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Prebiotic, Probiotic and Symbiotic as Alternative to Antibiotics on the Performance and Immune Response of Broiler Chickens

Author(s)

Murarolli VDA[I]
Burbarelli MFC[II]
Polycarpo GV[II]
Ribeiro PAP[II]
Moro MEG[III]
Albuquerque R[IV]

[I] Master in Animal Nutrition and Production – School of Veterinary and Animal Sciences, FMVZ – USP
[II] Graduate studentin Animal Nutrition and Production – School of Veterinary and Animal Sciences, FMVZ – USP
[III] Professor–School of Animal Sciences and Food Engineering, FZEA– USP
[IV] Professor, Department of Animal Nutrition and Production – School of Veterinary and Animal Sciences, FMVZ – USP

Mail Address

Corresponding author e-mail address:
Ricardo de Albuquerque
Faculdade de Medicina Veterinária e Zootecnia da USP - FMVZ VNP
Rua Duque de Caxias Norte, 225
13635-900 - Pirassununga – SP
E-mail: ricalbuq@usp.br

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ABSTRACT

This study aimed to evaluate diets supplemented with prebiotic, probiotic and symbiotic as an alternative to antibiotics on the performance and immune response against the virus of Newcastle disease in broiler chickens. 1,400 one-day old male Cobb 500 chicks were raised until 42 days old in a completely randomized design with 2x2+1 factorial scheme with seven replications. The treatments were: diet without supplementation (base diet - BD), BD + prebiotic, BD + probiotic, BD + symbiotic (prebiotic + probiotic), and BD + antibiotic. The parameters evaluated were performance and antibody serum titers against Newcastle disease. No antibiotic effect was observed on performance. The symbiotic provided better results for weight gain and feed:gain ratio until 21 days old than isolated additives. At 28 days old, the broilers fed diets with prebiotic presented better feed: gain ratio. In the same period (28 d-old), there was an antibody production increase against the Newcastle disease virus in the group supplemented with prebiotic. It can be concluded that the utilization of symbiotic in broiler chickens' diets can substitute performance enhancing antibiotics. The inclusion of prebiotic in the diet improves feed: gain ratio at 1-28 days old. The chickens' immune response increases at 28 days against the Newcastle disease virus in the group supplemented with prebiotic.

INTRODUCTION

In poultry production, the main objective is to obtain high yield and quality of the final products. Therefore, antimicrobial additives, including antibiotics, have been used in poultry diet (Loddi *et al.*, 2000). The main concern is that the continuous use of antibiotics may develop and disseminate resistant bacterial populations and that this resistance may be transferred to pathogenic microorganism, becoming a risk to human and animal health (Dawson & Pirvulescu, 1999; Menten & Loddi, 2003).

This has led researchers to develop alternatives that keep high yield without harming human and animal health. The utilization of prebiotics, probiotics and symbiotics (Furlan *et al.*, 2004) are among these alternatives. Probiotics, live culture of beneficial microorganisms, have beneficial actions on the host through the competition for linking sites and nutrients, production of antibacterial substances, suppression of ammonia production, neutralization of enterotoxins, in addition tostimulating the immune system (Fuller, 1989; Jin *et al.*, 1997; Andreatti Filho & Sampaio, 2000; Silva, 2000; Andreatti Filho & Silva, 2005).

Gibson & Roberfroid (1995) defined prebiotics as the food ingredients that provide beneficial effect to the host by selectively stimulating the growth and/or metabolism of a limited group of bacteria in the intestinal tract, acting closely to probiotics because it would constitute the "food" of probiotic bacteria and also blocking adherence sites, immobilizing and reducing the fixation capacity of pathogenic bacteria in the intestinal mucous (Silva, 2000; Andreatti Filho & Silva, 2005). This association favors the intestinal microbiota by the action of prebiotics that are able to link themselves to the fimbriae of pathogenic bacteria, conducting them along the fecal bolus, stimulating the growth and accelerating the metabolism of a limited number of non-pathogenic microorganisms. The action of probiotics is added to this mechanism, making easy the nutrition of cells (enterocytes) that recover the digestive tract and provide balance and intestinal health to birds (Gibson & Roberfroid, 1995).

The objective of this study was to evaluate the influence of supplementation with prebiotic, probiotic and symbiotic as an alternative to antibiotics on the performance and immune response against the Newcastle disease virus in broiler chickens.

MATERIAL AND METHODS

In this study, 1,400 one-day-old, male *Cobb 500* chicks were distributed in a completely randomized design in a 2x2+1 factorial arrangement with five treatments and seven replications with 40 birds per experimental unit.

The diets were provided *ad libitum* during all experiment period and formulated according to the recommendations of Rostagno *et al.* (2005) (Table 1). The treatments were: diet without supplementation (base diet - BD); BD + prebiotic, mannanoligosaccharide derived from the cell wall of *Saccharomyces cerevisae*eyeast at a dose of 2.000 ppm; BD + probiotic, *Enterococcus ssp.* (106 UFC/g) and *Lactobacillus acidophilus* (107 UFC/g) added to the diet at a dose of 400 ppm; BD + symbiotic,added to the diet at a dose of 2.000 ppm; and BD + antibiotic, 10 ppm avilamycin at a dose of 100 ppm of the product. All additives were utilized substituting the inert material (kaolin), adjusting the percentage composition of different experimental diets. The performance variables were qualified at 7,14, 21, 28, 35 and 42 days.

The chicks were vaccinated against Marek's disease in the hatchery and against coccidiosis through drinking water in the first week of life. At 14 days old, they were vaccinated against Newcastle's disease (LaSota strain) via conjunctiva using lyophilized vaccine with live and attenuated VG/GA strain. The utilized vaccine dose was recommended by the manufacturer (0.03 ml) and their diluent was utilized at the proportion of 30 ml/1000 vaccine doses. To evaluate the serum titers of antibodies against Newcastle, samples were collected at 14 days (before the vaccination) of two chicks per experimental unit, totalizing 14 birds per treatment that were individually identified, allowing the subsequent collection at 28 and 35 days by puncturing the ulnar vein. The samples were placed in *eppendorf* tubes without anticoagulant and centrifuged to obtain serum that was later submitted to immune enzymatic assay with ELISA (Enzyme-Linked Immune sorbent Assay) kit (Purchase *et al.*, 1989).

The results were analyzed through the Statistical Analysis System (SAS, 2001).The data were submitted to normality test by Shapiro-Wilk's testand to homogeneity of variances test by Hartley's test (Ott,

Table 1 – Composition and Calculated Values of Experimental Diets.

Ingredients,%	Phases		
	1 – 21 d	22 – 35 d	36 – 42 d
Corn	52.26	57.11	63.70
Soybean meal	40.13	34.00	28.00
Soybean oil	3.52	4.90	4.50
Salt	0.35	0.35	0.35
Kaolin	1.24	1.60	1.60
Dicalcium Phosphate	1.60	1.14	0.95
Methionine	0.24	0.21	0.18
Vit.-Min Supplement [1]	0.30	0.30	0.30
Additives			
Prebiotic	0.20	0.20	0.20
Probiotic	0.04	0.04	0.04
Symbiotic	0.20	0.20	0.20
Antibiotic	0.01	0.01	0.01
Inert	0.16-0.36	0.19-0.39	0.22-0.42
Total	100	100	100
Calculated Analysis			
Metabolizable energy (kcal/kg)	2950	3100	3150
Protein (%)	22.5	20.0	18.0
Methionine (%)	0.35	0.32	0.30
Methionine + cistine (%)	0.71	0.65	0.60
Calcium (%)	0.95	0.95	0.90
Available Phosporus (%)	0.45	0.35	0.30

[1] Vitamin and mineral supplement in the initial phase (kg by product): 35250 UI vit. A, 8513 UI vit. D3, 6.0 mg vit. K, 49.57 mg vit. E, 6.67 mg vit. B1, 15 mg vit. B2, 8.33 mg vit. B6, 40 mcg vit. B12, 100 mg niacin, 2.5 mg folic acid, 39.13 mg calcium panthotenate, 0.033 mg biotin, 144.80 mg iron, 144.50 mg zinc, 28.53mg copper, 186.67 mg manganese, 1.87 mg iodine, 1.13 mg selenium, 14 mg antioxidant.

Vitamin and mineral supplement in the growth phase (kg by product): 29351 UI vit A, 7088 UI vit D3, 5.0 mg vit K, 41.33 mg vit. E, 5.77 mg vit. B1, 12.50 mg vit. B2, 6.90 mg vit. B6, 33.33 mcg vit. B12, 83.33 mg niacin, 2.07 mg folic acid, 33.33 mg calcium panthotenate, 0.27 mg biotin, 144.80 mg iron, 144.50 mg zinc; 28.80mg copper, 186.50 mg manganese, 1.87 mg iodine, 1.13 mg selenium, 14 mg antioxidant.

Vitamin and mineral supplement in the finishing phase (kg by product): 23500 UI vit A, 5675 UI vit D3, 4.0 mg vit K, 28.00 mg vit. E, 4.47 mg vit. B1, 10.00 mg vit. B2, 5.53 mg vit. B6, 26.67 mcg vit. B12, 66.67 mg niacin, 1.13 mg folic acid, 26.67 mg calcium panthotenate, 0.15 mg biotin, 144.80 mg iron, 144.50 mg zinc; 28.80mg copper, 186.50 mg manganese, 1.87 mg iodine, 1.13 mg selenium, 14 mg antioxidant.

1983). The data that did not present normality distribution and/or homogeneity of variances were submitted to the logarithmic transformation to meet the statistical presuppositions. After the tests cited, the data were submitted to analysis of variance at 5% significance. The methodology of orthogonal contrasts was utilized to separate the effects of the treatments. The utilized contrasts were separated into effect of prebiotic, effect of probiotic, interaction (symbiotic effect), and antibiotic effect against the effect of treatments with alternative additives (prebiotic and/or probiotic).

RESULTS AND DISCUSSION

The averages of weight gain (WG), feed intake (FI) and feed:gain ratio (F:G) of broilers at 01-07, 01-14, 01-21, 01-28, 01-35 and 01-42 days old are shown in Table 2. For the intervals of 01-07, 01-14 and 01-21 days old, the significant effect of the additive interaction on WG and F:G was observed. The symbiotic treatment presented better results when compared to separately tested additives. In the interval of 1-28 days old, there was improvement only in F:G, and an isolated effect of the prebiotic was observed.

Under the conditions of this experiment, it was not possible to verify significant differences on FI of chickens or the effect of treatments for WG after 28 days of breeding. Chickens fed diets containing antibiotic presented similar performance to those that were fed diets containing alternative additives, showing that the utilization of symbiotic may represent a viable option to substitute antibiotics as performance enhancers.

On the other hand, Vargas Jr. *et al.* (2000), Maiorka *et al.* (2001) and Loddi (2003) did not observe a significant difference in the utilization of symbiotic in the diets when compared to other treatments in the 21-day-old period. Vargas Jr. attributes this result to the low sanitary conditions in which the experiment was carried out and that could be really crucial in such evaluations. However, Santin *et al.* (2001) observed that supplementation of 0.01% and 0.02% of the cell wall of *S. cerevisiae* as prebiotic provided higher values of F:G when compared to the non-supplemented diet, in the periods of 21 and 42 days old.

Table 2 – Performance of broiler chickens in the intervals: 01-07; 01-14; 01-21; 01-28; 01-35 and 01-42 days old, under different treatments.

Variables[1]	Treatments[2]						Probability			
	Without Probiotics		With Probiotics							
	Without Preb	With Preb	Without Preb	With Preb	Atb	CV (%)	Preb	Prob	Inter	Atb
01-07 days										
WG	120.14	118.43	108.14	120.28	118.57	5.17	0.0025	0.0031	0.0001	0.1155
FI	132.32	135.59	133.90	133.67	136.69	2.66	0.2497	0.8990	0.1889	0.1341
F:G	1.10	1.14	1.23	1.11	1.15	4.84	0.0007	0.0001	0.0001	0.3472
01-14 days										
WG	421.71	413.28	394.00	422.10	417.14	3.61	0.0282	0.0352	0.0002	0.1478
FI	525.07	530.72	540.15	526.18	537.01	2.83	0.4598	0.3505	0.0875	0.4727
F:G	1.24	1.28	1.37	1.24	1.28	4.03	0.0001	0.0001	0.0001	0.2363
01-21 days										
WG	885.85	876.28	850.71	888.71	875.85	2.21	0.0162	0.0508	0.0002	0.5443
FI	1193.97	1211.24	1211.13	1202.13	1204.77	2.55	0.7355	0.7420	0.2873	0.8101
F:G	1.35	1.38	1.42	1.35	1.37	2.81	0.1016	0.0424	0.0001	0.4078
01-28 days										
WG	1504.00	1510.86	1479.57	1494.71	1507.29	2.15	0.3726	0.1054	0.7355	0.3900
FI	2169.55	2162.33	2145.85	2149.40	2168.84	2.17	0.9214	0.3282	0.7719	0.4492
F:G	1.44	1.43	1.46	1.44	1.44	1.26	0.0158	0.0592	0.4022	0.5435
01-35 days										
WG	2198.29	2184.43	2151.43	2180.86	2199.29	2.32	0.6870	0.1975	0.2665	0.2304
FI	3365.28	3352.54	3314.48	3333.00	3357.71	2.28	0.9236	0.2482	0.6046	0.4852
F:G	1.53	1.53	1.54	1.53	1.53	1.25	0.6107	0.8502	0.2860	0.3742
01-42 days										
WG	2829.14	2817.86	2794.71	2801.57	2842.00	2.16	0.9254	0.2880	0.7015	0.1785
FI	4676.96	4687.67	4588.02	4646.41	4674.91	2.4	0.4233	0.1367	0.5796	0.4918
F:G	1.65	1.66	1.64	1.66	1.64	1.83	0.2618	0.5085	0.7908	0.4644

[1]WG, weight gain (g); FI, feed intake (g); F:G, feed:gain ratio (g/g). [2]Preb, prebiotic; Prob, probiotic; Inter, interaction; Atb, antibiotic.

The effects of additives on the average titers of vaccination antibodies against Newcastle disease, obtained by ELISA test, are presented in Table 3. At 14 days old, before the chickens' vaccination, the averages of analyzed antibody titers are considered passive antibodies (maternal origin). In this period, there was an effect of diets with antibiotic.

At 28 days, the chickens that were fed prebiotic-supplemented diets presented better vaccine response. After the hatching, there is a gradual decline of the maternal antibody levels, and after 28 days old they are not observed anymore. Thus, the antibodies detected are originated from the acquired immune response. At 35 days old, no significant difference was observed in any of the tested contrasts.

Zulkifli *et al.* (2000), when evaluating antibody titers against Newcastle disease, observed the highest titers values in the treatment with probiotic compared to antibiotics; however, these results were only seen after a period of high temperature exposure, reinforcing the argument that the benefits of this additive are intensified in situations where there is some kind of challenge. Vesna *et al.* (2007) observed higher antibody titers against Newcastle disease in the treatment supplemented with MOS when compared to the control treatment.

Darpossolo *et al.* (2010) observed no differences in antibody titers against Newcastle disease in the treatment supplemented with β-Glucans, one of the compounds of *Saccharomyces cerevisae* yest wall, disagreeing with the present study. In addition, Nikpiran *et al.* (2013) studying effects of prebiotic and probiotic in immune response to the Newcastle disease observed that probiotic improved immune response to the vaccine in comparison to the prebiotic and control groups.

CONCLUSION

Under the experimental conditions, it was inferred that the use of symbiotic in the diets of broiler chickens can substitute performance enhancing antibiotics. The inclusion of prebiotic improves F:G until 28 days. There is an increase of the birds' immune response against Newcastle disease in the group supplemented with prebiotic at 28 days.

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Table 3 – Averages of antibody titers against the virus of Newcastle Disease in broiler chickens at 14, 28 and 35 days old (d).

Periods	Treatments						Probability			
	Without Probiotics		With Probiotics							
	Without Preb	With Preb	WithoutPreb	WithPreb	Atb	CV (%)	Preb[1]	Prob	Inter	Atb
14 d	2.17	2.36	2.48	2.30	1.95	18.79	0.9420	0.2377	0.0851	0.0008
28 d	2.44	2.69	2.37	2.76	2.69	18.78	0.0149	0.9854	0.6013	0.5691
35 d	2.62	2.75	2.99	2.75	2.80	15.78	0.6316	0.1246	0.1206	0.8233

[1]Preb, prebiotic; Prob, probiotic; Inter, interaction; Atb, antibiotic.

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