TRENDS IN ANIMAL AND PLANT SCIENCES
https://doi.org/10.62324/TAPS/2024.061
www.trendsaps.com; editor@trendsaps.com
E-ISSN: 3006-0559; P-ISSN: 3006-0540

RESEARCH ARTICLE

Impact of Dietary Lysozyme and Acidifiers on Broiler Performance and Antibody Titer against Viral Diseases

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Article History: 24-54 Received: 27-Nov-2024 Revised: 29-Jan-2025 Accepted: 04-Feb-2025

ABSTRACT

The study aims to check the impact of supplemented Lysozyme and Acidifiers on growth performance and immunity in broilers. For this purpose, 160 broiler chicks obtained from a local company's hatchery, raised to the age of 42 days, and vaccinated against ND, IB, IBD, and H9 according to the commercially available vaccine schedule of that area. These chicks separated into four groups A, B, C, and D equally, each having two replicates (n=40). Group A will not receive any treatment and understood as the control group, group B treated with Lysozyme (lysozyme 50 %, 1ml/4 liter of drinking water), Group C treated with Acidifiers (combination of organic acids 1ml/4 liter of drinking water), and group D will be treated with Lysozyme (lysozyme 50 %, 1ml/4 liter of drinking water) and Acidifiers (combination of organic acids 1ml/4 liter of drinking water) in this group we were checked the combined effect of lysozyme and acidifiers. Starter feed offered from day 1 to the age of 21 days and finisher offered from 22 to the age of 42 days. Blood sampling done at ages 28 and 35 for antibody titers. The body weight of chicks was measured at age days 7, 14, 21, 28, 35 and 42 days. At end of the experiment recorded data of body weight gain, feed consumption, antibody titers, and hematological analysis statistically analyzed by using statistical techniques the variance technique under a completely Randomized Design, and mean values compared by using Tuckey's test. The result of this experiment set a direction to the use of lysozyme and acidifiers at the commercial level in the field.

Key words: Lysozyme, Acidifiers, Antibody titer, Growth performance.

INTRODUCTION

The Poultry Industry is the 2nd largest industry in Pakistan and is grooming day by day. Especially broiler sector is a flashing sector of the Poultry Industry due to its low start-up and quicker return of Finance. Pakistan's total meat production is 4.9 million MTs in FY21, which include 48% share from beef, 37 % from poultry, and 15% from mutton. The poultry industry's share of Pakistan's GDP is 1.3%. Broiler farming needs special attention because it reaches to harvesting age in a short period of time as compared to other livestock farming. To fulfill the meat' demand of the increasing population of the country, it is necessary to give more emphasis to enhance the meat production of broilers but due the prevalence of diseases and shortage of quality ingredients for poultry feed formulations are major issues, due to which broiler performance is continuously in decline direction. New techniques to enhance the profit margins from the broiler business have been persuaded with various approaches such as the use of antibiotics, probiotics, prebiotics, enzymes, and other feed additives as growth promoters.

The population of microbes in the intestine affects the host's health, any changes in the microbial flora of the intestine results in many diseases (Turnbaugh et al., 2006). The population of normal intestinal flora help in the digestion of food, production of many feed nutrients,

Cite This Article as: Siddiqui AA, Abbas A, Zubair I, Rehman A, Kashif M, Ahmad T and Nadeem M, 2025. Impact of dietary lysozyme and acidifiers on broiler performance and antibody titer against viral diseases. Trends in Animal and Plant Sciences 5: 20-23. https://doi.org/10.62324/TAPS/2024.061

and enhance immunity. The excessive use of antibiotics results in a disturbance of intestinal microbial flora both in humans and animals (Lozupone et al., 2012).

From a long period of time, antibiotics are being used in poultry and animal production. In the poultry industry in the broiler's diet antibiotics are being used to control the growth of gram-positive and gram-negative bacteria in the intestine (Kohanski et al., 2007). Now currently due to the development of antibiotics resistant in humans against many pathogens, Europe Union has banned the use of antibiotics in poultry diets as a growth promoter (Hofacre et al., 2003 and Casewell et al., 2003). Now it is necessary to use probiotics, prebiotics, herbs, and exogenous enzymes as an optional approach in place of antibiotics as a growth promoter in poultry diets to accomplish the gut health issues (Edens, 2003, Patterson & Burkholder 2003; Guo et al., 2004). From a prevention point of view, the inclusion of lysozyme in the diet or supplemented through water is considered to be safer than antibiotics. Mostly lysozyme is found in saliva secretions and milk where it provides protection against bacteria (Abdel-Latif et al., 2017 and Gong et al., 2017).

Acidifiers low the pH of water, foods, and intestine act as conserving agents and inhibit the growth of microbes. The supplementation of acidifier in the broiler diet without any antibiotic is a potential growth promoter, improves ADG, and decreases FCR (Ding et al., 2017). Lysozyme is abundantly available in egg white, extracted from it, and is used as a therapeutic drug for humans (Sava, 1996).

MATERIALS AND METHODS

A research was conducted at R&D farm Sadiq Feed Pvt Limited. In this trial 160 broiler birds reared and the duration of the trial was 42 days. After de-population the house scratch out all poultry manure by using scrapper and broom. Then performed 2-3 washing of ECH by using plain water. The washing was done by using detergent in water. Apply Caustic soda to scratch out remaining debris @ 5kg in 200ltr water. (450*45 shed water 400ltr and soda 10kg). White wash the house included Roof. Then applied a spray Lifejacket (Potassium mono per sulphate) 1% solution over floor and roof. Then add bedding material rice husk 2-3inch depth (0.400 kg / square feet of floor). Then thermal fogging was done by using bled of quaternary ammonium compounds and glutaraldehyde groups. Water line was disinfected by hydrogen per oxide 3 % solution for 24 hours then washed by using plain water. Feeder and shed partition was washed with plain water and then dipped in 1% lifejacket solution. After completing all Chick receiving arrangements before 36 hour of chick arrival apply Virkon or lifejacket @ 1%. (water 50ltr for 1000sq feet).

Then temperature was controlled by using diesel brooder and ventilation was managed by using SWF and tunnel fans, inlets and cooling pads. All environmental managemental parameter was controlled by agrologic controller. Strict biosecurity measures were adopted during the completion of trial. Entrance of irrelevant personnel strictly prohibited.

Experimental Birds and Treatment Plan

One hundred and sixty (160) day old broiler chicks (Ross-308) was purchased from Jadeed Chicks. These chicks were equally divided into eight pens. Each pen will contain twenty chicks. Basically, there was four treatment design and each treatment have two replications. In this trial Acidifier was used marketed by KBNP with the name of ACIDOSAN containing Formic acid 39.5%, Acetic acid 1.6%, Ammonium propionate 10%, Sodium (formate) 5.06 %, Mon propylene glycol 1%, and glycerine 1%. The lysozyme was used, marketed by KBNP with the name of Lysoimune Gold containing lysozyme 50%.

RESULTS

Overall results in this trial satisfactory, the use of Lysozyme as antibiotic replacer is a good choice to save the human beings to be antibiotic resistant. Results derived at the end of the trial will be discussed here.

Effect of Treatments on Feed Conversion Ratio

The FCR at the end of trial is given in Fig. 1. The highest FCR was observed in A group followed by B, C and D.

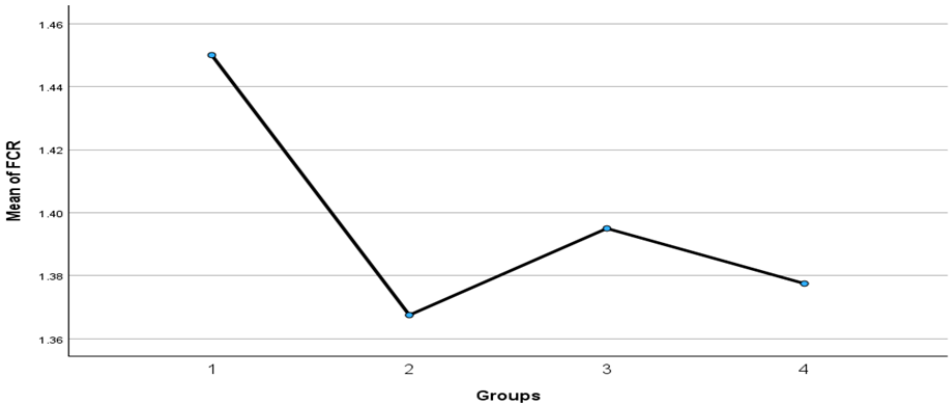

Fig. 1: Show the FCR of different groups at end of trial

Table. 1: ANOVA results for FCR

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	.003	3	.001	.754	.541
Within Groups	.015	12	.001		
Total	.018	15			

Table 2: Effect of treatments on Hematological values

Groups	Control	Lysozyme	Acidifier	Lysozyme Acidifier
Hb (g/dL)	7.98 ± 0.09^{a}	8.11 ± 0.09^{a}	9.52 ± 0.17^{b}	8.10 ± 0.12^{a}
TEC (million/uL)	2.33 ± 0.06^{a}	2.44 ± 0.04^{a}	2.95 ± 0.06^{b}	2.32 ± 0.10^{a}
PCV (%)	31.04 ± 0.73^{a}	32.00 ± 0.64^{a}	37.76 ± 0.35^{b}	31.68 ± 1.23^{a}
MCV (fl)	133.37 ± 3.47^{ns}	131.40 ± 2.21 ^{ns}	132.62 ± 3.39 ^{ns}	136.92 ± 3.36 ^{ns}
MCHC (%)	25.77 ± 0.53 ^{ns}	25.39 ± 0.51 ^{ns}	25.22 ± 0.61 ^{ns}	25.09 ± 0.78 ^{ns}
MCH (pg)	34.32 ± 0.78 ^{ns}	33.32 ± 0.52 ^{ns}	33.36 ± 0.46 ^{ns}	34.35 ± 1.37 ^{ns}

Table 3: Showed the treatment effects on different organs ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Chest weight	Between Groups	85561.375	3	28520.458	9.346	.028
	Within Groups	12206.500	4	3051.625		
	Total	97767.875	7			
Liver weight	Between Groups	2234.564	3	744.855	17.838	.009
	Within Groups	167.025	4	41.756		
	Total	2401.589	7			
Heart weight	Between Groups	86.564	3	28.855	42.669	.002
	Within Groups	2.705	4	.676		
	Total	89.269	7			
Gizzard weight	Between Groups	25.444	3	8.481	2.980	.159
	Within Groups	11.385	4	2.846		
	Total	36.829	7			
Neck weight	Between Groups	1059.240	3	353.080	29.350	.003
	Within Groups	48.120	4	12.030		
	Total	1107.360	7			
Drum Stick	Between Groups	4580.030	3	1526.677	4.246	.098
	Within Groups	1438.250	4	359.563		
	Total	6018.280	7			
Thigh weight	Between Groups	1084.535	3	361.512	3.459	.131
	Within Groups	418.060	4	104.515		
	Total	1502.595	7			

The statistical analysis showed non-significant effect of treatments in finisher phase as mentioned in ANOVA table.

Effects of Treatments on Haematological Parameters

Effects of dietary acidifier and lysozyme on hematological parameters in broiler chicken different superscript letters in a row denote significant difference at $p<0.05$ level, Mean ± SEM, Not significant ($p>0.05$).

Effect of Treatments on Carcass Weight

The highest weight of organs was observed in group D followed by C, B and A. The statistical analysis showed significant effect of treatments on finisher as mentioned in ANOVA table.

DISCUSSION

Liu et al. (2010) studied that lysozyme is considered a naturally occurring protein, having antimicrobial properties, and an important part of the innate immune system. When it is supplemented with 40mg/kg of diet to chicken result in the reduction of clostridium perfringens colonization in the intestine and boosts up the intestinal barrier function and growth performance. It also inhibits the overgrowth of Escherichia Coli and makes the feed conversion ratio of chicken better. Ibrahim et al. (1994) showed in their study that lysozyme has bacteriolytic properties by hydrolyzing the bacterial cell wall mainly gram-positive bacteria at the β-1,4-glycosidic linkage between N-acetyl muramic and N-acetyl glucosamine. The supplementing lysozyme to broiler chicken at a rate of 90 g lysozyme 10 %® per ton of diet results in improved bird growth performance. A positive change was observed in the intestinal flora of broiler chicken, gut antioxidant status, and nonspecific immunity. Zhang et al. (2006) experimentally proved that, in vitro, it is proved that hen egg white lysozyme inhibits the growth of Clostridium perfringens and its alpha toxin production at a minimum inhibitor concentration of 156 microgram/ ml. Zhang et al. (2010) studied that the inclusion of a blend of lysozyme 200g/metric ton of feed decreases the negative health effect in broiler chickens when the birds are challenged with necrotic enteritis, leading to better growth performance. Humphrey et al. (2002) studied show that the use of lysozyme in the diet results in the increased height of villi in the intestine which provide more absorption to chicken for feed nutrients. Khalil et al. (2020) studied that a remarkable weight gain percentage and improvement in other hematological factors were achieved in a trial where lysozyme was supplemented to broiler chicken via drinking water at a dose of 1ml/4ltr of DW. Adil et al. (2010) described that the European Union no have, any objection to the use of acidifiers and their salts in poultry production because they are esteemed safe. (Lückstädt and Mellor, 2011) described that the classification of feed preservatives by the European Union included formic acid, propionic acid, and several other organic acids, and their salts. Leeson et al. (2005) stated that remarkable improvement was achieved in broiler chickens in terms of increasing carcass weight and breast weight yield when they are supplemented with butyric acid. Fascina et al. (2012) experimentally proved that a combination of organic acids comprising acetic acid 6.5 %, citric acid 8%, formic acid 7%, benzoic acid 25.5%, and lactic acid 30% improves the broiler growth performance parameters. Pham et al. (2022) proved that feeding a blend of organic acids with essential oil 500mg/kg of broiler diet gives distinguished results in terms improve FCR, inhibiting the growth of Clostridium perfringens, and improving overall health status. Ebeid et al. (2022) studied that feeding of organic acids preserves healthy microbial balance leading to improve gut structural integrity, enhancing normal functioning of GIT, and

guaranteeing the best intestinal immunity. Ma et al. (2021) studied the use of mixed organic acids with 3000mg/kg in the diet to increase the immune characteristics and antioxidative response in the serum of broiler chicken.

Conclusion

In this trial it was concluded that the lysozyme and organic acids are good replacer for antibiotic, and have positive impact on weight gain, FCR, antibody titers, haematological factors and overall carcass yield. The lysozyme improves the overall health status of chickens. Antibiotic replacement via organic compounds and enzymes is a vast subject and need a lot research in this field.

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