages would release cytokines such as TNF- $\alpha$ , IL-1 $\beta$ , IL-6 and IFN- $\gamma$  to inhibit the growth of a wide variety of microorganisms with the help of nitric oxide (NO) cytolytic function and inducible nitric oxide synthase (iNOS) (Je and Kim, 2006; Dou *et al.*, 2007).

The expression of iNOS is essential for the killing of microbes and functions of NO in the regulation of immune responses (Xing and Schat, 2000). Chitosan enhanced iNOS mRNA expression in duodenum, jejunum, and ileum and thus improved the gut immune system and gut health (Li *et al.*, 2009). Similarly, the neem had antimicrobial effect against *E. coli* and *Staphylococcus aureus* (Sairam, 1997) and chitosan and neem combination had better bactericidal effect due to their synergistic effect on the microbes (Rajendran *et al.*, 2012).

Hence, it could be concluded that supplementing the broiler chickens with

chitosan and NLM in combination at both the levels caused a significant reduction in the total gut bacterial total Clostridial counts. The total Coliform count was significantly reduced in the group supplemented with 0.05 per cent chitosan and 1.0 per cent NLM as compared with other groups.

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