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The Effects of Protexin Probiotic and Aquablend Avian Antibody on Performance and Immune System of Broiler Chickens

Research Article

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ABSTRACT

This study was conducted to investigate the effects of protexin probiotic and aquablend avian antibody on the performance and immune system of broilers. In this experiment, 320 1-day-old male broiler chicks of the Ross 308, with 5 treatments and 4 replications in a completely randomized design were distributed in experimental units (cages). The treatments include: control (C), protexin (P1), protexin (P2) with double there commended dose, aquablend (A1) and aquablend (A2) aquablend twice there commended amount. The results showed that protexin probiotic and aquablend avian antibody in different doses had no significant effects on average weight gain and feed consumption of broilers at different week (P>0.05). A significant difference between treatments was observed in feed conversion ratio at the second and fifth weeks (P<0.05). Consumption protexin probiotic and aquablend avian antibody with different doses has significant effect on the relative weight of carcasses (P<0.01). Consumption protexin probiotic and aquablend avian antibody with different doses had no significant effect on antibody titers produced in primary and secondary challenge with sheep red blood cells (P>0.05). According to results of this research, it seems that the using of protexin probiotic and aquablend avian antibody has not importance effect on improving the performance of broilers except for carcass weight.

KEY WORDS aquablend avian, broilers, immune system, performance, protexin.

INTRODUCTION

Food safety and feed conversion ratio are very important in the production of foods of animal origin. The increased population sizes of farm animals, clustering of food animal production units and the intensive global transport of live animals and animal products facilitate the spread of zoonotic pathogens. Moreover, farm animal health is severely affected by gastro-intestinal infections that occur frequently under large sized farming conditions. Thus, strategies aimed to improve farm animal health may have impact on both animal and public health. In addition, improvement of intestinal health will lead to lower costs since animals with impaired intestinal health have a reduced appetite and / or diarrhea, resulting in a reduced nutrient uptake and, therefore, negatively affect the feed conversion ratio. Additionally, the following immune response may trigger muscle wasting by increasing catabolism to fulfill the excessive need for amino acids necessary to produce immune response effectors, such as cytokines and antibodies (Thomke and Elwinger, 1998). To promote growth, farm animal feed has been supplemented with sub therapeutical doses of antibiotics, so-called growth-promoting antibiotics (GPAs), since the mid-1940's (Dibner and Richards, 2005). During the first three decades of their use in feed, mean increases of body weight ≥ 8% for Penicillin and Tetracycline were reported (Graham *et al.* 2007). However, nowadays the magnitude of these effects is marginal, due to selective breeding, improved feed formulations and improved hygienic conditions in animal husbandry. Furthermore, the mechanisms involved in GPA-mediated enhanced growth are still under debate.

Foremost, GPAs are thought to inhibit subclinical infections (Gaskins *et al.* 2002), thereby preventing illness and thus maintaining the feed conversion ratio. Other proposed modes of action are suppression of carbohydrates and fat malabsorption, improved nutrient utilization by inhibiting the growth of normal GI tract flora, reduction of growth-depressing microbial metabolites, such as ammonia, aromatic phenols and bile degradation products, and enhanced nutrient uptake through the thinner intestinal wall in GPA-fed animals (Gaskins *et al.* 2002).

A more important point of concern was the relative ease at which microorganisms had demonstrated to be able to transmit antibiotic resistance genes via the exchange of transposons or plasmids and the possible transmission of this resistance to human pathogens. It became such a major concern that it resulted in a total European ban in 2006 on the use of antibiotics as a feed additive to promote growth (Phillips, 2007). A consequence of the ban is that pathogens, suppressed by the use of GPAs, can now reemerge. Because GPAs were almost entirely aimed at gram-positive bacteria (Witte, 2000), it can be expected that gram-positive bacteria, such as *Clostridium perfringens* will increasingly become a problem in the poultry sector. Regardless of a possible global ban on the use of GPAs, the many disadvantages involved with their use make it mandatory to search for alternative strategies to increase food safety and to promote growth in food animals. Not surprisingly, the body's natural defense mechanisms are now one of the focuses, in particular those of the digestive tract. The gastrointestinal (GI) tract comprises the largest mucosal surface in the body and is in direct contact with the external environment. A healthy GI tract harbors a wide variety of residential "non-pathogenic" and potential pathogenic microorganisms displaying complex symbiotic and competitive interactions (Verstegen and Williams, 2002). A disturbance in this balance could facilitate outgrowth of pathogenic microbiota, which will depress animal growth by competing with the host for nutrients and by producing toxic metabolites resulting in increased turnover of gut mucosa (Verstegen and Williams, 2002). Therefore, several strategies aim at shifting this delicate balance in the favor of beneficial microbiota by stimulation and/or activating growth of these subpopulations of intestinal microbiota via prebiotics, probiotics, organic acids, enzymes or herbs (Verstegen and Williams, 2002).

But in many cases due to the high cost of the introduced material or require high expertise for the production and consumption of these materials or absolute uncertainty of the effects of these products and their associated complications, products have not been able found to be commercially available (Rus *et al.* 2005). One of the cases that have been considered is the production of effective materials on the digestive system of animals. Stomach and intestinal mucosal surfaces are the body's major systems and are directly linked to the external environment, which is the balance and performance in natural state (Verstegen and Williams, 2002).

Any change in this area would facilitate and stimulate the growth of pathogens that causes loss and delay of the animal grow due to impaired absorption of nutrients (Verstegen and Williams, 2002). One of the main alternatives for antibiotics in poultry industry is probiotics. One of these probiotics is protexin (Ayasan, 2013). Also, Aquablend Avian® is a blend of beneficial bacteria and antibodies of natural origin extracted from dehydrated egg, both designed to provide the animal with adequate microflora accompanied with the presence of antibodies specific against predominant pathogens in the poultry. Animal needs a minimum amount of beneficial bacteria (microflora) in order to digest a normal digestion and also be protected against pathogens. Normally, when pathogens enter the intestinal tract, they tend to compete with the microflora in occupying inner intestinal surface (competitive exclusion). This creates an imbalance of the intestinal microflora reducing the dominant presence of lactic acid producing bacteria. In this situation, desired microbial in Aquablend Avian® provide the animal with both, beneficial desired microbial to recover the normal balance in the intestinal microflora and natural antibodies, specific against predominant pathogens in the animal. These antibodies attach themselves to their specific receptors in the inner intestinal surface. In this way, the antibodies inhibit the infectious action of the pathogens, which are then excreted, thus preventing animal infection. Because of the importance of poultry as an economic and nutritious form of animal protein and the fast growing characteristics of this animal, research workers have devoted studies to the use of probiotics in poultry. This study was conducted to investigate the effect of Protexin and aquablend avian antibody on the immune system, performance and carcass characteristics of broiler chickens.

MATERIALS AND METHODS

320 Ross broilers (308), 1 day-old male broiler chicks (mean weight 140.36±5.26 g) were equally allocated to five treatments containing 20 pens in each. In each pen were included 16 birds. Diets were formulated to meet the nutrient requirements for poultry (NRC, 1994); Tables 1 and 2. The birds were fed a starter diet from 1 to 10 d, grower diet from 11 to 22 d and finisher diet from 23 to 35 d.

Table 1 Nutrient content of the basal diet over different periods of production

Ingredients (%)	Starter (1-10 d)	Grower (11-22 d)	Finisher (23-35 d)
Corn	55.59	61.27	63.19
Soybean meal (44% CP)	36.74	31.04	28.31
Soybean oil	3.56	4.07	5.05
Common salt	0.30	0.30	0.35
Dicalcium phosphate	1.85	1.79	1.66
Limestone	1.25	1.22	1.15
DL-methionine	0.21	0.22	0.23
L-lysine HCl	0.00	0.09	0.06
Mineral and vitamin premix	0.50	0.50	0.50

Table 2 Nutrient content of the basal diet over different periods of production

Analysis results	Starter (1-10 d)	Grower (11-22 d)	Finisher (23-35 d)
Dry matter (%)	89.1	89.1	89.2
Crude protein (%)	21.4	19.3	17.4
Metabolizable energy (kcal/kg)	2915	2955	3009
Lysine (%)	1.16	1.03	0.91
L-methionine (%)	0.56	0.51	0.45
L-methionine + cystine (%)	0.85	0.77	0.70
Calcium (%)	0.9	0.88	0.89
Available phosphorus (%)	0.44	0.4	0.35

Birds received diets which were supplemented with 1 gram per liter (P1) and 2 gram per liter (P2) protexin in water, and supplemented with 5 gram per liter (A1) and 10 gram per liter (A2) Aquablend Avian® in water. The experiment lasted for 6 weeks. Feed intake was recorded by replicate every week. Feed efficiency was calculated as: feed consumption weight/body weight. During 42 days of experimental period, mean weight, feed consumption, feed conversion and carcass characteristics was measured as below (mortality was recorded as it occurred):

Feed consumption= (feed weight at beginning period-feed weight at the end of period) / age
Feed conversion= (feed consumption during period/total weight increases during period)

For preparation injectable suspension SRBC, blood from sheep jugular vein using a syringe containing EDTA anticoagulant was used. Sheep red blood cells were washed three times by phosphate buffered saline (Munns and Lamont, 1991).

Then 1% SRBC suspension at a rate of 2.0 mL at the age of 21 and 35 days to two chicks from each replicate through breast muscle injection, and seven days after the chicks via wing vein blood samples were taken. 16 hours after blood coagulation, serum samples were isolated at a temperature of 37 ˚C. Then total SRBC antibody titers were measured (Vander, 1980). All analyses were carried out with one-way analysis of variance of SAS (SAS, 1996). Means were compared by using Duncan’s test.

RESULTS AND DISCUSSION

Result showed that protexin and aquablend avian antibody has not significant effects on mean weight of broilers in different ages (P>0.05); (Table 3). Result showed that protexin and aquablend avian antibody has not significant effects on feed consumption of broilers at different week (P>0.05); (Table 4). Result showed that protexin and aquablend avian antibody has significant effects on feed conversion ratio of broilers in 2 and 5 week ages at 95% confident level (Table 5), but in total has not significant effect. Result showed that protexin and aquablend avian antibody has significant effects on carcass weight of broilers at 95% confident level (Table 6), but in other factors not showed significant effect. Result showed that protexin probiotic and aquablend avian antibody with different doses had no significant effect on antibody titers produced in primary and secondary challenge with sheep red blood cells (P>0.05); (Table 7).

The results showed that the antibody and protexin had no significant effect on average weight and feed consumption of broilers at different week (P>0.05). Significant difference between treatments was observed in feed conversion ratioat the second and fifth weeks (P<0.05). Consumption of protexin probiotic and aquablend avian antibody with different doses has significant effect on relative weight of carcasses (P<0.01). Consumption protexin probiotic and aquablend avian antibody with different doses had no significant effect on antibody titers produced in primary and secondary challenge with sheep red blood cells (P>0.05).

Table 3 Protexin and aquablend avian antibody effects on mean weight of broilers (g)

Items	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Total
Control	148.8	297.8	465.7	542.7	528	753.5	2736.7
P1	136.6	301.8	466.5	526	550	733.2	2714.2
P2	140.9	309.4	443.2	547.2	534.2	701.7	2676.9
A1	140.7	312.4	471.2	542.2	615.2	754.5	2836.4
A2	134.8	301.9	456.5	535.2	554.2	732.7	2715.4
Significant	0.18	0.32	0.71	0.91	0.20	0.94	0.36
SEM	5.26	2.43	6.49	7.05	12.78	17.59	25.84

P1: protexin; P2: protexin with double there commended; A1: aquablend and A2: aquablend twice there commended.
SEM: standard error of the means.

Table 4 Protexin and aquablend avian antibody effects on feed consumption of broilers (g)

Items	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Total
Control	188.1	447.2	935	968.5	1047.7	1217.8	4804
P1	174.7	448.7	928.8	970	1099.1	1344.8	4966.7
P2	175.7	444	929.1	966	1091	1144.2	4750.5
A1	173.5	443.5	890.5	1005.4	1053.2	1301.4	4858
A2	170.0	441.0	907.5	965.3	1041.5	1366.6	4892.2
Significant	0.06	0.05	0.37	0.76	0.68	0.26	0.82
SEM	2.14	4.49	6.86	10.62	14.88	35.91	110.1

P1: protexin; P2: protexin with double there commended; A1: aquablend and A2: aquablend twice there commended.
SEM: standard error of the means.

Table 5 Protexin and aquablend avian antibody effects on feed conversion ratio of broilers

Items	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Total
Control	1.26	1.5	2.0	1.78	1.98	1.62	1.76
P1	1.28	1.49	2.0	1.84	2.01	1.85	1.83
P2	1.25	1.44	2.11	1.76	2.06	1.63	1.77
A1	1.23	1.38	1.89	1.88	1.71	1.72	1.71
A2	1.26	1.46	1.98	1.8	1.87	1.89	1.80
Significant	0.43	0.05	0.33	0.77	0.04	0.42	0.25
SEM	0.17	0.03	0.03	0.02	0.04	0.05	0.03

P1: protexin; P2: protexin with double there commended; A1: aquablend and A2: aquablend twice there commended.
SEM: standard error of the means.

Table 6 Protexin and aquablend avian antibody effects on carcass characteristics of broilers

The main effects	The relative weight of the body organs								
	Live weight (g)	Carcass weight %	Digestive system %	Liver %	Bursa %	Thymus %	Spleen %	Heart %	Gut (m)
Treatment									
Control	2710	82.37[a]	3.85	1.85	0.04	0.17	0.05	0.33	1.84
P1	2980	79.08[b]	4.17	1.99	0.04	0.17	0.05	0.36	1.93
P2	2720	84.36[a]	3.12	1.73	0.04	0.16	0.06	0.32	1.67
A1	2820	83.79[a]	3.39	1.97	0.03	0.16	0.06	0.36	1.76
A2	2830	82.03[ab]	3.9	1.78	0.05	0.22	0.07	0.39	1.86
P-value	0.29	0.01	0.21	0.59	0.73	0.38	0.63	0.07	0.21
SEM	0.04	0.58	0.25	0.05	0.002	0.009	0.003	0.009	0.03

P1: protexin; P2: protexin with double there commended; A1: aquablend and A2: aquablend twice there commended.
SEM: standard error of the means.

Table 7 Protexin and aquablend avian antibody effects on immune system characteristics of broilers

Treatment	Immune system						
	Control	P1	P2	A1	A2	P-value	SEM
Main effects							
IgM 21 days	1.75	1.12	1.5	1.25	1.75	0.44	0.14
IgG 21 days	2.37	2.12	1.75	2	1.37	0.33	0.22
SRBC 21 days	4.12	4.25	3.25	3.25	2.87	0.39	0.26
IgM 35 days	2.25	2.37	2.33	2.37	2.62	0.22	0.36
IgG 35 days	2.25	2.87	3.5	2.75	3.62	0.31	0.26
Sheep red blood cells (SRBC) 35 days	4.5	5.25	6.16	5.12	6.25	0.38	0.32

P1: protexin; P2: protexin with double there commended; A1: aquablend and A2: aquablend twice there commended.
SEM: standard error of the means.

Kabir *et al.* (2003) showed that with antibiotic consumption, live weight gains obtained were significantly higher in experimental birds as compared to control ones at all levels during the period of 2nd, 4th, 5th and 6th weeks of age, both in vaccinated and non-vaccinated birds. A significantly (P<0.01) higher carcass yield occurred in broiler chicks fed with the probiotics on the 2nd, 4th and 6th week of age both in vaccinated and non-vaccinated birds. The weight of leg was found significantly (P<0.01) greater for experimental birds as compared to control ones on the 2nd, 4th and 6th week of age. A significantly (P<0.01) higher breast weight in broiler chicks fed with the probiotics was observed on the 4th and 6th week of age. Analogously a significantly (P<0.05) higher breast portion weight was found in experimental birds as compared to control ones during the 2nd week of age. The antibody production was found significantly (P<0.01) higher in experimental birds as compared to control ones. Significant differences were also observed in the weight of spleen and bursa due to probiotics supplementation. The results of the study thus revealed that probiotics supplementation promoted significant influence on live weight gain, high carcass yield, prominent cut up meat parts and immune response. Shahsavari (2006) reported that the probiotic (protexin) on the function (egg production, feed conversion and weight, egg mass) and quality characteristics of broiler breeder eggs were not affected. Balevi *et al.* (2000) investigated the effects of dietary supplementation of a commercial probiotic (protexin feed consumption, egg yield, egg weight, food conversion ratio and humoral immune response in layer hens. In 7 replicates, a total of 280 40-week-old layers were given diets containing either 0, 250, 500 or 750 ppm for 90 d 2. When compared with the controls, the food consumption, food conversion ratio and the proportions of damaged eggs were lower in the group consuming 500 ppm probiotic (P<0.05). There was no significant difference between the controls and the groups receiving 250 and 750 ppm probiotic in food consumption, food conversion ratio and proportion of damaged eggs. Similarly, the egg yield, egg weight, specific gravity, and peripheral immune response showed no statistically significant differences between the groups) on daily.

Ayasan *et al.* (2006) investigate the effects of grower diets, dietary three different levels of probiotic (protexin) in grower diet on Japanese quail (*Coturnix coturnix Japonica*). Results showed that age and body live weight of quails at the first laying was found significant different between groups. During the egg production period, probiotic supplementation to the diet did not affect feed intake and feed conversion. Probiotics and prebiotics alter the intestinal microbiota and immune system to reduce colonization by pathogens in certain conditions. Feeding birds in a report Protexin increased antibody titers against the Newcastle disease vaccine (Zakeri and Kashefi, 2011). Also, chickens treated with protexin higher antibody titers against avian influenza viruses showed (Ghafoor *et al.* 2005). Some studies showed that parameters related to immune (antibody titer against SRBC, antibody titer against Newcastle disease and immunoglobulins IgG, IgM) were not affected by different levels of probiotics. However most SRBC and IgG antibody titers were obtained by applying the most probiotics, more immunoglobulin IgG were related to probiotic treatments were statistically significant difference between them and the other groups.

As with increase the use of antibiotics, environmental and stress status influence efficacy of prebiotics and probiotics, these products show promise as alternatives for antibiotics as pressure to eliminate growth promotant antibiotic use increases by improving the microbial balance of the intestine bird probiotics and digestive enzymes increase the activity of digestive enzymes and enabling increased nutrient availability indigestible and beneficial changes in the metabolism of food consumed, thus improving feed efficiency (Chen and Nakthong, 2005). Due to the fact that each of these additives and active ingredient are different compounds, dose and components used in the experiment can be obtained different results in the use of these substances are effective growth promoters (Lee *et al.* 2004). Defining conditions under which they show efficacy and determining mechanisms of action under these conditions is important for the effective use prebiotics and probiotics in the future.

CONCLUSION

The purpose of using protexin and antibodies at the same time is increasing the quality and quantity, while protexin and aquablend has not importance effect in improving the performance of broilers. Probiotics with increased feed intake and the efficiency of feed intake increase the weight. Overall increasing performance due to use of antibodies and probiotics may be due to many reasons including existence various chemical compounds and improve the efficiency of food consumption and eliminate the annoying factors including harmful microorganisms in the digestive tract and food. However, according to the survey results, it is recommended that more studies and promising for the study of the materials used in poultry diets.

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