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Persistence of passive immunity against some important viral diseases and feed conversion in chickens fed diet supplemented with probiotic

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Abstract

The ban of antibiotic growth promoters due to increased bacterial resistance and drug residues in poultry production, together with consumer's demand for "natural" products, have encouraged increase in the use of alternative growth promoters like probiotics. This study evaluated the effects of probiotic supplemented diet on the feed conversion ratio (FCR) and passive immunity against Newcastle disease (ND) and Infectious Bursal Disease (IBD) in chickens. Forty, one day-old broiler chicks were randomly selected into two groups: No probiotic and probiotic fed groups. The weight gained and FCR were monitored while blood samples for serum were collected from five birds per group weekly, for four weeks. Haemagglutination inhibition (HI) and passive HI tests were conducted to evaluate the levels of passive immunity (maternally derived antibody) in the different groups. There was a significant improvement in weight gain and FCR of birds fed the probiotics. Maternally derived antibody (MDA) against ND and IBD in the chicks were high at week 1. There was a delay in the decay of MDA against ND and IBD in the probiotic fed groups although MDA levels against both diseases were almost the same at week 3 in both probiotic fed and no probiotic groups. It could be concluded that probiotics can facilitate increased body weight, FCR and persistent passive immunity in chickens.

Keywords: Passive immunity, Newcastle disease, infectious bursal disease, feed conversion, probiotics

Introduction

Probiotics are feed additives that contain live microorganisms and promote beneficial effects to the host by favoring the balance of the intestinal microbiota (Mousavi *et al.,* 2015). Probiotics act by adhering to the binding sites of the intestinal epithelium, producing bactericidal substances, stimulating the immune system, suppressing ammonia production and neutralizing enterotoxins (Panda *et al.,* 2006; Dallout *et al.,* 2003). Nowadays, the use of probiotics in order to reduce drug and antibiotic inclusion into the feeds, especially in poultry production, is widely practiced (Tomar *et al.,* 2011; Smith, 2014; Dibaji *et al.,* 2015). Probiotics are introduced as feed supplements that can beneficially affect the intestinal microbial balance, resulting in improved body weight gain, improved feed conversion ratio (FCR) and reduced mortality in broiler chickens (Haung *et al.,* 2004; Panda *et al.,* 2006). Probiotics also protect chickens against avian pathogens (Biggs and Parsons, 2008), stimulate systemic immune responses (Kabir, 2009) and enhance the production of natural and specific antibodies (Talebi *et al.,* 2008).

Newcastle Disease and Infectious Bursal Disease are important viral infections of poultry that have been incriminated as causes of massive economic losses in poultry (FAO, 2013). They affect birds of all ages, especially ND, while IBD affects more of young birds and vaccination has been used over the years to prevent outbreaks on poultry farms in Nigeria. In a bid to maintain a high antibody titer against these diseases, routine and multiple vaccinations are done bearing in mind that the consequence of immunosuppression is lowered resistance to disease (*Minalu et al*., 2015). Among factors that affects immune response to vaccination is high levels of passive immunity, also known as maternal derived antibody (MDA) in chicks.

For ND virus and IBD virus, as with other agents, MDA are naturally passed from the hen to the chick through the egg yolk (Hamal *et al.,* 2006) by hyper immunized dams. The type and amount of MDA transferred depends on the circulating antibodies in the hen at the time the egg was laid. They have a characteristic half-life usually between 2 and 3 weeks of age (Hamal *et al.,* 2006) before they naturally degrade in the chick. Although MDA can prevent or reduce clinical disease by passive immunization during the first weeks of the chick's life (Al-Natour *et al.*, 2004), they can also hinder the immune response to vaccination (Kapczynski and King, 2005; Rauw *et al.,* 2009). With the increase in the use of probiotics by farmers, it is important to evaluate its effect on the weaning of MDA. The beneficial effect of some probiotics on growth parameters. egg production of laying hens, feed intake, feed conversion ratio (FCR), mortality rate and antibody responses to routine vaccination with live Avian influenza (AI), NDV and infectious bursal disease vaccines in broiler chickens has been reported (Davis and Anderson, 2002; Talebi *et al.,* 2008; Dibaji *et al.*, 2015; Talebi *et al.*, 2015). This study evaluated the effect of a probiotic that contained *Lactobacillus acidophilus, Lactobacillus casei, Enterococcus faecium and Bifidobacterium bifidium,* on weight gain, feed conversion ratio (FCR) and the weaning of MDA against ND and IBD.

Materials and methods

Forty day-old chicks were randomly placed into two groups: No probiotic fed (A) and the probiotics fed (B) groups. The chicks were labelled appropriately, housed in separate groups and fed *ad-libitum* with commercial diet. Water-soluble PrimaLac® (Star-Labs, USA) was administered orally at 12g/L for 42 days. The water used to prepare the probiotic treatment each day was non-chlorinated well water. Feed conversion was assessed by measuring the weekly feed consumption and weight gain. One thousand doses each of one commonly used lyophilized IBD vaccines in Nigeria and Lyophilized ND Vaccine (Lasota strain) produced by the National Veterinary Research Institute, Vom, Jos plateau State was the source of antigen used for the tests. Blood was randomly collected from five chicks from each group every week until they were four weeks old. Blood was collected from the jugular vein and the sera were used to assess maternally-transferred antibodies of the chicks against Newcastle disease and infectious bursal disease. Haemagglutination inhibition (HI) and passive haemagglutination inhibition tests (Allan and Gough, 1974; Ezeibe *et al.,* 2012) were used to determine antibody titres of the chickens against Newcastle disease and infectious bursal disease respectively.

Haemagglutination (HA) test

50 µl of normal saline was dispatched into each well of the first three rows (A-C) of a 96-well microtitre plate. A vial of 1000 doses of ND vaccine (Lasota) was reconstituted with 1ml of normal saline. The reconstituted ND vaccine (50 µl) was then added to the first well in each of the three

rows and serially diluted up to the 11th well of each of rows (A-C). Finally, 50 µl of 0.5% washed chicken red blood cells (RBC) suspension was added into each well of the rows and the plate was allowed to sit for 45 minutes at room temperature. A uniform layer of agglutinated RBC covering the bottom of well of the plate was considered as positive HA and negative when a sharp buttoning of RBCs formed at the bottom of the wells. The vaccine antigen titre was determined by recording the highest dilution of vaccine that prevented the agglutination activity of RBCs.

Haemagglutination inhibition (HI) test

50 µl of normal saline was dispatched into each well of the 96-well microtitre plate. Then 50 µl of the sera sample was serially diluted across each row (triplicate test was done on each sample) and then 50 µl of reconstituted ND vaccine at 4HA unit was added into each well of microtitre plate. This mixture was then incubated at room temperature for about 15 minutes. To each well, 50 µl of 0.5% washed chicken RBC was added and mixed thoroughly by shaking. The plate was incubated at room temperature for 30 minutes and the result of HI test was read. The antibody titre of each serum sample was determined by recording the highest dilution of serum which inhibited the agglutination activity of the RBCs. The average of the antibody titre of each sera sample was taken as the titre of a particular sera sample.

Passive haemagglutination inhibition tests

One (1%) washed human erythrocytes (blood group O+) were sensitized with IBD antigen by mixing the prepare red cells with the IBD vaccine virus and incubating this at 4 °C overnight. 50 µl of normal saline was dispensed into each well of the 96-well microtitre plate. Then 50 µl of each serum sample was serially diluted across each row to the 11th well (triplicate test was done on each sample) and the last well on the microtitre plate was used as the control group. Thereafter, 50 µl of the sensitized erythrocytes was added to each well on the microtitre plate. The mcirotitre plate was tapped gently to ensure even dispersion of the erythrocytes and then incubated at 37°C for about 45 minutes. After incubation, the plates were read and the reciprocal of the last dilution showing complete agglutination of the erythrocytes was recorded as the passive HA titre of the serum sample.

Statistical analysis

Data were analysed using SPSS 16 software by employing T-test to analyze the difference in the variables (weight gain, feed consumption and maternal antibody titre) between the two groups. Significance difference was taken at $p < 0.05$ level.

Results

The average body weight of the chickens at day 1 was 41g which increased steadily over the weeks in all the group. The rate of weight gained in the groups fed with probiotics was significantly higher than those not fed with probiotics at weeks 1, 2 and 4 (Figure 1).

There was no significant difference ($p < 0.05$) in feed consumption between the two groups but the FCR of the probiotic fed group was lower than the groups not fed probiotic (Table 1).

The MDA against ND and IBD was very high in the chicks at week 1. There was no significant difference in the rate of weaning of MDA against ND and IBD (Figure 2 and 3) between the two groups. The MDA weaned to its lowest titre at week three for both ND and IBD. There was a steady decline of MDA against ND and IBD for the groups not fed PrimaLac but there was a delay in its decline in the probiotic fed group.

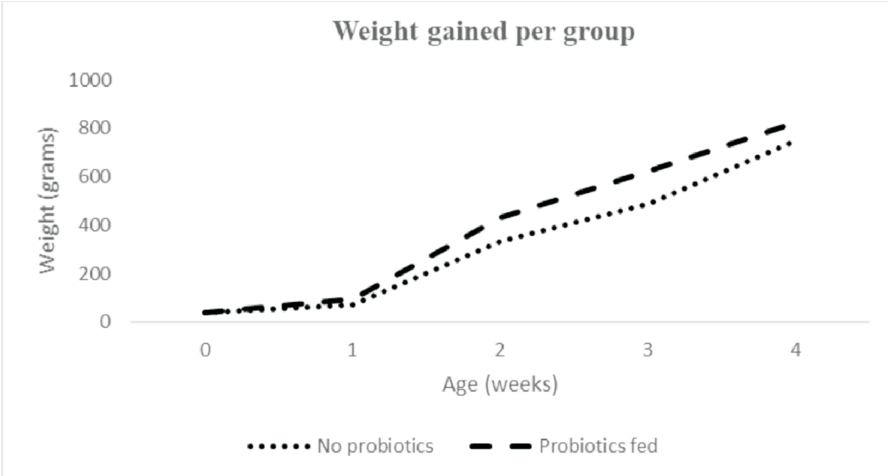

Figure 1: Weight gained by chickens in 4 weeks

Table 1: Feed intake and Feed conversion ratio in chickens fed diets supplemented with probiotics

	No Probiotic (per week)				Probiotic Fed (per week)			
	1	2	3	4	1	2	3	4
Mean feed consumption (g)	54	304	510	924	54	328	540	929
Feed conversion ratio (FCR)	0.73	0.91	1.04	1.23	0.56	0.76	0.87	1.12

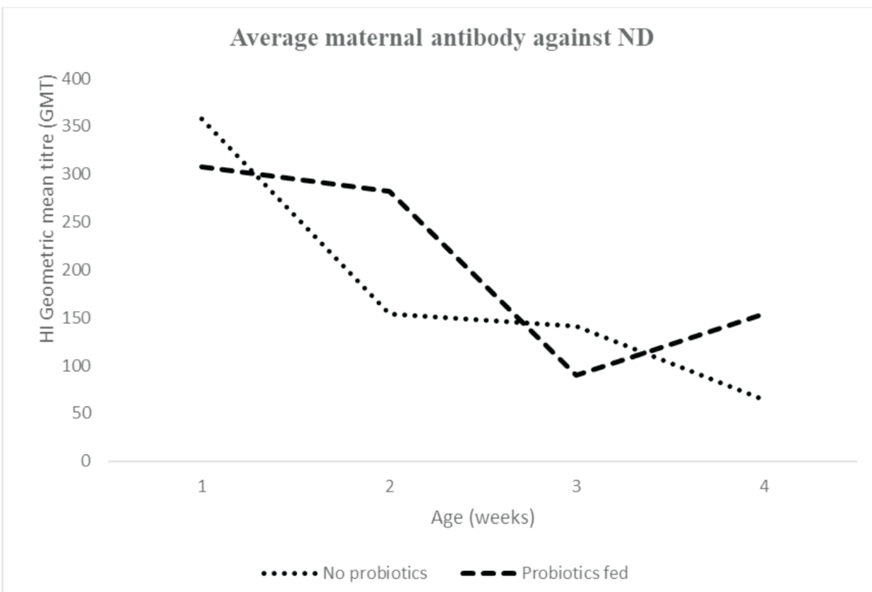

Figure 2: Passive immunity levels against ND in the chicks

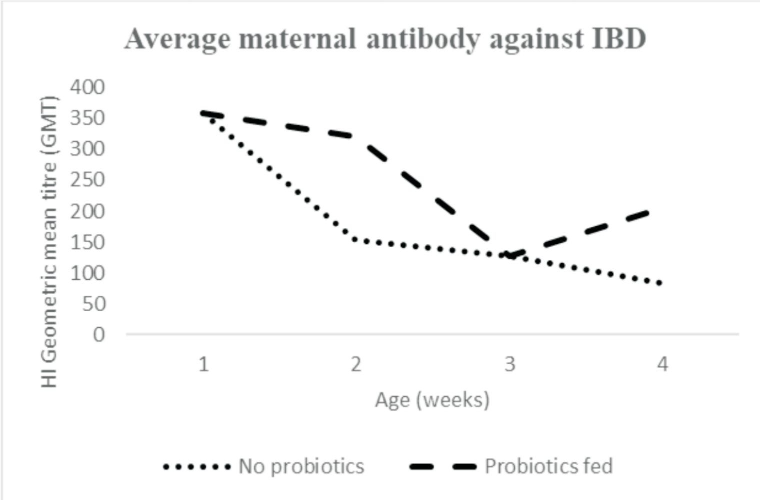

Figure 3: Weaning pattern of MDA against IBD in the chickens

Discussion

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 'good' bacteria of the gastrointestinal tract (GIT) which play important role in nutrient metabolism and absorption throughout the GIT. In this study, the significant improvement in the weight gain obtained in the birds fed probiotics supports the results of Panda *et al.* (2006) and Talebi *et al.* (2008) that probiotics improved broiler performance. Studies by Mokhtari *et al.* (2015); Idoui and Karam (2016); Odefemi (2016); Pourakbari *et al.* (2016) have shown that the administration of probiotic in broiler diets have significant positive effect on body weight gain (BWG) and feed conversion ratio (FCR) which aligned with our observation in this study.

Furthermore, in this study, feeding probiotics had no significant effect on feed intake. This observation supports previous studies that the addition of probiotics did not have any significant effect on feed intake of broiler chickens (Aliakbarpour *et al.,* 2012; Zhang and Kim, 2014). On the other hand, there are reports that supplementation of the diet with probiotic, significantly improved the feed intake in broiler chickens (Odefemi, 2016; Mukthar *et al.,* 2017). The inability of the probiotic to improve feed intake may be due to its taste or flavor. Additives with good tastes and flavor has been reported to help improve feed intake in poultry. Although feed intake was not improved, the body weight gained was significantly improved. The improvement may be due to beneficial effects of probiotics, its mechanism of action, which inhibits the growth and proliferation of pathogenic bacteria that can compromise the integrity of the GIT, thus limiting nutrient intake (Goldin, 1998). High levels of MDA suppresses active immune response to early vaccination in chicks (Kumar *et al.,* 2000), thus different researches have been carried out to determine the titre of MDA that can interfere with active immunity. The MDAs have a characteristic half-life usually between 2 and 3 weeks of age (Hamal *et al.,* 2006) before they naturally degrade in the chick. In this study, the MDA against ND was high at week one which is similar to the findings of Jacobs *et al.* (2004) and Jalil *et* *al.* (2009) but did not decline to zero at day 25 as reported in both groups by Jalil *et al.* (2009). it has been reported that MDA higher than log_2 2.5 HI unit will interfere with active immunity (vaccination) (Underwood *et al.,* 2004). Observation in this study, indicated that the MDA against ND was still higher than log_2 2.5 HI unit at week three in both groups. Likewise, the MDA against IBD in this study was also high at week one similar to the findings of Zaheer and Saheed (2003) but contrary to their findings it did not decline to zero at day 28. Talebi *et al.* (2015), on the other hand, reported that MDA against IBD degraded completely at day 42 which may be the case in this study although this study ended at week 4. There was no difference in the rate of degradation of MDA against ND and IBD between both groups in this study but there was a delay in the rate of degradation in all the probiotic fed groups which was similar to the findings of Talebi *et al.* (2008) who also fed their birds with PrimaLac®. In this study, MDA against both diseases in the probiotic fed group declined to its lowest at week 3 before a surge at week 4 unlike in the groups not fed probiotics where MDA continued to degrade at week 4. The surge in immune response against both ND and IBD in the probiotic fed group may be as a result of quick response to environmental contamination by ND virus and IBD virus, since both diseases are endemic in Nigeria. Probiotics have been reported to enhance systemic immune responses against diseases like ND, IBD and AI among others (Haghighi *et al.,* 2005; Gill and Prasad, 2008; Talebi *et al.,* 2015) and stimulate the production of natural antibodies (antibodies secreted by B-1 cells) in unimmunized chickens (Haghighi *et al.,* 2006).

Conclusion

In conclusion, dietary supplementation of broiler fed with the probiotic improved the chickens' weight gain and feed conversion ratio. Also, the MDA against NDV and IBD did not diminish rapidly in chickens fed with diet supplemented with probiotic.

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