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Effect of probiotic and prebiotic supplementation on performance and immune response of broiler chickens vaccinated against Newcastle disease

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Abstract

This study was carried out to evaluate the effects of two different commercial products Bactofort® and Azix plus®) used in feed and water respectively on growth performance and immune response of broiler chickens vaccinated against Newcastle disease (ND). A total of 120 day old broiler chickens were used for this experiment. The birds were randomly allotted to five treatments: group A (Control: given basal diet without additives), group B and C were administered probiotics in feed at 0.5and 1.0g/kg of feed while group D and E birds were administered prebiotic in drinking water at an inclusion rate of 2-5ml/2L. Weekly weight gain and feed conversion rate of each group of birds were recorded for the six weeks experimental period. Humoral immune response was assayed by Haemagglutination inhibition test. There was significant difference ($P \leq 0.05$) in the weight gain between the four experimental groups when compared to the control group from the second week to the end of the experiment. Group B, C, D and E birds had higher live weight than group A (Control) while feed conversion ratio was higher in group B and C at the end of the experiment. Although administration of the probiotic appeared to improve the antibody responses to Newcastle disease virus after vaccination, the antibody titres of the probiotic-treated group were not significantly different from that of the control. The ND virus antibodies produced was inadequate to give complete protection against the disease. Therefore the study concludes that inclusion of prebiotic and probiotic in poultry diet and drinking water improved weight gain and feed conversion ratio but had no significant effect on the immune status of the birds.

Keywords: Broiler chickens, Feed conversion ratio, Live weight, Prebiotic, Probiotic, Newcastle disease

Introduction

The poultry sector is believed to contribute up to 25% of the agricultural gross domestic products of the Nigerian economy and currently Nigeria is rated as the leading country in Africa in commercial egg production and the fourth in broiler production (FAO, 2009). Feed additives are products used in animal nutrition for purposes of improving the quality of feed and the quality of food from animal origin and to improve the animal performance and health (Hashemi and Davoodi, 2010). Antibiotics as feed additives have been used for many years in poultry diets (Engberg *et al*., 2000). Due to the potential of bacterial resistance and antibiotic residues in animal products, attempts are being made to replace them with prebiotics and probiotics (Goodarzi and Nanekarani, 2014). These replacement ingredients are claimed to enhance growth and the immune status against all infectious agents (Al-Khalaifah, 2018).

Probiotics are live microbial feed supplements, which beneficially affect the host animal by improving its microbial intestinal food balance (Fuller, 1989). Prebiotics are non-digestible feed ingredients that beneficially affect the host by selectively stimulating the activity of one or a limited number of bacteria in the colon (Gibson and Roberfroid, 1995). Prebiotics influences on intestinal bacteria and immunity of chickens have been reported (Bozkurt *et al*, 2014). The predominant prebiotics tried in chickens include types of oligosaccharides like fructooligo-saccharides (FOS), Inulin, mannonoligo-saccharides (MOS) and Xylooligosaccharides (Patterson and Burkholder, 2003). In animal production systems, probiotics are frequently used for improving the health status as well as production performance, feed conversion efficiency and immune responses especially in cattle, swine and poultry (Doyle and Erickson, 2006; Willis *et al.*, 2007).

Newcastle Disease is an important viral disease of poultry owing to its wide distribution and high fatality rate. The disease is highly contagious and death can be abrupt and whole flocks can be lost (Eze *et al.*, 2012, Eze and Ike, 2015), either through 100% mortality rates or condemnation of carcasses (Miller *et al.*, 2013). The Office International des Epizooties/world Organization for Animal Health (OIE) regards ND as a notifiable disease (in the list A Group of disease) (Liu *et al.*, 2016). In-feed antibiotics are not directly effective against Newcastle disease virus (NDV), but they may assist in preventing associated production problems. With in-feed antibiotics being controlled or banned, probiotics, particularly those sourced from spices, and probiotics have been investigated as potential alternatives for maintaining seroconversion in poultry vaccinated against NDV (Bansal *et al.*, 2011).

Contemporary biosecurity threats arising from the increasing resistance of pathogens to antibiotics and the accumulation of antibiotics residues in animal products and the environment (Barton, 2000, Snel *et al.*, 2002) elicit a call for a worldwide antibiotic growth promoter (AGP) ban. The use of antibiotics as growth promoters was banned in 2006 by the European Union (Castanon, 2007).

In commercial poultry production, antimicrobial compounds are commonly included in poultry feed by farmers for promoting growth and control of disease (Demir *et al.*, 2005). The search for alternative to antibiotic growth promoters (AGPs) has been intensified following concerns regarding the development of resistant group of pathogenic and opportunistic pathogens and the breakdown of the symbiosis existing between animals and desirable gut flora (Alloui *et al.*, 2014).

Feed efficiency, feed conversion ratio, survival rate and weight gain rate are considered as the main parameters for evaluating effectiveness of non-AGPs in scientific studies, either via comparison with AGPs or using them alone (Olnood*et al.*, 2015 and Zhang *et al.*, 2015)

Supplementation with probiotics and prebiotics can improve the performance of broiler chickens (Bozkurt *et al.*, 2014). However, there are still indications that the results of using these products in poultry diets are quite inconsistent. Hence, the aim of the current study was to evaluate the effects of probiotic and prebiotic supplementation in broiler diets on performance and immune response of broiler chickens vaccinated against Newcastle disease.

Materials and Methods

Study Location

This study was carried out in Makurdi the capital of Benue state, Nigeria. The State is located in the North Central Geopolitical zone of Nigeria (NPC, 2006). Makurdi is located in the North Eastern part of Benue state and lies on latitude 7°30'N and longitude 8°35'E. It is located within the flood plain of lower River Benue valley (Tyubee, 2009).

Commercial Products

Bactofort® probiotics was used, a mixture of feed additive obtained from the combination of dried yeast culture and dried bacterial fermentation products and extracts, containing active dry yeast, yeast culture, *Bacillus subtilis*, dried *Aspergillus oryzae* fermentation extract, and dried *Aspergillus niger* fermentation extract. It was added to the ration in two concentration levels 0.5 and 1 g/kg feed for six successive weeks. Azix plus(R) a prebiotic containing Sodium chloride, formic acid, propionic acid, Ammonium propionate and other additives was used in drinking water. The water used to prepare the prebiotic treatment each day was non-chlorinated water. The commercial products were used according to the manufacturer instruction.

Experimental Design

A total of 120 day-old broiler chicks purchased from a commercial hatchery and brooded for two weeks in a deep litter system were used for this experiment. The temperature in the flock was 32°C at the beginning of the experiment and was gradually reduced to between 21°C and 26°C. The birds were fed with a commercial feed and provided with water ad libitum for the 6 weeks experimental period and all necessary routine management practices were observed. No antimicrobial agent was applied to the birds. The broiler chicks were divided randomly into five groups (A-E) of 25 and 20 birds each. Probiotic and prebiotic feeding was from the 1st day of age up to 42 days.

Group A: Birds were fed with commercial feeds only with no additive and served as the control.
Group B: Birds were administered probiotics in feed at an inclusion rate of 0.5g/kg
Group C: Birds were administered probiotics in feed at an inclusion rate of 1g/kg
Group D: Birds were administered prebiotics in water at an inclusion rate of 2ml/2L
Group E: Birds were administered prebiotics in water at an inclusion rate of 5ml/2L

Assessment of Performance

During the experiment, birds were weighed weekly and feed intake per pen was recorded at the same time. Feed intake was determined for each repetition as the difference between the amount of feed supplied and the remaining feed at the end of each week. Body weight and body weight gain were calculated as the difference between the final and initial bird weight. Feed conversion ratio (FCR) was calculated as the ratio between feed intake and body weight gain at the end of each week.

Sample Collection

The birds were vaccinated against Newcastle disease on days 7 and 21 and infectious bursal disease on day 14. Blood samples were collected from the chicks at different intervals between June to August 2021. On day 7 before the first vaccination blood samples were taken from each group to assess the maternal antibody level. Blood samples was also taken at weekly intervals after each vaccination and challenge. About 1-2ml of blood was collected from the wing vein of each bird using 23 gauge sterile hypodermic needle and syringe. Serum was separated after centrifugation at 1000g for 10 min and stored at -20° C until analysis. Hemagglutination inhibition (HI) test was performed on sera and humoral immunity was assessed as antibody production to Newcastle disease virus. A total of 469 sera were collected.

Determination of Newcastle Disease Antibodies

The serological test was conducted at the National Veterinary Research Institute (NVRI), Vom Plateau State. Haemagglutination (HA) and Haemagglutination inhibition (HI) test were conducted according to the method described in OIE manual, (2012). The Lasota strain of NDV and antibody positive serum obtained from the NVRI, Vom were used as the positive antigen and control serum. Briefly, the 4 HA units of Lasota strain of NDV in equal volume (25 µl) were added to each serum dilution in a V-shaped microtitre plate and incubated at 37°C for 45 min. Thereafter, 1% of chicken RBC in 25 µl volume was added to each well and incubated at 37°C for 15 min. The reciprocal of the last serum dilution showing inhibition of hemagglutination of the 4 HA units of the NDV was considered as the HI antibody titre of the serum (log_2 value of HI titre).

Challenge Experiment

The birds were challenged two weeks after the second dose of ND vaccine. One vial of the lyophilized NDV Kudu 113 strain with a titre of $10^{7.6}$/0.1ml was diluted with 9 ml of sterile phosphate buffer saline (PBS). A sterile hypodermic needle was used to inject 0.2ml of the challenge virus intramuscularly to the breast muscle of the birds. Clinical signs and mortality were recorded daily.

Data Analysis

All data obtained from the experiment were subjected to Analysis of Variance (ANOVA) using SPSS 20 software (SPSS Inc. IL, USA).Significant difference between control and treatment means were separated using Duncan Multiple Range Test, p value ≤0.05 was considered significant.

Results

The result of the average body weight gain and FCR of all the experimental birds are presented in (Table 1). There was no statistical significance difference in the body weight gain among the five experimental groups in the first three weeks (p≥0.05). However, there was statistical significance difference in the fourth to sixth week (p≤0.05). The body weight gain of broiler birds in group B and C was significantly higher than group D and E compared to the control which had lower weight (Table 1). There was no significant difference in feed consumption between the treatment groups and the control but there was statistical significance difference in the feed conversion ratio (p≤0.05) between the experimental groups and the control (Table 1). However, the FCR of the four treatment groups was lower than that of the control group, birds in groups B and C fed probiotics in feed had higher FCR than groups D and E which were given prebiotics in drinking water (Table 1).

Serum antibody titre for maternal immunity against NDV in the first week of age was significantly high across all the groups with (71.0%) of birds having protective HI antibody titre of ≥4Log2and (29.0%) were non-protected against ND (Table 2). The overall result of the HI antibody test showed that (76.8%) of the birds were not protected and (23.2%) were protected against Newcastle disease (Table 2). The reported protective antibody titre for ND vaccines are HI ≥4Log2 (OIE, 2000) with reference to conventional ND vaccine designed for intensively reared commercial chickens. The results showed that 2 weeks after the first vaccination at 3 weeks of age, there was increase in antibody titre across all the groups when compared with the control group, group C had the highest HI titre followed by group E and B (Figure 1). Two weeks after the second vaccination at 5 weeks of age, results showed that majority of the birds in groups B, C and D had low antibody titre ≥L-3Log2 with only few birds in group B having antibody titre ≥4Log2 which was considered protective (Figure 2). Significant influence on the immune response to ND virus through supplementation in feed and water was observed. The HI antibody titre did not differ significantly (p≥0.05) among the different treatment groups till the 14 and 21 days post vaccination. However, the titre started decreasing in all the treatment groups, and this trend continued until the birds were challenged at 5 weeks of age (Table 2).

The result after challenge of the experimental birds showed that the highest mortality was recorded in group C (96%) and group E (80%) with an overall mortality of 74% (Table 3).

Table 1: Effect of probiotic and prebiotic on live body weight (Mean ±SEM) and feed conversion ratio (FCR) in broiler chickens.

Age(days)	Index	A	B	C	D	E
7	Live body weight	0.29±0.64[a]	0.45±0.69[b]	0.46±0.50[b]	0.45±0.69[b]	0.46±0.68[b]
	FCR	1.38	1.27	1.25	1.21	1.20
14	Live body weight	0.54±0.19[a]	0.80±0.82[b]	0.83±0.54[b]	0.75±0.69[b]	0.81±0.94[b]
	FCR	1.46	1.35	1.35	1.28	1.25
21	Live body weight	1.24±0.15[a]	1.44±1.12[b]	1.41±1.12[b]	1.40±1.60[b]	1.40±1.11[b]
	FCR	1.55	1.40	1.40	1.30	1.28
28	Live body weight	1.42±0.93[a]	1.70±0.12[b]	1.70±1.20[b/c]	1.64±0.99[c]	1.60±1.04[c]
	FCR	1.70	1.56	1.55	1.44	1.40
35	Live body weight	1.60±0.73[a]	2.10±1.34[b]	2.30±1.30[c]	2.13±1.20[c]	1.90±0.97[c]
	FCR	1.85	1.78	1.75	1.70	1.71
42	Live body weight	1.74±0.82[a]	2.43±0.90[c]	2.70±1.30[d]	2.42±0.80[c]	2.02±0.93[b]
	FCR	2.24	2.05	2.04	1.95	1.92

*SEM=standard error or means
In each row, means with different superscript letters are significantly different (P≤0.05)

Table 2 Newcastle disease antibody titre after vaccination and challenge of experimental birds

Age (Weeks)	Non protected (%) ≥1-3Log2	Protected (%) ≥4Log2	Total
1.	9(29.0)	22(71.0)	31(100)
2.	99(88.4)	13(11.6)	112(100)
3.	66(62.9)	39(37.1)	105(100)
4.	94(94.9)	5(5.1)	99(100)
5.	92(98.9)	1(1.1)	93(100)
6.	0(0.0)	29(100)	29(100)
Total	**360(76.8)**	**109(23.2)**	**469(100)**

Table 3: Mortality rate according to groups after challenge with Kudu 113 NDV

Group	Total number per unit	No. dead after challenge virus	No. survived after challenge virus	Percentage mortality (%)
A	25	15	10	60.0
B	25	18	7	72.0
C	25	24	1	96.0
D	20	12	8	60.0
E	20	16	4	80.0
Total	**115**	**85**	**30**	**74.0**

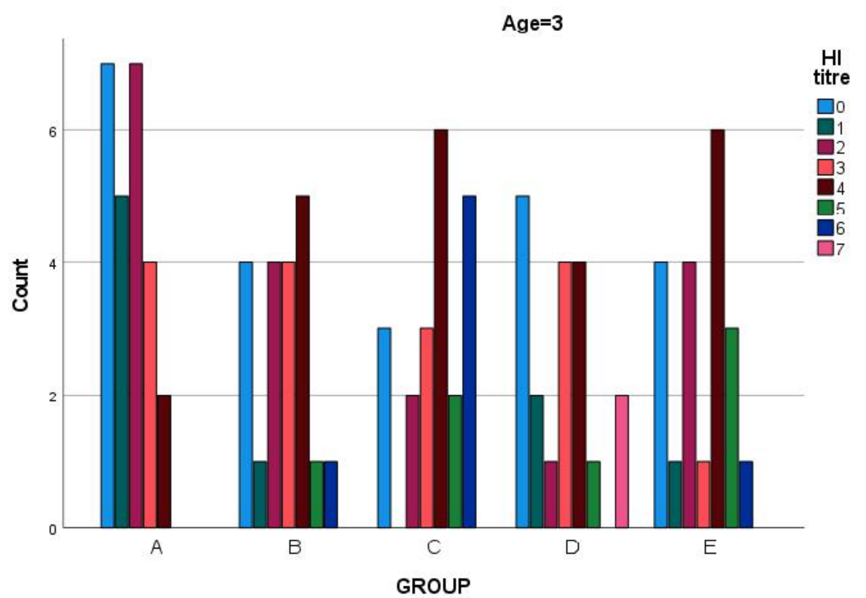

Fig.1: Antibody titre of experimental birds at 3 weeks

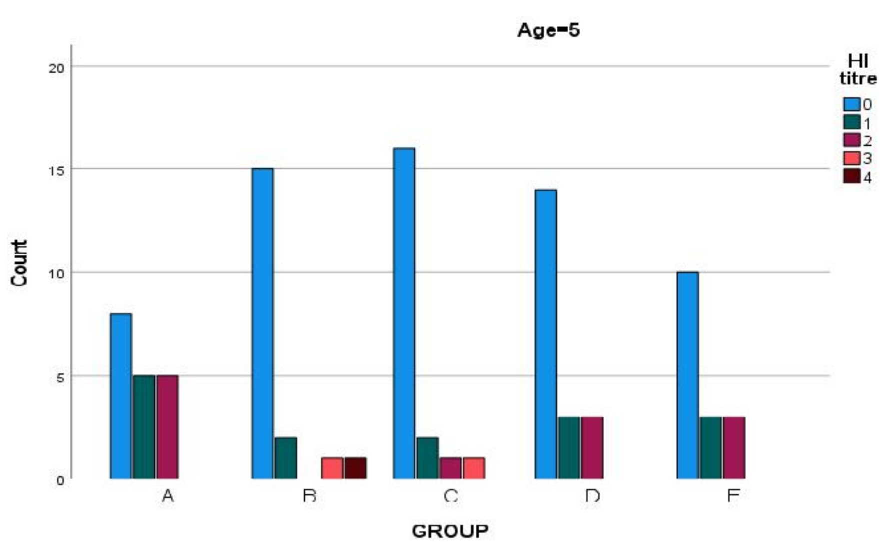

Fig.2: Antibody titre in experimental birds at 5 weeks

Discussion

The results of this study showed a significant improvement in the weight gain of the birds fed probiotic and prebiotic. This finding supports the results of Panda *et al.* (2006) and Talebi *et al.* (2008) that probiotics improved broiler performance. Weight gain in poultry production is a function of the feed conversion rate of the individual bird that makes up the flock and this can be affected by many other factors including the gut health. Probiotics have been reported to improve gut health by preserving the beneficial bacteria of the gastrointestinal tract (GIT) which play important role in nutrient metabolism and absorption throughout the GIT (Oluwayinka*et al*., 2020).

The result of the feed intake was low in both the treatment group and the control birds. This observation is similar to previous report by Zhang and Kim (2014) that the addition of probiotics did not have any significant effect on feed intake of broiler chickens. The inability of the probiotic to improve feed intake may be due to its taste or flavor, also Yang *et al*. (2008) reported that feed additives could affect nutrient utilization of birds. On the other hand, there are reports that supplementation of the diet with probiotic, significantly improved the feed intake in broiler chickens (Odefemi, 2016). It was also reported that the mixture of probiotics when administered creates a favourable micro flora in the gut of birds through competitive exclusion such as competition for adhesion site and nutrient with pathogenic microorganism thereby enhancing the uptake and utilization of available nutrients and invariably improving live weight and feed conversion ratio of the birds (Nilson *et al.*, 2004). Similarly, Murshed and Abudabos (2015) found that dietary inclusion of probiotic and prebiotic had beneficial effects on body weight gain and feed conversion ratio.

The reported protective antibody titre for ND vaccines are HI ≥4Log2 (OIE, 2000) with reference to conventional ND vaccine designed for intensively reared commercial chickens. The ND antibody titre levels of all the treatment groups were found higher than that of the control group. The percentage of vaccinated birds with HI titres ≥4Log2 showed the highest increase (37.1%) at three weeks of age. The antibody titre peak in this study was achieved at 21 days after the first dose of the vaccine. This is similar to the findings of Rahman *et al.* (2004) that HI antibody titres attain the peak between 2 and 3 weeks after ND vaccination. Flock immunity reported by Van Boven *et al*. (2008) as the only means to prevent the transmission of NDV when ≥ 85% of vaccinated birds have antibody titres of ≥ 3log2 was not achieved in this study. Prior to challenge at five weeks of age, the percentage dropped with none of the groups having percentage mean HI antibody titre sufficient to protect the birds from challenge. High level of HI antibody titre due to the feeding of probiotic and prebiotic in broilers was reported by several authors (Mahdavi *et al*., 2013; Srinvas *et al*., 2015). The primary function of the immune system is to identify and eliminate pathogens (Waititu *et al*., 2014), and this may be enhanced by administering probiotics that stimulate the local immune system (Fuller, 1989). The most likely reasons for the increase in antibody titer in the present study might be due to the competitive exclusion of pathogens through competition of receptor sites, production of volatile fatty acids that are inhibitory of certain enteric pathogens or stimulation of host innate immune response (Gaggia *et al*., 2010). Reports from (Cross, 2002) indicated that some probiotic could stimulate a protective immune response sufficiently to enhance resistance to microbial pathogens. Similarly, Haghighi *et al.* (2005) reported that probiotic-treated birds had significantly more serum antibody than the birds that were not treated with probiotics. The gut and its resident microbiota play an essential role in shaping the immune system (Nover and Huffinagle, 2004). The report by Mountzouris (2007) failed to show improvement in the overall broiler humoral status at systemic level in response to probiotic supplementation. Higher anti-NDV antibody titre as a result of feed supplementation with probiotics have also been reported by Chughtai *et al*. (2015). However, a recent report by Shirisha *et al*. (2017) suggested that there was no significant influence on anti-NDV antibody titre with probiotic supplement. Manafi *et al.* (2018) reported that the inclusion of a commercial preparation of S. boulardii in the feed of broilers improved antibody titres against NDV. According to this trial, the best immune result was obtained when the probiotic was used at a concentration of 100 g/ton.No significant differences in mortality rate was found between probiotic and prebiotic fed and the control group in this study. This result is in disagreement to the findings of Lalev *et al.* (2011), and Arpasova *et al*. (2012); who observed that probiotics increases resistance to infectious diseases and reduces risk of mortality caused by the presence of infectious diseases. The study carried out by Al-Sultan *et al*. (2016) showed that poultry that were supplemented with probiotics had a higher antibody response to ND vaccine compared to those whose feed were supplemented with organic acids or prebiotics as well as the control. They observed a reduction in mortality by 6.66% compared to the control.

Conclusions and Recommendations

Inclusion of dietary prebiotic and probiotic supplementation of broiler feed and water improved the weight gain and feed conversion ratio. Feed supplements used in this study stimulated humoral immune response of vaccinated birds to produce some additional amount of antibodies but the NDV antibodies produced was inadequate to give complete protection against Newcastle disease. Further studies are needed to identify eventual differences among the commercially available prebiotics and probiotics in each country, determine their optimum level of inclusion in broiler ration as well as the immune-modulatory effects of feed and water supplementation with these products

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Conflict of Interest

The authors declare that they have no competing interests.

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