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Effects of a multi-strain probiotic (PrimaLac) on performance and antibody responses to Newcastle disease virus and infectious bursal disease virus vaccination in broiler chickens

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Abstract

The effects of the probiotic PrimaLac (*Lactobacillus acidophilus*, *Lactobacillus casei*, *Enterococcus faecium* and *Bifidobacterium bifidum*) was investigated on performance and immune responses to vaccination against Newcastle disease and infectious bursal disease in broiler chickens. Use of PrimaLac significantly ($P < 0.05$) enhanced broiler performance by improving body weight and decreasing feed conversion ratio. Although administration of the probiotic appeared to improve the antibody responses to Newcastle disease virus and infectious bursal disease vaccination the antibody titres of the probiotic-treated group were not significantly different to those not receiving probiotics.

Introduction

Bacterial resistance to antibiotics and the risk of residues in poultry products for human consumption, have encouraged the use of probiotic microorganisms, prebiotic substrates, or synbiotic combinations of prebiotics and probiotics as alternative approaches to the use of sub-therapeutic antibiotics in poultry (Patterson & Burkholder, 2003). Probiotics have been defined as a live microbial feed supplement that can beneficially affect the intestinal microbial balance resulting in improved body weight gain, feed conversion ratio and reduced mortality in broiler chickens (Haung *et al.*, 2004; Panda *et al.*, 2006). Probiotics may protect chickens against certain avian pathogens by colonization in the gastrointestinal tract (Pascual *et al.*, 1999), by activating immunocytes and stimulating systemic immune responses (Panda *et al.*, 2000), by promoting the endogenous host defence mechanisms (Isolauri *et al.*, 2001) and by modulating the mucosal immune system (Dallout *et al.*, 2003). Furthermore, probiotics have been used for improving immunity to infections with salmonellas (Perdigón *et al.*, 1990), *Candida albicans* (Wagner *et al.*, 2000), coccidia (Dallout *et al.*, 2003) and *Escherichia coli* (Medici *et al.*, 2005). Probiotics have been reported to stimulate production of natural antibodies (Haghighi *et al.*, 2006) and to reduce lung damage (Racedo *et al.*, 2006).

Lactobacillus and *Bifidobacterium* are the genera most frequently used as probiotics (Isolauri *et al.*, 2001) and different strains may affect their efficiency. Oral administration of lactobacilli exerts a strong adjuvant activity, which is responsible for the enhanced host immune responses (Perdigón *et al.*, 1988). Different strains of *Lactobacillus* induce distinct mucosal cytokine profiles showing different intrinsic adjuvant capacity (Maassen *et al.*, 2000; Perdigón *et al.*, 2002). Antibody production against Newcastle disease virus (NDV) in a group of broiler chicks treated with probiotic was significantly higher 10 days post

immunization than an untreated group (Khaksefidi & Ghoorchi, 2006). The lipid profiles of broilers were also affected by *L. sporogenes* supplementation (Panda *et al.*, 2006).

Recently, the beneficial effect of PrimaLac (*Lactobacillus acidophilus*, *Lactobacillus casei*, *Enterococcus faecium* and *Bifidobacterium bifidum*) on growth parameters and egg production of laying hens was reported (Davis & Anderson, 2002) and the present study was designed to evaluate the effects of PrimaLac on performance (feed intake, feed-conversion ratio, mortality rate) of broiler chickens and on their antibody responses to routine vaccination with live NDV and infectious bursal disease virus (IBDV) vaccines.

Materials & Methods

Chickens. Two hundred and 88 one-day-old broiler chicks (Ross-308 strain) were allocated randomly to four groups: vaccination + probiotic (V+P), vaccination (V), probiotic (P) and control (C). Three replicates were used for each group (24 chickens/replicate). The chicks were labeled individually and then housed in separate groups and fed *ad libitum* with a Ross-308 recommended diet. Ambient temperature, lighting, ventilation and other environmental conditions fully met the requirements for management of Ross-308 birds. Great care was taken to prevent any cross-contamination between groups.

Probiotic. Water soluble PrimaLac (Star-Labs, USA) was administered throughout the study as recommended by the manufacturer. The water used to prepare the probiotic treatment each day was non-chlorinated well water.

Vaccination. Vaccination was carried out according to the routine regional vaccination programme and based on optimal timing of maternal antibody level. Groups V+P and V were vaccinated with NDV clone 30 (Nobilis®ND Clone 30, Intervet) on days 9 and 21 of age by eye-drop and drinking water routes respectively and with IBDV D78 (Nobilis®Gumboro D78, Intervet) via the drinking water on days 15 and 25 of age.

Assessment of performance. Performance was assessed by measuring weekly feed consumption and weight gain. Feed conversion ratio (FCR) was also calculated for each replicate and treatment. The European efficiency factor was calculated as:

$$\frac{\text{average weight (kg)} \times \text{livability (\%)} \times 1000}{\text{FCR} \times \text{age (days)}}$$

Sampling. On day 0 blood samples were collected from half the chicks of each replicate as previously described for this age of chick (Alcorn, 2001) and the samples were used to assay the maternal immunity for the best time for vaccination of the remaining chicks. On day 7 and then at weekly intervals until 42 days, blood samples were collected from jugular veins or brachial veins and sera used to assess maternally-transferred antibodies of the chicks and to determine the humoral immune response derived from vaccination against ND and IBD. Haemagglutination inhibition (HI) tests (Allen & Gough, 1974) and ELISAs (IDEXX Laboratories, B.V., The Netherlands) were used to determine antibody titres of the chickens against ND and IBD, respectively.

Statistical analysis. Data were analyzed using SPSS software by completely randomized design employing one-way analysis of variance and the means of different treatments were

compared with Duncan multiple range tests and repeated measure test. Significance differences were taken at $P < 0.05$ level.

Results

Performance and FCR. Average weights of the chickens at the end of the experiment are shown in Table 1. There was no significant difference in feed intake among the groups but FCR and body weight in group P (probiotic) differed significantly ($P < 0.05$) from corresponding birds in vaccinated and control groups (V and C). Although the average weight of the chickens in group V+P (vaccinated + probiotic) was higher than those of groups V and C, the differences were not significant. There was no significant difference among groups in terms of European efficiency factor ($P = 0.095$) and mortality rate ($P = 0.119$) in the starter, grower or whole experimental period.

ND antibody titres. Figure 1 shows that reduction of maternal antibody titres in probiotic-treated groups was slightly lower than the corresponding groups (i.e. group V+P vs V or group P vs C). After day 7 the maternal antibody titre in the control group was below $\log_2 3$, as considered appropriate for first vaccination against NDV.

After vaccination the antibody response to NDV vaccine was higher in the V+P group than the V group, but the difference was not significant ($P = 0.236$).

IBD antibody titres. As shown in Figure 2, maternal antibodies had declined to negligible levels by 21 days but reduction in titre was slower in probiotic-treated groups than the vaccinated or control groups (V+P vs V and P vs C).

After vaccination, antibody titres gradually increased in both vaccinated groups but the level was higher in the group receiving vaccine and probiotic than in the group receiving only vaccine (V+P vs V), although the difference was not significant ($P = 0.236$).

Discussion

Significant ($P < 0.05$) improvement in weight gain and FCR obtained in this study (Table 1) contradicts some previous reports (Ergun *et al.*, 2000; Kumprechtova *et al.*, 2000) that probiotics have no effect on the performance of broilers but support the results of others (Lan *et al.*, 2003; Ahmad, 2004; Panda *et al.*, 2006) that probiotics improve broiler performance. The differences between investigations might be attributed to the strains of microorganisms used. The ideal probiotic (Patterson & Murkholder, 2003; Reid, 2006) changes the bacterial community structure in the avian gastrointestinal tract (Netherwood *et al.*, 1999) and modifies mucin biosynthesis and/or degradation, which in turn influences gut function resulting in improved nutrient uptake (Smirnov *et al.*, 2005). Probiotics can also improve broiler performance by increasing the villous height in the small intestine (Gunal *et al.*, 2006; Panda *et al.*, 2006).

In our study maternal antibody titres of the chicks on day 0 did not differ significantly ($P > 0.05$). The slower decline of maternal antibodies in probiotic-treated chicks may indicate that probiotics can influence the half-life of these antibodies. Comparison of antibody titres of group V+P vs V and group P vs C suggests that the probiotics may have had an immunomodulatory role. The observation, although not statistically significant, is in agreement with that of others (Gill *et al.*, 2000). Oral administration of *Lactobacillus* strains is reported to significantly enhance the IgG response (Maassen *et al.*, 2000) and administration of lactic acid bacteria produced higher levels of anti-pneumococcal serum IgG and bronchoalveolar lavage IgA in mice (Racedo *et al.*, 2006). The antibody isotypes involved were not specifically determined in our study but such a study might shed more light

on the underlying mechanisms involved and whether probiotics have a differential effect on IgM and IgG(Y) antibodies.

The exact mechanisms of immunomodulation by probiotics have not been fully explained but they may stimulate different subsets of immune system cells (Maassen *et al.*, 2000; Christensen *et al.*, 2002; Dalloul *et al.*, 2003; Bal *et al.*, 2004). Oral administration of probiotics can significantly affect both the systemic and mucosa-associated immune responses resulting in disease prevention (Dalloul *et al.*, 2003).

It is now well recognized that bioactive peptides released during fermentation by lactic acid bacteria could contribute to the known immunomodulatory effects of probiotic bacteria (Leblanc *et al.*, 2004) and interactions between host cells and pathogens or their structural components may, either locally or systemically, lead to modulation of T- or B-cell-mediated immune responses (Haghighi *et al.*, 2006). Probiotics also enhance the systemic antibody response to some antigens in chickens (Haghighi *et al.*, 2005) and stimulate production of natural antibodies (antibodies secreted by B-1 cells and may be of isotype IgM, IgG, or IgA) in unimmunized chickens (Haghighi *et al.*, 2006). Functionally, B-1 cells participate in innate immunity by producing the majority of the natural IgM in serum protecting against invading pathogens before the onset of the adaptive immune response (Tung & Herzenberg, 2007).

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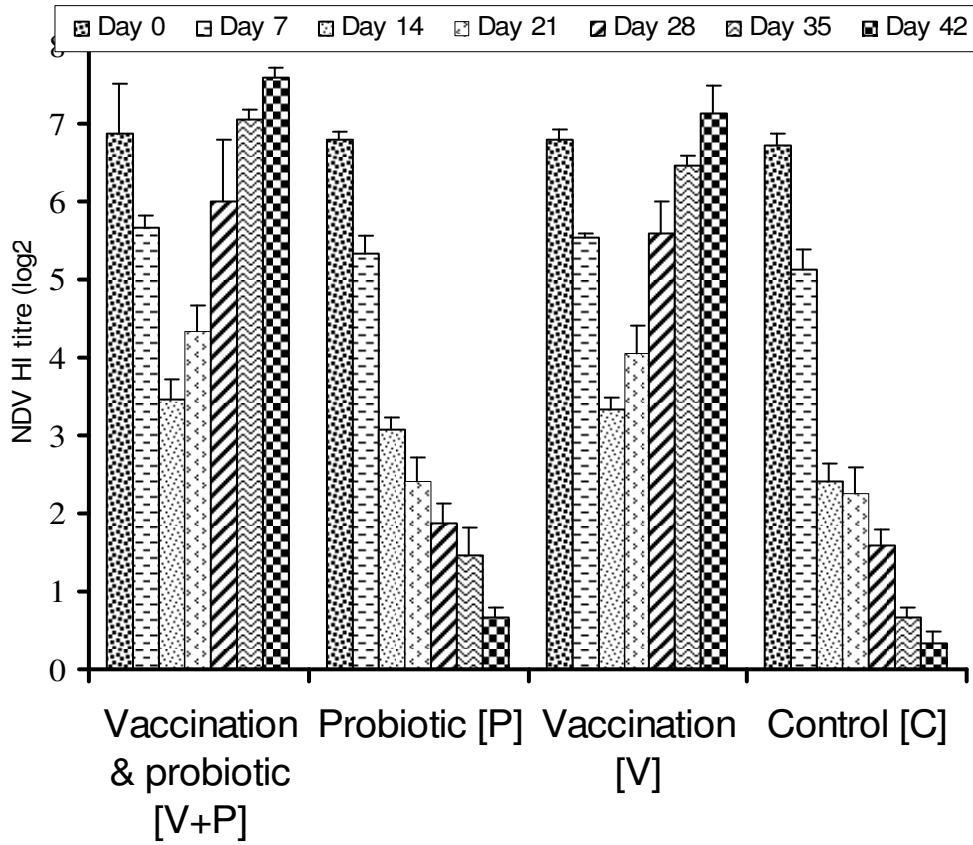


Figure 1. Mean Newcastle disease virus (NDV) HI antibody titres of vaccinated and unvaccinated broiler chickens with or without treatment with probiotics.

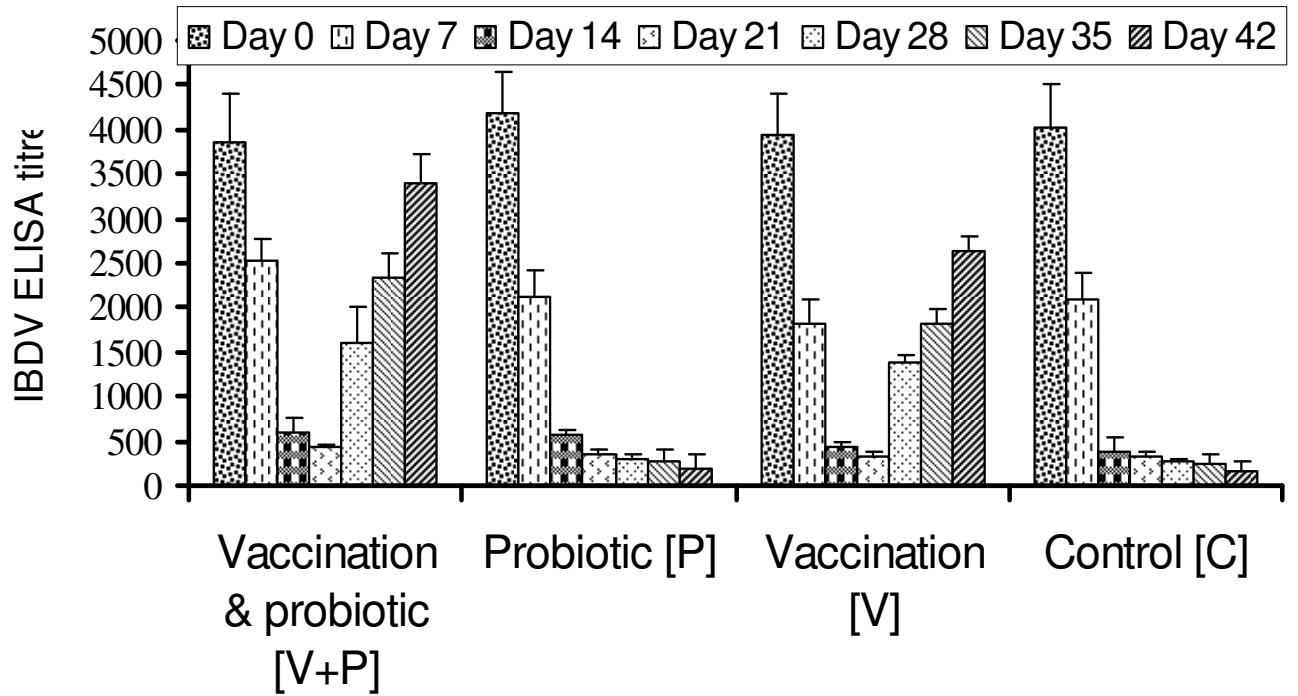


Figure 2. Mean infectious bursal disease virus (IBDV) ELISA antibody titres of vaccinated and unvaccinated broiler chickens with or without treatment with of probiotics.