Revista Brasileira de Ciência Avícola
ISSN: 1516-635X
revista@facta.org.br
Fundação APINCO de Ciência e Tecnologia Avícolas
Brasil

Boostani, A; Mahmoodian Fard, H R; Ashayerizadeh, A; Aminafshar, M

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Revista Brasileira de Ciência Avícola, vol. 15, núm. 1, enero-marzo, 2013, pp. 1-6
Fundação APINCO de Ciência e Tecnologia Avícolas
Campinas, SP, Brasil

Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

ISSN 1516-635X Jan - Mar 2013 / v.15 / n.1 / 1-6

Growth Performance, Carcass yield and Intestinal Microflora Populations of Broilers Fed Diets Containing Thepax and Yogurt

Author(s)

Boostani A[1]
Mahmoodian Fard H R[1]
Ashayerizadeh A[2]
Aminafshar M[3]

[1] Fars Education Center of Jahad-e-Agriculture, Shiraz, Iran

[2] Faculty of Animal Science, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran

[3] Department of Animal Science, Faculty of Agriculture and Natural Resource, Science and Research Branch, Islamic Azad University, Tehran, Iran

Mail Adress

Corresponding author e-mail address
Amin Ashayerizadeh
Postal Address: Faculty of Animal Science, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran. PC: 4918943464
Tel: +98-9173099064
Fax: +98-7297262150
E-mail: amin.ashayerizadeh@yahoo.com

Keywords

Ross 308, Probiotic, Carcass yield, E. coli.

Submitted: October/2011
Approved: September/2012

ABSTRACT

The present study aimed at evaluating the effect of the probiotic thepax and yogurt (as probiotic) on the growth response and intestinal microflora results of broiler chickens. Two hundred forty day-old Ross 308 broilers were equally distributed into 12 floor pens and reared for 42 day. The treatments consisted of yogurt (10, 5 and 2.5% during starter, grower and finisher periods in the drinking water, respectively) and thepax (1000, 500, 250 g/ton^{-1} in the starter, grower and finisher diets, respectively), resulting three experimental diets and a control group. Each dietary treatment was fed *ad-libitum* to four replicate group of 20 birds at the beginning of rearing period. Birds and feed were weighed on days 21 and 42. The results of experiment indicate that diets containing feed additives improved broiler performance. The body weight gain and feed conversion ratio improved significantly more ($p \leq 0.05$) with the thepax treatment compared with the control broilers during the total rearing period. The highest ($p \leq 0.05$) carcass and thigh values were recorded for broilers fed the diet supplemented with thepax and yogurt, respectively. The lowest abdominal fat pad value was obtained in broilers fed the diet supplemented with thepax. On d 21, thepax and yogurt significantly reduced ($p \leq 0.05$) cecal *Escherichia coli* and *Clostridium perfringens* populations compared with the control group. In conclusion, thepax and yogurt improved broilers growth response and conferred intestinal health benefits to chickens by improving their microbial ecology.

INTRODUCTION

Probiotics are defined as a live microbial feed supplement that beneficially affects the host animal by improving its microbial intestinal balance (Fuller, 1989). The efficacy of probiotics may be increased by several methods: selection of more efficient strains, gene manipulation, combination of several strains, and combination of probiotics and synergistically-acting components. Lactobacilli and enterococci are among the wide variety of microbial species that have been extensively used as probiotics (Patterson & Burkholder, 2003). Improvements in growth performance and feed efficiency have been reported after feeding probiotics to broiler chickens (Cavazzoni *et al.*, 1998; Jin *et al.*, 1998; Zulkifli *et al.*, 2000; Kabir *et al.*, 2004; Mountzouris *et al.*, 2007; Samli *et al.*, 2007).

The proposed modes of action of probiotics in poultry are: 1) maintenance of a beneficial microbial population by competitive exclusion and antagonism (Fuller, 1989), 2) improvement of feed intake and digestion (Nahanshon *et al.*, 1992, 1993), and 3) changing bacterial metabolism (Cole *et al.*, 1987; Jin *et al.*, 1997). Thepax is a manipulated product of *S. cerevisiae* yeast cells with controlled reproduction and supplemented with amino acids, minerals and vitamins. These supplementary ingredients have positive effects on growth and provide a favorable intestinal flora. Yogurt, a dairy product, can be effectively used as probiotic. Yogurt mainly contains lactobacilli and other beneficial bacteria that have strong positive health effects. Yogurt may aid digestion and inhibit the development of pathogens by improving the balance of the microbes living in the digestive tract (Metchnikoff, 1998).

The objective of this study was comparing the effects of the probiotic product and yogurt on the performance, carcass traits and intestinal microflora of broiler chickens.

MATERIALS AND METHODS

Birds and housing

Two hundred and forty 1-d-old male broiler chicks (Ross 308) were obtained from a commercial hatchery. Birds were randomly divided into three groups (80 bird per group) and housed in identical size pens (130 × 130 cm) in a deep litter system using wood shavings as substrate. Each group had three replicates (20 birds/ pen). Birds were reared under a 24h light lighting schedule, and had free access to water and feed. Environmental temperature during the first week of life was 34°C and decreased to 20°C until the end of the experiment.

Diets

A basal diet, considered as control, was formulated according to the NRC recommendations (1994) for the starter, grower and finisher periods. Three dietary treatments were applied: control diet; yogurt group: 10, 5 and 2.5 % yogurt (contain 1×10^8 CFU of *Lactobacillus delbrueckii* and *Lactobacillus thermophilus*) in the drinking water; and the thepax probiotic (contain 1×10^{10} cells of *Sacchromyces cervisiae* and *Lactobacillus acidophilus* mg^{-1}) group: 1000, 500 and 250 g/ton^{-1} in the starter, grower and finisher diets, respectively. The compositions of the diets are shown in Table 1.

Growth performance evaluation

All birds were individually weighed after their arrival from the hatchery to the experimental farm (initial weight) and on d 21 and 42. Body weight gain for each dietary treatment was calculated. Feed intake was recorded during the entire starter and grower periods for each treatment, and the feed conversion ratio was subsequently calculated.

Table 1 – Ingredient composition (as dry matter percentage) and calculated nutritional levels of the basal diets

Ingredients	Starter	Grower	Finisher
Yellow corn	495.3	530.4	536.4
Soybean meal (44%)	383.1	315.1	300.2
Wheat grain	50.2	80	100
Fish meal (66%)	25	25	0
Vegetable oil	10.3	16	28
Dicalcium phosphate	15.3	14.4	15.2
Oyster shell	9.5	8.4	10.2
Salt	1.5	1.4	1.8
Vitamin-mineral suplement[1]	6	5.5	5
Coccidiostat	0. 4	0.4	0.4
DL-Methionine	2.3	2.2	2
L-Lysine	1.1	1.2	0.8
Calculated chemical composition			
Dry matter (%)	90.9	90.1	90.2
ME (MJ/kg)	12.14	12.56	12.97
Crude protein (%)	22.2	20.2	18.2
Calcium (%)	1	0.97	0.92
Available phosphorus (%)	0.5	0.48	0.45
Methionine	0.68	0.57	0.52
Lysine	1.37	1.18	1.05

Vitamin and mineral provided per kilogram of diet: vitamin A, 360,000 IU; vitamin D3, 800,000 IU; vitamin E, 7,200 IU; vitamin K3, 800 mg; vitamin B1, 720 mg; vitamin B9, 400 mg; vitamin H2, 40 mg; vitamin B2, 2,640 mg, vitamin B3, 4,000 mg; vitamin B5, 12,000 mg; vitamin B6, 1,200 mg; vitamin B12, 6 mg; choline chloride, 200,000 mg, manganese, 40,000 mg, iron, 20,000 mg; zinc, 40,000 mg, copper, 4,000mg; iodine, 400 mg; selenium, 80 mg.

Carcass traits

At the end of experiment, six birds per treatment were randomly selected for carcass trait evaluation. Chicks were fasted for approximately 12 h and then individually weighed, sacrificed, de-feathered and eviscerated. Breast, thigh, heart, liver and abdominal fat pad weights were recorded. The percentage (%) of carcass parts and organs relative to live body weight was calculated.

Microbiological analysis

On 21 d, ceca of the sacrificed birds (n = 2 per replicate) were aseptically removed, placed into sterile stomacher bags (Spiral Biotech Inc., Norwood, MA), and kept on ice. Fresh cecal contents were diluted 10-fold by weight in buffered peptone water and mechanically homogenized using a stomacher (Model 400 Lab Blender, Seward Medical, London, UK) for 30

s. Samples were then serially diluted in 0.80% sterile saline solution. All microbiological analyses were performed in duplicate and the average values were used for statistical analysis.

Clostridium perfringens was enumerated in Perfringens Agar Base (Oxoid Limited, Basingstoke, Hampshire, England) supplemented with TSC selective supplement B and Egg Yolk and incubated in a controlled-environment chamber at 35° C for 48 h. Rapid E. coli 2 agar (Bio-Rad Laboratories, Mississauga, Ontario, Canada) and E. coli supplement (Bio-Rad Laboratories) were used to quantify *Escherichia coli* after 24h culture at 37°C, as recommended by the supplier. After the incubation periods, colonies of the respective bacteria were counted.

Statistical analysis

All data were analyzed using the one-way analysis of variance (Anova procedure) of SAS® (SAS, 1998) statistical package. Significant differences among treatments were identified at 5% probability level by Duncan's multiple range tests (Duncan, 1955).

RESULTS AND DISCUSSION

Growth performance

Performance results are presented in the Table 2. In all rearing periods evaluated in this experiment, birds supplemented with thepax and yogurt presented significantly higher ($p \leq 0.05$) body weight gain (BWG) compared with control group, except for yogurt during the period of 1-21d of age. Feed conversion ratio (FCR) improved ($p \leq 0.10$) in birds supplemented with thepax compared with the control treatment, but this difference was only significant compared with yogurt during the period of 1-42d of age. Feed intake was not different among treatments ($p > 0.05$).

In general, the positive effects on performance obtained with the evaluated additives are consistent with previously reported results. Sultan *et al.* (2006) showed that yogurt has favorable effects on broiler weight gain. In the present study, the beneficial effects of thepax and yogurt on broiler performance parameters, including BWG and FCR are in agreement with previous studies (Zulkifli *et al.*, 2000; Pelicano *et al.*, 2003; Cavit, 2004; Aftahi *et al.*, 2006; Falaki *et al.*, 2010). In contrast, Shoeib *et al.*, (1997), Gunal *et al.*, (2006), and Willis *et al.*, (2007) reported that the inclusion of these additives in broiler feeds had no significant effects on broiler growth performance. This inconsistency among research reports may be related to differences in probiotic types, management practices and environmental conditions among the experiments. It is suggested that under favorable management and/or environmental conditions, the effect of such feed additives may be worthless.

The improvements in BWG and FCR of broilers fed thepax and yogurt (contains mainly lactobacilli) were probably due to the lactobacilli present in yogurt. Also, by improving intestinal microflora microbial balance, probiotics beneficially influence growth performance. Those improvements could be ascribed to better nutrient digestion and absorption due to the presence of enzymes derived from lactobacilli. In addition, the probiotics are able to increase available energy levels by increasing carbohydrate digestibility by improving organic matter digestibility, increasing amylase enzyme

Table 2 – The main effects of treatments on growth performance of broiler chickens

	Control	Thepax	Yogurt	SEM	P-value
Body weight gain (g)					
1-21	924.13 ^{b}	988.81 ^{a}	942.64 ^{ab}	16.78	0.049
1-42	2546.86 ^{b}	2662.93 ^{a}	2627.90 ^{a}	22.93	0.019
Feed consumption (g)					
1-21	1633.52	1545.69	1515.07	34.47	0. 111
1-42	5181.7	4988.5	4930.8	79.76	0. 145
Feed conversion ratio (g/g)					
1-21	1.74 ^{a}	1.56 ^{b}	1.60 ^{ab}	0.04	0.026
1-42	2.02 ^{a}	1.86 ^{b}	1.90 ^{b}	0.02	0.004

a,b means in each row with different superscripts are significantly different ($p \leq 0.05$).

activity, and reducing bacterial β-glucuronidase, β-glucosidase and urea enzymes activities (Jin *et al.*, 1997; Jin *et al.*, 2000).

Carcass characteristics

The effects of the dietary treatments on carcass and internal organ weights of 42-day-old broilers are shown in Table 3. The thepax- and yogurt-supplemented groups presented higher ($p \leq 0.05$) carcass and thigh percentages compared with the other groups, respectively. Breast percentage was higher ($p \leq 0.05$) in the thepax and yogurt treatments than in the control group. Birds under the thepax treatment presented the lowest abdominal fat pad percentage as compared to the other treatments ($p \leq 0.05$). There were no significant differences in mean heart and liver yield among treatments.

The benefits of probiotics and yogurt use on carcass characteristics have been reported by other researchers (Haj Ayed *et al.*, 2004; Sultan *et al.*, 2006; Chumpawadee *et al.*, 2008). Kabir *et al.* (2004) reported that adding 2g probiotic per each of water consumed by broiler chickens increased thigh and breast yield compared with the control treatment. However, our findings on carcass composition were in contrast to those of Pelicano *et al.* (2003), Karaoglu & Durdag (2005), and Willis *et al.* (2007). As pointed out before, these differences between reported results could be related to the mode of action of those feed additives, which may be quite different, particularly regarding their antimicrobial activity. However, they may exert similar physiological effects by modifying intestinal pH, altering the composition and balance of intestinal flora, enhancing nutrient digestibility and improving growth rate and carcass characteristics. Also, as shown by the reduction in fat pad percentage in birds fed the probiotic product, it is suggested that this product may interfere in fat deposition in the carcass.

Microbial culture

Table 4 shows the results of the effect of the above mentioned additives on the cecal microflora. These results indicate that the birds under yogurt treatment had the lowest *E. coli* population in the ceca than control or the probiotic treatments ($p \leq 0.05$). The use of probiotic and yogurt determined significantly smaller *C. perfringens* population in the ceca when compared to the control treatment ($p \leq 0.05$). *E. coli* strains cause a number of diseases in domestic poultry, reducing egg and meat production, increasing carcass condemnations, and eventually causing disease and death (Sackey *et al.*, 2001). Djouvinov *et al.* (2005) showed that the use of probiotics reduced the *E. coli* population in the cecal digesta of 54-day-old ducklings compared with the control treatment ($p \leq 0.05$). Lin *et al.* (2011) reported that the use of probiotics in the feed reduces total *E. coli* population in the intestinal lumen. *Clostridium perfringens* is a normal inhabitant of the intestinal tract of chickens, as well as a potential pathogen that causes necrotic enteritis and colangiohepatitis (Engstrom *et al.*, 2003). Murry *et al.* (2006) reported that, in broilers under probiotic treatment, the populations of *C. perfringens* and *E. coli,* recovered respectively from cloaca and cecal contents, were lower than those of the control group. However, Jin *et al.* (1998) and Ceylan *et al.* (2003) did not observe any changes in the gastrointestinal microbial flora by adding probiotics to broiler diets. When probiotics are consumed, a large amount of useful microorganisms enters the animal's gastrointestinal tract. These microorganisms produce acids (such as acetic acid and lactic acid) and other compounds that inhibit pathogenic bacteria growth and aid beneficial bacteria to adhere and rapidly colonize the intestinal mucosa of the animal (Fuller, 1989).

Table 3 – The main effects of treatments on carcass characteristic of 42d broiler chickens

	Control	Thepax	Yogurt	SEM	P-value
Carcass (g)	2249.8^{b}	2518^{a}	2384.5^{ab}	70.14	0.04
Breast (%)[c]	27.11^{b}	29.49^{a}	29.06^{a}	2.74	0.02
Thigh (%)	27.65^{b}	29.77^{ab}	30.69^{a}	2.41	0.02
Heart (%)	0.61	0.54	0.68	0.17	0.57
Liver (%)	3.13	2.71	2.94	0.43	0.95
Abdominal Fat pad (%)	2.1^{a}	1.27^{b}	1.9^{ab}	0.54	0.01

[a,b] means in the same row with different superscripts are significantly different ($p \leq 0.05$).

[c] As percent of live body weight

Table 4 – Main effects of treatments on intestinal microbial population[1] of chickens at 21 day

	Control	Thepax	Yogurt	SEM	p-value
E. coli	3.11 [a]	2.37 [b]	2.23 [c]	0.02	0.0001
C. perfringens	3.44 [a]	3.11 [b]	2.96 [b]	0.05	0.0006

a,b,c means in each row with different superscripts are significantly different (p≤0.05).

The results are mentioned as log cfu/g of cecal contents.

CONCLUSION

Competitive exclusion is a popular strategy for preventing intestinal infectious diseases in poultry due to the effective inhibition of pathogenic bacteria in the gut. The tested probiotic product, with defined bacteria, had a significant effect on growth performance. Based on the results of the present study, it may be interesting to focus on using these feed additives (thepax or yogurt) in broilers nutrition in order to reduce pathogenic microflora levels, thereby improving growth performance and carcass yield of the host instead of using antibiotics.

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