



COMPARATIVE ANTI-BACTERIAL ACTIVITIES OF *NIGELLA SATIVA* AND LINCOMYCIN IN THE GUT OF BROILER CHICKS

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ABSTRACT

Nigella sativa (*N. sativa*) is an important herb with multiple pharmacological activities. Therapeutic properties of *N. sativa* based on the availability of thymoquinone (TQ) which is an essential oil and an active chemical component. Current study was designed to investigate the antibacterial effect of *N. sativa* in comparison with lincomycin, as growth promoter in the gut of broiler chicks. Activity of *N. sativa* powder on feed conversion ratio in broiler chicks was also recorded. Day old birds were taken and divided into three groups and having three biological replicates in each treatment group (6 chicks). First group was control group that received normal diet; second group received the normal diet with growth promoter and third group fed with routine diet and powder of *N. sativa*. Birds were slaughtered at the end of study period (day 36th). Blood and tissue samples were collected on 28th and 36th day of trial. The results of this study showed that addition of 1% *N. sativa* powder appeared to have a positive impact on growth performance of broiler chicks as it improved body weight gain and feed conversion ratio (FCR) of broilers at finisher phase ($P < 0.05$) and it could be considered as a substitute of antibiotic growth promoter (lincomycin) for broiler chicks.

1. INTRODUCTION

Antibacterial feed additives have been widely used since many years, to improve the production of broiler chicks in terms of feed conversion ratio and weight gain. The growth performance of broiler chicks could be better by controlling the infections in the gut mucosa. As resistance to these antibacterial substances is high, therefore the use of these feed additives in animals and poultry feed was banned throughout the world in 2006. Therefore, there is need to use some alternatives such as, from plant origin, for example plant extracts, essential oils and whole plant as antibacterial feed additives. However, for promoting growth in farm animals, these feed antibiotics have shown negative results on animal's profitability. Research is being conducted on various active substitutes for antibiotics for example black cumin. Black cumin is also known as black seeds or *N. sativa*. Black seeds are mostly growing in Mediterranean and Asian countries [1]. *Nigella sativa* (*N. sativa*) is a medicinal plant, belongs to Family *Ranunculaceae*, is an emerging miracle herb having a rich historical background. *N. sativa* is a flowering plant that grows annually up to 20-90 cm tall. Leaves of *N. sativa* segmented, thread like to narrowly linear. Endosperm of *N. sativa* is composed of thin walled cells that are rectangular and polygonal; these cells have filled with oil globules [2]. Microscopic view of *N. sativa* seed powder shows that these are parenchymatous cells with oil globules in it [3]. *N. sativa* is much richer in active compounds [4]. Therapeutic properties of *N. sativa* based on the availability of thymoquinone (TQ) which is an essential oil and an active chemical component [5]. Thymoquinone has a gastro protective effect, through the mechanism of inhibiting proton pump, neutrophil permeation and acid secretion [6]. The powder of *N. sativa* is used for antibacterial activity and it is compared with other antibiotics such as, tetracycline, ampicillin, levofloxacin, gentamycin and streptomycin. This antibacterial activity performed by disc diffusion method using *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* [7]. Seeds of *N. sativa* could be used as alternatives of feed antibiotics, because it has antimicrobial activity. As antitumor agent and for the treatment of asthma these black seeds have been used for many centuries in the Middle East and in Asia. Studies have shown that the black cumin's oil extract have a positive effect on performance of poultry chicks

in terms of feed intake and body weight. It was proved that 10% black cumin has no harmful effect on poultry's performance when it is use as dietary supplements. The seeds and oil extract of black cumin have shown to be effective against coliform count and to inhibit the monocytes, respectively [1].

Worldwide, Infectious Bursal Disease (IBD) has a wide distribution, but there is no reported case of IBD in Ethiopia. This is due to indigenous chicken's resistance to Infectious Bursal Disease Virus and other viruses as well. It was observed that disease emergence has large concern because of rising chicken farming on commercial scale. This reported case observed in 20-45 days old layer and broiler chicks. In these chicks, the level of water and feed consumption has dropped rapidly and diarrhea was a common problem. The prominent clinical symptoms that have observed area rapid drop of water and feed intake, white creamy diarrhea, mass death, weight loss and soiling of vent while recovered broilers remained underdeveloped. After observable symptoms, high mortality started on day 3rd and remains continue up to day 15th. For both, the layers and broiler chicks, the overall mortality rate is 49.89% at the end of 8th week. While mortality rates in layer is 25.1% and 56.1% in broiler chicks. In the 3rd week of age, the disease's onset and appearance of symptoms, both were compatible with the clinical type of the disease [1].

Now a day, growing public health problem is the development of bacterial resistance due to excessive use of antibiotics. Excessive exposure to antibiotics is the main reason for development of antibiotic resistance. In both animals and humans, it is same. Source of antibiotic exposure in human is animal husbandry and food additives. This results in the development of resistant bacterial strains. In fact, there are two types of antibiotic resistance, in one type, resistance develops when bacteria resist to antibiotics due to their natural ability of resistance to a certain type of antibiotics and in second type, it inhibits the enzymes [8]. In the current study, natural herb *N. sativa* is going administered in poultry to avoid the excessive use of antibiotics and it will result no development of antibiotic resistance. Because of anti-microbial activity of *N. sativa*, micro biota of poultry gut remains protected; it will improve the bird's performance. Because poultry chicks commonly used in human diet for protein source, thus human will be protecting from development of resistance to

antibiotics. The aim of the current study is to compare the antibacterial effect of *N. sativa* and lincomycin in the gut of poultry chicks if any. As the gut health of poultry plays an important role in their feed absorption and ultimately influences their growth rate.

2. MATERIALS AND METHODS

The experiment was conducted to study the comparative Anti-bacterial activities of *Nigella sativa* and Lincomycin in the gut of broiler chicks. The total duration for this project was 36 days. The shed was white washed, disinfected and fumigation was performed a week before the arrival of chicks at University of Agriculture, Faisalabad. The temperature maintained at 95°F for first week. Drinkers washed on a daily basis. Ground lime and formalin solution placed at the entrance of experimental shed. Lighting program followed according to Hubbard classic management guide. From day one to day four, light provided for 23 hours and kept off for 1 hour. From day 5 to day 9, light provided for eighteen hours and from day ten to nineteen 8 hours per day. From day twenty to twenty-nine light kept on for 18 hours and off for 6 hours. From day 30 to onward lights was on for 22 hours and off for 2 hours. The temperature maintained at 95 °F for first week and then it reduced by 5°F each week until 75 °F attained.

2.1 Preparation of *N.sativa* seed powder

N.sativa seeds were taken from a local market of Faisalabad. The seeds coarsely powdered by a mechanical grinder and then directly mixed with manually prepared diets in appropriate doses according to schedule.

2.2 Feeding schedule and treatments

On arrival, chicks first let them free for half an hour then served with a 250gm/liter sugar solution to provide the energy and to ease the birds from stress of transportation. On the first day, the chicks randomly divided into three treatment groups of six chicks in each group. Feed and water provided *ad libitum*. In which one group received normal diet while the second group received lincomycin (6.25g/500g of feed) and the third group received *N.sativa* (6.25g/500g of feed) added feed Table 1.

Table 1: Schedule of feeding and drug administration to experimental birds

Groups	Diet + Dose
Group I	Receiving normal diet + Water (<i>ad libitum</i>)
Group II (n = 6)	Receiving normal diet + Oral dose of lincomycin (0.025% = 250g / ton of feed) + Water
Group III (n = 6)	Receiving normal diet + Oral dose of <i>N. sativa</i> (0.025% = 250g / ton of feed) + Water

2.3 Experimental birds

Day-old broiler chicks reared in a group on broiler starter diet for one week (adaptation period). These chicks were divided into three groups with six birds in each group (6 chicks). Group 1 was negative control one to which negative feed provided. Second group was positive control (lincomycin) in which lincomycin in feed provided and third group was *N.sativa* in which *N.sativa* added in feed.

2.4 Experimental feed

Four experimental diets for 1-21 days (starter) and 22-36 days (finisher) formulated. Starter diets had crude protein 20% and metabolizable energy 2780 kcal/kg while finisher diets had crude protein 18% and metabolizable energy 2880 kcal/kg.

2.5 Vaccination

The experimental birds vaccinated against various viral diseases like Newcastle Disease (ND) and infectious Bursal Disease (IBD). For intraocular vaccination, vaccine was prepared with standard recommended diluents in a sterile dropper bottle. The dropper bottle was used to vaccinate the birds by pouring a drop of vaccine in their eyes. Before and after the vaccination, birds were giving vitamins and electrolytes as an anti-stressor Table 2.

Table 2: Vaccination schedule for broilers

Age (days)	Vaccine	Method
5	ND	Intraocular
10	IBD	Intraocular

18	IBD	Drinking water
22	ND	Drinking water

2.6 Statistical analysis

Data collected regarding different traits analyzed by Analysis of Variance technique (ANOVA) in a Completely Randomized Design (CRD). Duncan's New Multiple Range Test partitioned the means for various parameters [9].

3. RESULTS

3.1 Anti-bacterial activity of *Nigella sativa*

The antimicrobial activities of studied species were determined against both gram-positive and gram-negative bacteria. Aqueous extracts showed the zone of inhibition against all microorganisms. The observed zone of inhibition was measured in mm and summarized in table (3). Zone of inhibition against *E. coli* (12.5mm) was observed by using aqueous extract of *N. sativa* while no (0mm) zone of inhibition by lincomycin was observed against *E. coli* and other tested microorganisms, because microbes are highly resistant to lincomycin.

Table 3: Zone of Inhibition (mm) of *N.sativa* and Lincomycin

Plant/Drug	Extract	Pathogens		
		<i>S. typhus</i>	<i>E.coli</i>	<i>Staph. aureus</i>
<i>Nigella sativa</i>	Aqueous	0.1mm	12.5mm	0.0mm
Lincomycin	Aqueous	0.0mm	0.0mm	0.0mm

3.2 Growth performance in starter phase

3.2.1 Feed intake (0-21days)

The average feed intake per bird during the starter phase is 811.6, 815.8 and 777.8 g in the control group, lincomycin and *N.sativa* group, respectively. This is greater in lincomycin group and lower in *N. sativa* group. Statistical analysis of data revealed non-significant difference in groups ($p>0.05$). Feed intakes in all three groups were almost similar shown in figure 1.

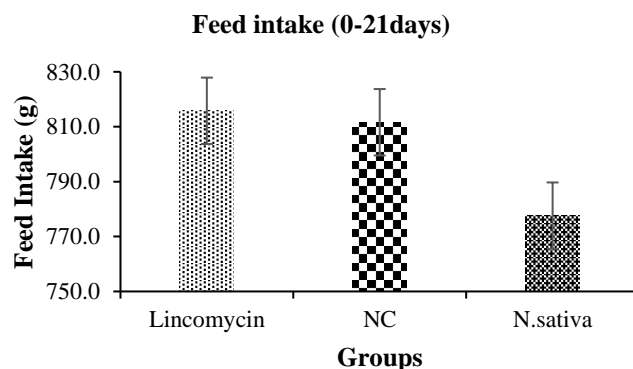


Figure 1: Feed intake during starter phase

3.2.2 Weight gain (0-21days)

The average weight gain per bird during the starter phase is 594.9, 512.9 and 503.0g in lincomycin, control group and *N.sativa* group, respectively. This is greater in lincomycin group and lower in *N. sativa* group, but difference was non-significant. Statistical analysis of data revealed non-significant difference in groups ($p<0.05$), as shown in figure 2.

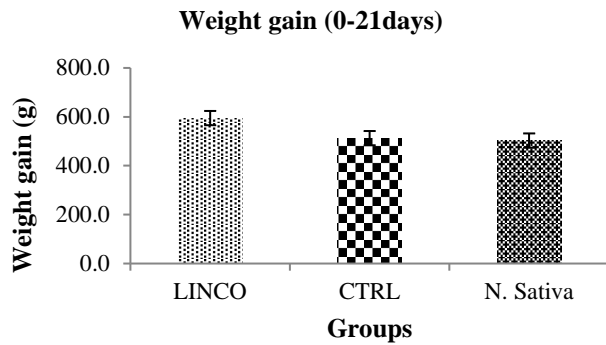


Figure 2: Weight gain (g) during starter phase

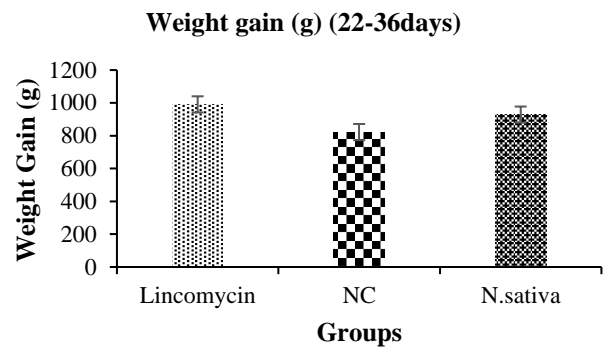


Figure 5: Weight gain (g) during finisher phase

3.2.3 Feed Conversion Ratio (FCR) (0-21days)

The average feed conversion ratio per bird during the starter phase is 1.37, 1.58, and 1.55, in the lincomycin, control group and *N.sativa* group, respectively. This is lower in lincomycin group and higher in *N. sativa* group (Figure 3). Statistical analysis of data revealed significant difference in groups ($p < 0.05$). Maximum FCR was in control group and minimum FCR in *N.sativa* group.

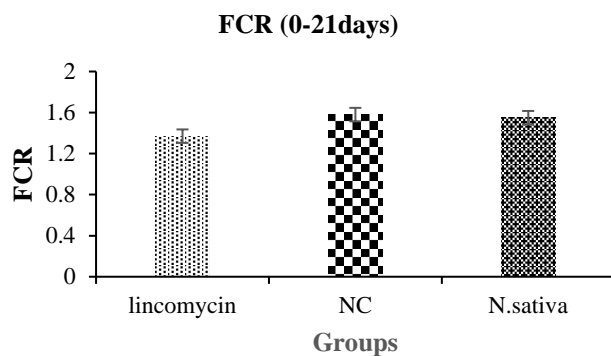


Figure 3: Feed Conversion Ratio during starter phase

3.3 Growth performance in finisher phase

3.3.1 Feed intake (22-36days)

The average feed intake per bird during the finisher phase is 1683.5, 1539.7 and 1342.9 g in the lincomycin, control group and *N.sativa* group, respectively. This is greater in lincomycin group and lower in *N. sativa* group (Figure 4).

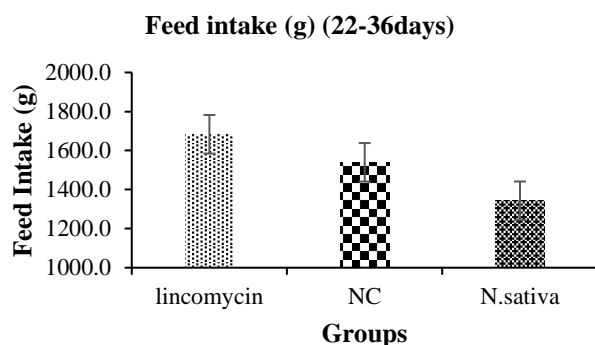


Figure 4: Feed intake during finisher phase

3.3.2 Weight gain (22-36 days)

The average weight gain per bird during the finisher phase is 990.9, 822.2 and 928.5g in the lincomycin, control and *N.sativa* group, respectively. This is greater in the lincomycin group and lower in *N. sativa* group. Statistical analysis of data revealed significant difference in groups ($p < 0.05$). Maximum weight gain was in *N. sativa* group and minimum weight gain in control group (Figure 5).

3.3.3 Feed conversion ratio (FCR) (22-36 days)

The average feed conversion ratio per bird during the finisher phase is 1.70, 1.87, and 1.45, in the lincomycin, control group and *N. sativa* group, respectively. This is higher in the lincomycin group and lower in *N. sativa* group. Statistical analysis of data revealed significant difference in groups ($p < 0.05$). Maximum FCR was in control and minimum FCR in *N. sativa* group (Figure 6).

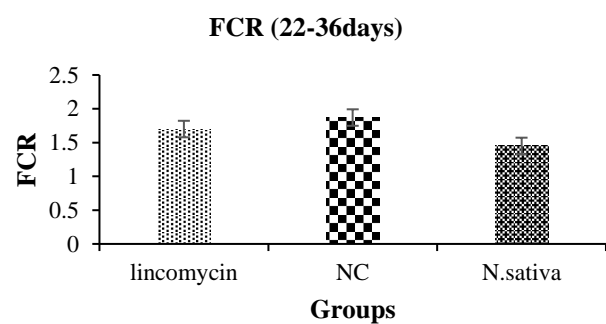


Figure 6: Feed Conversion Ratio (FCR) during finisher phase

3.4 Growth Performance (0-36 days)

Average feed intake (gm) of 5 weeks is 2499.3, 2351.3 and 2120.5g in lincomycin, control and *N.sativa* group respectively. This is greater in lincomycin and negative control group as compared to *N. sativa* group, respectively. Average weight gain (gm) is 1585.8, 1335.1 and 1431.5g, which is greater in lincomycin group and lower in *N.sativa* group, respectively. However, difference was non-significant. Average FCR of 36 days is 1.57, 1.76 and 1.48, which is higher in negative control and lincomycin group and lower in *N.sativa* group (Table 4).

Table 4: Average feed intake (g), weight gain (g) and feed conversion ratio (FCR) per bird fed diets containing Lincomycin and *N.sativa* group (0-36 day)

Diets	Parameters		
	Feed intake (g)	Weight gain (g)	FCR
A (Control)	2351.3 ^B	1335.1 ^B	1.76 ^B
B (Lincomycin)	2492.3 ^B	1585.8 ^A	1.57 ^C
C (<i>N.sativa</i>)	2120.5 ^A	1431.5 ^A	1.48 ^A

Mean with different superscripts within the same column reflect significant differences ($p < 0.05$)

Diet A (control) without growth promoter and whereas B and C, contain Lincomycin @ 6.25g/500g of feed and *N.sativa* @ 6.25g/500g of feed, respectively.

3.5 Hematological analysis

The results of hematological values of blood cells of all three different groups revealed that all mean values of blood cells possessed the normal blood values for normal growth of chicken and they fitted within a range of the values as reported by except the value of platelets (Table 5) [10].

Table 5: Overall Mean \pm SE values of HB, HCT, MCV, MCH, MCHC, RBCs, WBCs and PLT in control, lincomycin and *N. sativa* group

Hematological parameters	Reference values	Negative control	Lincomycin	<i>N. sativa</i>
HB (g/dL)	11.60-13.68	2.66 \pm 0.06 ^c	8.33 \pm 0.13 ^b	10.28 \pm 0.14 ^a
HCT (PCV; %)	29.8-31.6	24.06 \pm 0.30 ^b	32.23 \pm 0.24 ^c	38.91 \pm 0.22 ^a
MCV (mm ³)	115-125	121.06 \pm 0.4 ^{3A}	142.06 \pm 0.2 ^{4C}	134.26 \pm 0.4 ^{9B}
MCH (pg)	25.0-27.0	12.45 \pm 0.28 ^b	37.08 \pm 0.08 ^a	36.46 \pm 0.25 ^a
MCHC (g/dL)	21-23	9.85 \pm 0.14 ^c	25.81 \pm 0.28 ^b	27.50 \pm 0.33 ^a
RBCs (10 ⁶ /μL)	4.21-4.84	2.03 \pm 0.04 ^c	2.26 \pm 0.33 ^b	2.90 \pm 0.09 ^a
WBCs (10 ³ /μL)	20-30	262.15 \pm 1.3 ^{2C}	273.25 \pm 0.1 ^{3B}	283.78 \pm 0.3 ^{6A}
PLT (10 ³ /μL)	25-40	12.66 \pm 0.17 ^b	14.92 \pm 24.4 ^{7A}	11.58 \pm 14.3 ^{8C}

Mean values in a row sharing similar alphabets do not differ significantly ($P > 0.05$)

3.5.1 Hemoglobin

Overall, mean concentration of hemoglobin in different groups shown in table 5; figure 7. Overall mean of level of hemoglobin significantly increased in *N. sativa* treated group as compared to lincomycin group while change in negative control group was non-significant.

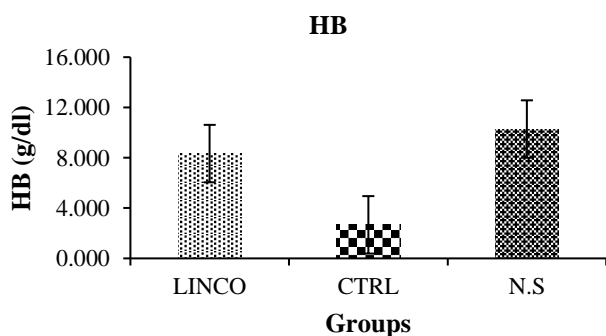


Figure 7: Hemoglobin (Hb; g/dL) level in control and experimental groups

3.5.2. Hematocrit or Packed Cell Volume

Overall, mean concentration of HCT in different groups shown in table 5 and figure 8. Overall mean of level of HCT significantly increased in *N. sativa* treated group and lincomycin treated group as compared to control group while change in *N. sativa* treated group was more significant than lincomycin treated group.

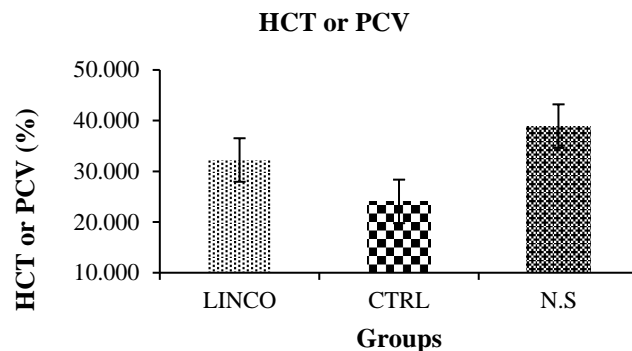


Figure 8: Hematocrit or Packed Cell Volume (HCT or PCV; %) in control and experimental groups

3.5.3 Mean Corpuscular Volume

Overall, mean concentration of MCV in different groups shown in table 5; figure 9. Overall mean of level of MCV significantly increased in lincomycin treated group and *N. sativa* treated group as compared to control group while change in lincomycin treated group was more significant than *N. sativa* treated group.

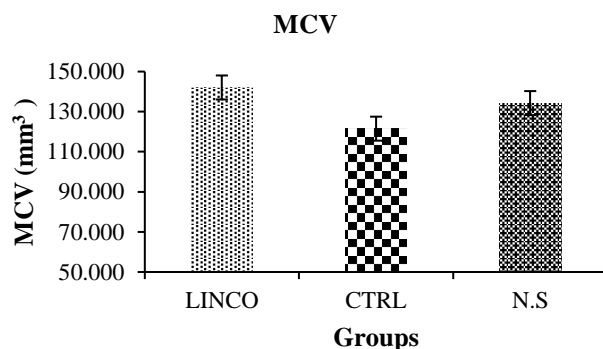


Figure 9: Mean Corpuscular Volume (MCV; mm³) in control and experimental groups

3.5.4 Mean Corpuscular Hemoglobin

Overall, mean concentration of MCH in different groups shown in table 5; figure 10. Overall mean of level of MCH significantly increased in lincomycin treated group and *N. sativa* treated group as compared to control group while change in lincomycin treated group was more significant than control group. There was non-significant difference between lincomycin and *N. sativa* treated group.

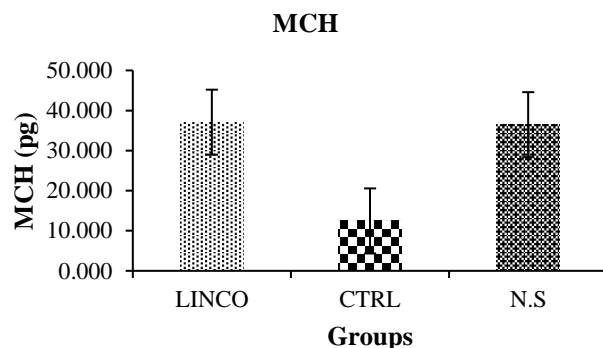


Figure 10: Mean Corpuscular Hemoglobin (MCH; pg) level in control and experimental groups

3.5.5 Mean Corpuscular Hemoglobin Concentration

Overall, mean concentration of MCHC in different groups shown in table 5; figure 11. Overall mean of level of MCHC significantly increased in *N. sativa* treated group and lincomycin treated group as compared to control group while change in *N. sativa* treated group more significant than lincomycin

treated group.

group while change in lincomycin treated group was more significant than to *N. sativa* treated group.

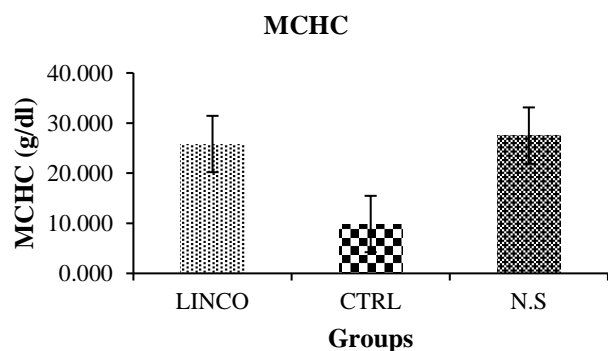


Figure 11: Mean Corpuscular Hemoglobin Concentration (MCHC; g/dL) in control and experimental groups

3.5.6. Total Red Blood Cells Count

Overall, mean concentration of TRBC in different groups shown in table 5; figure 12. Overall mean of level of TRBC significantly increased in lincomycin treated group and *N. sativa* treated group as compared to control group while change in *N. sativa* treated group was more significant than lincomycin treated group. There was significant difference between lincomycin and *N. sativa* group.

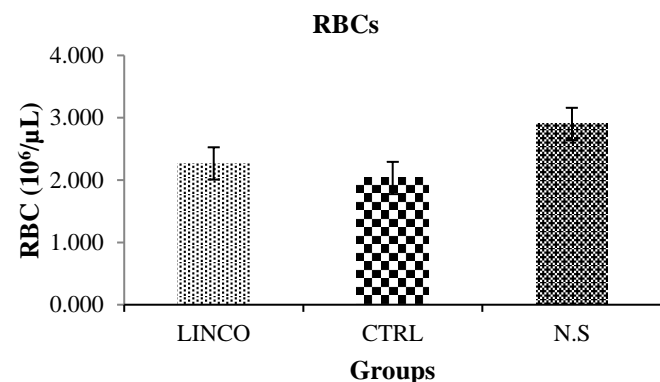


Figure 12: Total Red Blood Cells Count (10⁶/μL) level in control and experimental groups

3.5.7 Total White Blood Cells Count

Overall, mean concentration of TWBC in different groups shown in table 5; figure 13. Overall mean of level of TWBC increased in lincomycin treated group and *N. sativa* treated group as compared to control group while change in *N. sativa* treated group was more significant than lincomycin treated group.

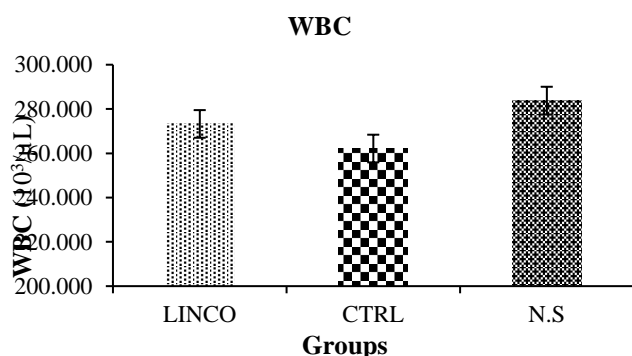


Figure 13: Total White Blood Cells Count (10³/μL) level in control and experimental groups

3.5.8 Total Platelet Count

Overall, mean concentration of TPC in different groups shown in table 5; figure 14. Overall mean of level of TPC significantly increased in lincomycin treated group and control group as compared to *N. sativa*

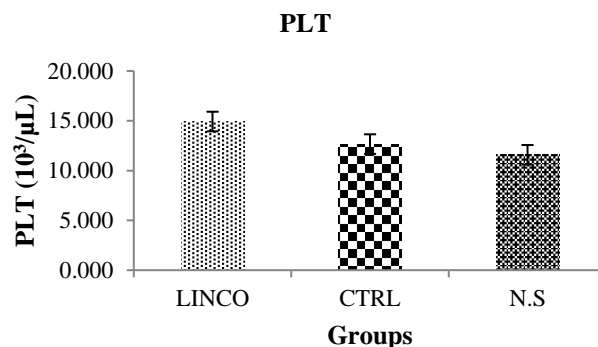


Figure 14: Total Platelet Count (10³/μL) level in control and experimental groups

3.6 Immune titer against ND and IBD

Immune titer for New Castle Disease was improved by given dose of *N. sativa*. However, results of immune titer were significant as compared to lincomycin and control group (Figure 15).

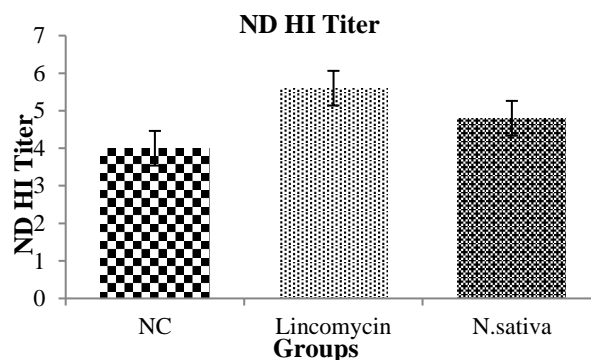


Figure 15: Immune titer against ND

Immune titer for Infectious Bursal Disease was not in the normal range by given dose of *N. sativa*. However, results of immune titer were non-significant as compared to lincomycin and control group (Figure 16).

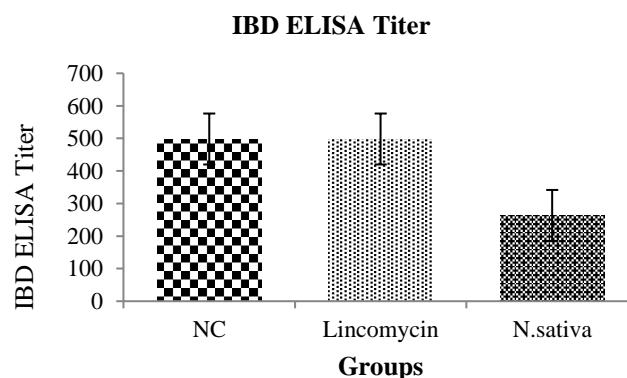
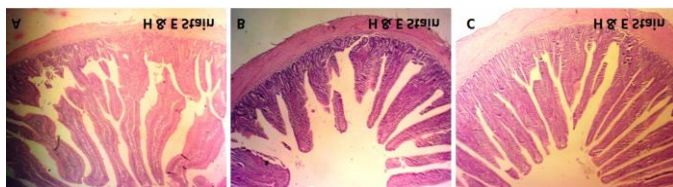


Figure 16: Immune titer against IBD

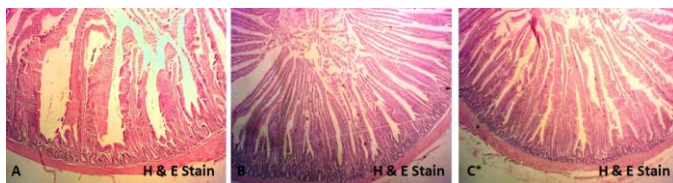
3.7. Histopathological Examination

Histopathological examination of small intestine of broiler chicks of different treatments groups is shown in photomicrograph 1. Length of villi in different treatment showed that length increased in *N. sativa* treatment group when compared with lincomycin treatment group.



Photomicrograph 1: Photomicrograph of small intestine of broiler chicks of negative control, lincomycin and *N. sativa* (A, B & C), respectively. Length of villi of small intestine has increased in the *N. sativa* group as compared to the control and lincomycin group. Epithelium was remained intact in *N. sativa* group as in control and lincomycin group.

Histopathological examination of large intestine of broiler chicks of different treatments groups is shown in photomicrograph 2. Length of villi in different treatment showed that length increased in *N. sativa* treatment group when compared with lincomycin treatment group and epithelium remained intact in *N. sativa* group as observed in control group.



Photomicrograph 2: Photomicrograph of large intestine of broiler chicks of negative control, lincomycin and *N. sativa* (A, B & C), respectively. Length of villi of large intestine has increased in *N. sativa* group as compared to control and lincomycin group. Epithelium was remained intact in *N. sativa* group as in control and lincomycin group.

4. DISCUSSION

Various effects of *N. sativa* have been reported on different bacterial isolates. Both oil and its extracts have been reporting to have a broad spectrum of activity against various microbes. Such as, pronounced antibacterial activity in vitro has been observed against *Shigella* spp., *Vibrio cholera*, *Staphylococcus albus*, *Escherichia coli*, and *Salmonella typhi*, even in 1:100 dilutions. However, the oil of *N. sativa* has activity against Gram-positive microbes as compared to a gram-negative microorganism. The black seeds have strong antifungal activity, especially in *Aspergillus* species [11]. Both water and crude alkaloid extracts of black seeds were effective in different types of microorganisms. Such types of organisms were isolated from human patients suffering from septic arthritis. Even resistant organisms to antibiotics found to be sensitive to extracts of black seeds. Gram-negative microorganisms were found to be more sensitive than gram positive. Black seeds exerted their antibacterial action at lower dose than the high concentration of antibiotics. After intraperitoneal administration of *N. sativa*, it exhibited the strong activity against infection caused by cytomegalovirus in mice. An improvement in innate immunity was the mechanism for the antibacterial action of black seeds [12].

It is found that volatile oils of *N. sativa* exhibit 67 constituents capable of inducing beneficial and pharmacological effects against bacteria such as *Staphylococcus* and *E. coli*. The active components of black seed possessing antibacterial, antioxidant, and anti-inflammatory activities induced positive effects on the immunity and organs involved [13]. However, in the current study *N. sativa* have shown the zone of inhibition only against *E. coli* with 1% dose of *N. sativa*. Feed efficiency (FE) is the prime factor in assessing the feed quality. Research regarding the effect of *N. sativa* on FE is neutral as well as positive. Feed efficiency can be improved by incorporating black seeds in the broiler rations. Researchers have reported the significant change in dietary intake of broiler by consuming feed containing black cumin and antibiotics. Similar results have found in the current study. In other research, it has found that diets with 4% grounded black cumin resulted in less feed intake but better FE as compared to control diet [14]. In the current study, feed efficiency, improved by feeding the diet supplemented with 1% black cumin seeds as compared to lincomycin and control groups.

The present commercial farming is becoming challenging for obtaining the desired weight without the use of antibiotics as growth promoters; therefore, natural products are capable of meeting the desired challenge. Different studies on the effect of *N. sativa* seed on broiler performance have been carried out. Researchers have reported an increased body weight by incorporating grounded *N. sativa* seeds in broiler feed. Similar results have been observed in the current study. Birds have gained more weight as compared to lincomycin group as they consumed the same amount of feed.

Improved average daily weight gain and better feed conversion ratio (FCR) in broilers was achieved with 1% *N. sativa* seed supplemented in broiler diet. Feed conversion ratio improved by using a feed containing 12.5g/kg of *N. sativa* powder as compared to lincomycin and control group. The favorable effects of *N. sativa* on performance thought to be due to high nutritive value as well as pharmacologically active substances present in the seeds. Black seeds contain a mixture of essential fatty acids, particularly oleic, linoleic and linolenic acids that cannot synthesize in the body. There are fifteen amino acids comprising the proteins of *N. sativa* out of which eight are essential. Studies have shown that stimulating effect of black seed on the digestive system, resulting in better absorption and performance. Therefore, in the current study, the broiler chicks of *N. sativa* group have more weight during the finisher phase as compared to lincomycin. The addition of *N. sativa* powder in broiler feed increased bile flow rate, which results in increased emulsification that activates the pancreatic lipases, which then aid in fat digestion and absorption of fat-soluble vitamins. Black seed oil and thymoquinone have hepato-protective effects, so these seeds have been using traditionally in wide range of gastrointestinal disorders.

The increased performance might also be due to antimicrobial effects of the active ingredients of black seed. Antimicrobial activities of *N. sativa* inhibit the growth of following bacteria; *Shigella dysenteriae*, *Vibrio cholera*, *Shigella sonnei*, *Escherichia coli*, *Bacillus pumilus*, *Bacillus subtilis*, *Staphylococcus lutea*, *Shigella flexneri*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* [14]. Findings related to the performance of broiler have shown that no significant effect on body weight gain, average daily weight gain and FCR by the addition of 1% *N. sativa* seed. Similar results have observed in the present study; *N. sativa* group has no significant weight gain during the starter phase as compared to lincomycin and control groups. The reduced weight gain during the starter phase by *N. sativa* attributed to high fiber contents of broiler feed. However, in case of finisher phase significant results have found as *N. sativa* group has more weight gain as compared to lincomycin and control groups.

The dietary *N. sativa* at the level of 1 and 3%, significantly increased final body weight of laying hens and caused negative impact on egg production. However, in contrast, 27 weeks old laying hens, fed diets supplemented with 1, 2, and 3% black cumin seeds, had no significant effects on body weight and FCR. Broiler chicks fed diet having 1 and 2 % black seeds showed a significant decrease in feed consumption, body weight gain and live body weight whereas, non-significant results are obtained regarding FCR. The reduced weight gain due to *N. sativa* meal attributed to high fiber contents of the meal [15]. In the present study, improved FCR found in *N. sativa* group as compared to lincomycin and control group. However, no significant effect on FCR observed during the starter phase due to lower body weight gain in *N. sativa* group. A significant increase in RBC, WBC, PCV and HB of *Trypanosoma brucei* infected rats when treated with black seed oil as compared to control rats [13]. Similar results were observed in the current study. *N. sativa* showed the significant increase in WBC while HB and RBCs remained within the normal range correlated the findings of Orawan and Aengwanich [10]. As the membrane of erythrocytes was intact, no hemolytic action has occurred. Therefore, value of hemoglobin was normal in *N. sativa* group. However, the significant difference found in control, lincomycin, and *N. sativa* group. MCV, MCH and PCV (%) values showed a significant increase in *N. sativa* as compared to lincomycin and control group. While MCHC values showed no significant difference between lincomycin and *N. sativa* groups, while a significant difference between *N. sativa* and control group. The results indicated that the health conditions of the poultry breeds used for this investigation could possibly be classified as normal conditions for normal growth of the poultry.

Black cumin showed an excellent potential as an alternative to antibiotics and vaccines to improve immunity and to reduce mortality in poultry. Mortality decreased from 16.67 to 4.17% by supplementation of layer diet with 1.5% black cumin and from 3.5% in the control group to 2% in the group fed the diet containing 1% powdered *N. sativa* seeds in broilers. New Castle and Infectious Bursal Disease (IBD) improved significantly by replacing bacitracin methylene disalicylate with grounded *N. sativa* seed in broiler diets; however, antibody titer against Infectious Bronchitis (IB) not affected significantly. The current study has also shown that antibody titer not affected with 1% *N. sativa* dose in broiler chicks. The improvement in ND and IBD titer attributed to *N. sativa* oil components such as thymoquinone, carvacrol, nigellimine, thymol and nigelline [16]. An increase in the lymphoid organ weight was observed when broilers fed the diet supplemented with 0.2 and 0.4 % black seeds. However, the present findings reveal that incorporation of *N. sativa* seeds failed to induce any significant impact on antibody titers against IBD and ND virus at 28 and 32 days of age. Supplementation of broiler diet with *N. sativa* strengthened the immunity by preventing liver damage and lipid peroxidation. It has found that broilers under heat stress condition behaved well with reduced

mortality in black cumin treated groups as compared to control group. Reduced mortality rate attributed to the antimicrobial effects that helped the birds to overcome bacterial diseases, increased immunity and promote health [17]. Length of villi of small intestine has increased in the *N. sativa* group as compared to the control and lincomycin group. Epithelium was remained intact in *N. sativa* group as in control and lincomycin group. Length of villi of large intestine has increased in *N. sativa* group as compared to control and lincomycin group. Epithelium was remained intact in *N. sativa* group as in control and lincomycin group.

5. CONCLUSION

The administration of *N.sativa* at the given dose rate (1%) resulted in improved body weight and lower feed intake. This was resulting in improved Feed Conversion Ratio of broiler chicks as compared to lincomycin group. Increased body weight by *N.sativa* was attributed due to its major active ingredient thymoquinone, it reduces the gut motility, maintains the beneficial gut micro-biota. Increased weight gain by *N.sativa* was also due to its anti-microbial activity against various pathogens. The hematological results indicated the health conditions of the poultry breeds used for this investigation could classify as normal conditions for normal growth of the poultry. Studies revealed that the *N.sativa* improves antibody titer against ND and IBD. However, at 1% dose of *N.sativa*, the antibody titer against IBD not affected. Further study can be done for determination of toxicity, side effects and pharmacokinetic properties of isolated antimicrobial compounds.

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