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The Effects of Biotic Additives on Growth Performance and Meat Qualities in Broiler Chickens

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Abstract: Commercial broiler chickens were treated with five diets containing probiotics (*Bacillus subtilis*), prebiotics (mannan oligosaccharide-MOS), synbiotics (*Saccharomyces cerevisiae*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium bifidum*, MOS and FOS (fructooligosaccharides), Avilamycin, or a control treatment (no additives). Performance parameters including total weight, daily weight gain, feed intake, viability production efficiency index and yield of carcasses and cuts were evaluated. In addition, meat quality parameters including the proportion of PSE meat (Pale, Soft, Exudative) and lipid oxidation were measured. The results indicated that the biotic treatments did not cause significant differences in any of the parameters evaluated. With regard to the meat quality, birds fed biotic diets showed a reduction in the development of PSE meat and also a decrease in lipid oxidation. These additives are therefore nutritionally feasible replacements for growth promoters and the animal husbandry indices of animals treated with these additives were similar to those of animals fed the normal rations and the use of additives contributed to improvements in the meat quality.

Key words: Organic additives; poultry; meat quality; performance parameters

INTRODUCTION

During the evolution of modern chicken production, there have been several changes in the nutritional requirements associated with a healthy feed product. In fact, in recent years, several food additives have been applied as replacements for antibiotic growth promoters. The most recent of these additives are prebiotics, probiotics and synbiotics (Santos *et al.*, 2002; Hajati and Rezaei, 2010). The use of bioactive nutritive components is becoming an attractive option because these additives can achieve results similar to those of antibiotics-based growth promoters. The high frequency of bacterial pathogens in animals, the antimicrobial resistance caused by the use of growth promoters and the increase of human pathogenic bacteria present in animal products have led consumers to question of the indiscriminate use of antibiotic ingredients in animal feed. However, animal production at the current levels of technology and productivity will be difficult to achieve without the aid of growth promoters or food additives for the prevention of diseases. Thus, the use of biotic additives appears as an attractive alternative to antimicrobial therapies (Revington, 2002; Patterson and Burkholder, 2003).

Prebiotic, probiotic and synbiotic materials are used in food additives and their addition to animal feed as growth promoter can be an asset to improve the animal

health and performance (Fuller, 1989; Menten, 2002). Their beneficial effects are noticeable in different regions of the gastrointestinal tract, especially in the small and large intestines. Because these biotics inhibit pathogen growth by competitive exclusion and the production of bactericidal substances, they are also able to provide substrates for the development of health-promoting microorganisms (Menten, 2002). In addition, there have been reports stating that these additives improve carcass and meat quality (Jensen and Jensen, 1992; Maruta, 1993; Santos *et al.*, 2002).

PSE (Pale, Soft and Exudative) meat is a consequence of rapid glycolysis and dramatic reduction of muscle pH while the carcass is still warm, resulting in poor functional properties due to muscle protein denaturation (Kissel *et al.*, 2009). The incidence of PSE meat was reduced by administering vitamin E in the birds' diets (Olivo *et al.*, 2001). Because biotics positively affect gastrointestinal tract integrity, it is believed that they might improve the absorption of feed ingredients, such as micro and macronutrients and that they may inhibit the development of PSE meat. Therefore, this study aims to evaluate the influence of organic additives such as probiotics, prebiotics and synbiotics in the diet on the results of the broiler chicken husbandry indices and meat quality, especially in controlling the incidence of PSE meat.

MATERIALS AND METHODS

Experimental design: It was adopted a completely randomized design with five treatments and six replicates of 50 birds per plot, totaling 1500 male birds. Each cage had size of 2x2 m, resulting in a total of 4 m² and density of 12.5 birds/m². A total number of 1500 commercial broilers lineage were distributed according to a completely randomized experimental design into five trials: trial 1 (T1) as control group without addition of any of the experimental additives; trial 2 (T2), growth promoter avilamycin (10 g/ton); trial 3 (T3), prebiotic (2 kg/ton), mannan oligosaccharide; trial 4 (T4) consisted of probiotic, *Bacillus subtilis* (30 g/ton) and finally trial 5 (T5) synbiotic composed of *Saccharomyces cerevisiae*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium bifidum*, mannan oligosaccharide and fructooligosaccharide (3 kg/ton at phase 1 (initial ration, 1-21 days), 2 kg/ton at phase 2 (growth ration, 22-28 days) and 1 kg/ton at phase 3 (final ration, 29-42 days). The feeding trial was conducted under the supervision of the Animal Care and under approval of Ethic Committee of Londrina State University (Proc. # 104/2008).

Characteristics evaluated: The following performance data were evaluated: feed intake per bird by bird final weight, weight gain per bird, feed conversion, production viability and Productive Efficiency Index (PEI) determined by the formula $PEI = DWG \text{ (daily weight gain)} \times V \text{ (availability)} / FC \text{ (feed conversion)} \times 10$.

Carcass and cut yields: At the end of the experiment, two birds were collected from each experimental replicate. A total of 12 birds per treatment were individually weighed and selected to represent the average weight of the experimental plot. Birds were identified by rings located on the legs, kept in crates and transported to the slaughterhouse while fasting for 8 hours. Subsequently, the birds were weighed to obtain the slaughter weight which served as a reference for the calculation of carcass yield. The carcass samples were weighed after the following treatments: stunning, bleeding, scalding, defeathering, evisceration, chiller storage and drip loss. Finally, the commercial cuts (chest, leg and wings) were weighed.

Meat quality: pH Measurement: pH value was measured by inserting the electrodes of a pH meter system (Testo 205) into the breast muscle. Analyses were performed in triplicate on refrigerated samples 24 h postmortem, as reported in Olivo *et al.* (2001).

Color measurement: A Minolta CR400 model colorimeter was used to evaluate the color parameters L*(lightness), a* and b* (CIELAB color system) as described in Olivo *et al.* (2001) and samples were classified as PSE and non-PSE meat as reported in Wilhelm *et al.* (2010).

Analysis of lipid oxidation: Breast samples from each treatment were analyzed for lipid oxidation after 90 days of freezing at -20°C. This measurement was carried out according to Tarladgis *et al.* (1964), as described in Soares *et al.* (2009). The results were expressed in mg TBARS (thiobarbituric acid reactive substances)/kg sample.

Statistical analysis: The results of every determination were analyzed using the program STATISTICA for Windows 6.0 to verify the effects of each treatment on these variables. Analysis of variance and Tukey's test were applied to compare results between groups, at a 5% probability.

RESULTS AND DISCUSSION

Animal performance: Weight Gain (WG), Feed Conversion (FCv), Feed Consumption (FCs), Availability (AV), Productive Efficiency Index (PEI), Carcass and cut yields: The performance results presented in Table 2 and 3 show that the use of various additives from day 1 to day 21 and day 1 to day 42 of age did not affect ($p > 0.05$) the feed intake, weight gain, feed conversion, production viability or productive efficiency index.

These results were most likely due to the low sanitary challenge faced by the birds because the negative control treatment presented results similar to that used for the additives. Similar results were obtained by Loddi *et al.* (2000), Rocha *et al.* (2010), Al-Barwary *et al.* (2012), Houshmand *et al.* (2012), who all showed that adding these alternative additives instead of growth promoters in broiler diets was not associated with a significant difference in broiler performance. On the other hand, other authors (Macari and Furlan, 2005; Neto *et al.*, 2007; Awad *et al.*, 2009) observed improvements in bird performance after using organic additives in the birds diets.

Carcass and cuttings yield: The results presented in Table 4 show there were no differences ($p > 0.05$) in the results of carcass yield or cuts among treatments. These results were most likely the consequence of the absence of significant differences in broiler performance among treatments. Furthermore, the nutritional values offered to the birds were similar, thus facilitating similar carcass and cut development under different diet treatments.

However, in contrast to these results, Pelicano *et al.* (2003) and Loddi *et al.* (2000) reported that chickens fed a probiotics diet presented higher yields of chicken legs and carcasses, although other reports showed results similar to those presented in this work (Carão, 2011).

Meat quality of broilers fed different additives: Table 5 shows the breast fillet pH and L*, a*, b* values taken at 24 hours post mortem for chickens fed under various diets; no significant differences between groups were

Table 1: Composition and nutrient content of broiler diets (initial, growth and final phases) offered from 1 to 42 days of age

Ingredients (%)	Phase 1: Initial (1-21 days)	Phase 2: Growth (22-28 days)	Phase 3: Final (29-42 days)
Com meal	54.96	64.13	65.46
Integral soy	11.88	10.69	22.99
Meat and bone meal	6.71	6.61	5.55
Feather meal	-	-	3.00
Soy meal	24.01	16.73	1,24
Premix ¹	0.42	0.42	0.42
Mycotoxin sequestrant	0.25	-	-
L-Lysine	0.32	0.32	0.33
Salt	0.30	0.35	0.31
DL-Methionine	0.38	0.29	0.27
Calcitic limestone	0.04	-	0.12
L-Treonine	0.14	0.10	0.10
Choline chloride	0.08	0.06	0.07
Sodium bicarbonate	0.21	-	-
Inert (caulin)*	0.3	0.3	0.10
Total	100	100	100
Calculated composition %			
Protein (%)	21	19.50	18.50
ME kcal/kg	3.100	3.160	3.200
Available phosphorus (%)	0.52	0.50	0.53
Calcium (%)	1.01	0.98	1.05
Met + cystine (%)	0.95	0.88	0.83
Met (%)	0.64	0.59	0.56
Lys (%)	1.32	1.21	1.12

¹Premix: Trial 1: Initial phase 1: 273 mg/kg, Se 59.28 mg/kg, Mn 15.500 mg/kg, Zn 18.250 mg/kg, vitamin A 1.900.000 UI/kg, vitamin D 600.000 UI/kg, vitamin E 2.500 mg/kg, vitamin K 98 mg/kg, vitamin B1 356 mg/kg, vitamin B2 1.600 mg/kg, vitamin B6 693 mg/kg, vitamin B12 2.200 mg/kg, pantothenic acid 1.710 mg/kg, niacin 15.840 mg/kg, biotin 32 mg/kg, folic acid 148 mg/kg, choline 144.000 mg/kg, Cu 25.000 mg/kg and S 2.33 %. Trial 2: Growth phase 2: 260 mg/kg, Se 54.72mg/kg, Mn 18.600 mg/kg, Zn 18.250 mg/kg, vitamin A 1.400.000 UI/kg, vitamin D 600.000 UI/kg, vitamin E 2,000 mg/kg, vitamin K 98 mg/kg, vitamin B1 356 mg/kg, vitamin B2 1.600 mg/kg, vitamin B6 693 mg/kg, vitamin B12 3.200 mg/kg, pantothenic acid 1.900 mg/kg, niacin 5.940 mg/kg, biotin 32 mg/kg, folic acid 40 mg/kg, choline 144.000 mg/kg, Cu 25.000 mg/kg, Na 1.5 %, S 3.90 % and Fe 5.400 %. Trial 3: Final phase: I 195 mg/kg, Se 118.56 mg/kg, Mn 30.720 mg/kg, Zn16.060 mg/kg, vitamin A 1.400.000 UI/kg, vitamin D 100.000 UI/kg, vitamin E 400 mg/kg, vitamin K 196 mg/kg, vitamin B2 672 mg/kg, vitamin B12 2.000 mg/kg, pantothenic acid 3.800 mg/kg, choline 144.000 mg/kg, Cu 3.200 mg/kg and S 2 %). These rations were based on Rostagno *et al.* (2005). *Caulin was used as an inert product

Table 2: Broiler Feed Consumption (FC), Weight Gain (WG), Feed Conversion (FCv) and Availability (AV) in response to feeding with different additives from days 1 to 21 of age

Treatments	FCs (g)	WG (g)	FCv	AV (%)
Negative control	1.164±0.06	781.61±24.92	1.56±0.03	96±3.35
Avilamycin	1.170±0.05	804.80±36.09	1.52±0.05	98±0.82
Prebiotic	1.156±0.03	782.92±20.63	1.55±0.03	96±5.28
Probiotic	1.189±0.04	789.45±42.84	1.57±0.06	98±2.66
Synbiotic	1.190±0.03	781.80±21.22	1.59±0.03	98±1.97
P-value	0.528305	0.645440	0.171251	0.4233

Table 3: Broiler Feed Intake (FCs), Body Weight at 42d (BW), Daily Weight Gain (WG), Feed Conversion (FCv), Availability (AV) and Index of Productive Efficiency (IPE) in response to feeding with different additives from days 1 to 42 of age

Treatments	FCs (g)	BW at 42d old	WG (g)	FCv	AV (%)	IPE ¹
Negative control	4.549±0.14	2.561±120.68	60.97±2.87	1.79±0.07	94±4.63	322.63±35.17
Avilamycin	4.686±0.16	2.673±42.51	63.65±1.01	1.75±0.03	95±2.76	344.68±15.53
Prebiotic	4.595±0.09	2.616±69.29	62.28±1.65	1.76±0.02	94±4.63	331.21±25.93
Probiotic	4.643±0.15	2.612±132.92	62.19±3.16	1.78±0.04	97±3.01	336.73±30.13
Synbiotic	4.722±0.17	2.642±60.20	62.89±1.43	1.77±0.02	95±3.72	337.03±14.69
P-value	0.264711	0.329771	0.329771	0.533334	0.779244	0.655887

±standard deviation; The averages of the treatments did not differ statistically according to the Tukey test with a 5.0% probability (p<0.05)

observed. However, by evaluating each treatment for the incidence of abnormal color in breast fillet meat individually (Olivo *et al.*, 2001), a high incidence of PSE meat was detected in the Control group, giving the

sequence of control > synbiotic > avilamycin = prebiotic > probiotic (Table 6).

Interestingly, the TBARS index presented a somewhat similar sequence; the breast fillets from birds fed a

Table 4: Carcass and parts yields of birds fed with different additives and slaughtered at 42 days old

Treatments	Carcass Yield (%)	Wings (%)	Legs (%)	Breast (%)	Back (%)
Control	72.89±4.75	11.76±0.48	31.32±1.26	38.12±1.50	18.80±0.70
Avilamycin	71.47±3.48	11.24±0.51	31.50±1.43	38.08±1.56	19.18±0.87
Prebiotic	73.60±6.49	11.40±0.49	31.66±1.30	37.42±1.09	19.09±0.59
Probiotic	70.84±2.75	11.50±0.60	31.58±1.56	38.06±2.12	18.86±0.73
Synbiotic	72.27±5.40	11.29±0.60	31.46±1.39	38.32±1.78	18.92±0.90
P-value	0.589898	0.110089	0.822804	0.736632	0.817013

The results did not differ significantly among treatments according to the Tukey test (p= 0.05), ±standard deviation

Table 5: Values of pH, L*, a* and b* for chicken breast fillets (Pectoralis major m.) from animals subjected to different treatments and growth promoter replacements, 24 h after slaughter

Treatments	pH _{24h}	Color		
		L*	a*	b*
Control	5.80±0.14	56.17±3.27	2.98±1.17	7.10±1.92
Avilamycin	6.04±0.59	52.27±2.51	4.20±2.11	7.19±1.72
Prebiotic	5.92±0.19	52.17±4.48	3.35±2.05	6.61±2.81
Probiotic	5.87±0.07	53.32±2.82	3.66±1.99	7.23±2.33
Synbiotic	5.86±0.09	54.49±2.32	3.72±1.87	8.53±2.98
P Value	0.359	0.071	0.684	0.365

The results did not differ significantly among treatments according to the Tukey test (p <0.05), ±standard deviation

Table 6: Incidence of PSE and lipid oxidation in meat from chickens fed different additives as growth promoters

Treatments	Incidence of PSE (%)	Lipid oxidation (TBARS)
Control	83	0.029±0.015a
Avilamycin	33	0.014±0.007b
Prebiotic	33	0.013±0.003b
Probiotic	25	0.016±0.005ab
Synbiotic	50	0.014±0.004b
Value of p	-	0.0100238

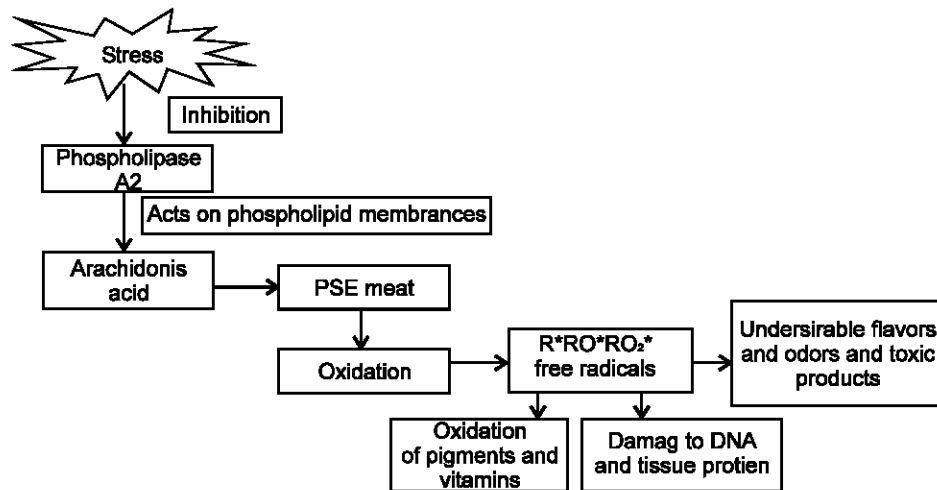


Fig. 1: Flowchart illustrating the use of biotic additives as tools to chemically reduce stress and explain the relationship between PSE meat and lipid oxidation. Adapted from Soares *et al.* (2009)

control diet were most oxidized samples and those from animals fed the avilamycin, probiotic, prebiotic and synbiotic diets had similar oxidation levels, equivalent to half of the lipid oxidation of the control samples (Table 6).

It should be noted that the occurrence of PSE meat depends on at least two factors: management and genetics (Marchi *et al.*, 2009). Under the management

strategies used, the birds are particularly subjected to thermal stress during the pre slaughter period. This is the main cause of the formation of PSE meat and can eventually lead to death, the so-called Dead on Arrival (DOA) (Oba *et al.*, 2009). However, there are reports indicating that birds fed a synbiotic diet presented 17.0% less broiler fearlessness in relation to control group (Ghareeb and Böhm, 2009). Aksu *et al.* (2005) and Zhang and Barbut (2005) evaluated the effect of

Saccharomyces cerevisiae (SC) in the diet and showed a decrease in TBARS values without an increase in color abnormality. The association of lipid oxidation with the appearance of PSE meat was first reported by Cheah and Cheah (1981) in pigs and by Soares *et al.* (2003) in broilers. In both cases, the authors reported that the PSE symptoms were the consequence of elevated phospholipase A2 activity in affected muscles and hypothesized this enzymatic reaction was the triggering factor that initiated the biochemical reactions leading to the meat color abnormalities. Fig. 1 shows a flowchart of the pathway from the stressor to the resulting lipid oxidation, mediated by an increase in PLA2 activity due to the impairment in calcium efflux from the Sarcoplasmic Reticulum (SR) to the sarcoplasm by calcium channel proteins which brings about the appearance of these abnormal meat qualities. As shown by Soares *et al.* (2009), PSE meat exhibited 27.0% higher lipid oxidation compared to normal meat. Furthermore, the fatty acid profile was also significantly different, i.e., arachidonic acid levels increased by 38.6% in PSE meat compared with normal meat. These observations, in addition to the fact that the PUFA/SFA ratio was much higher in PSE meat relative to control samples (Soares *et al.*, 2009), could explain the increase in oxidation because they show that there was a relative increase in polyunsaturated fatty acid availability, thus promoting the greater formation of free radicals, as shown in Table 6. All of these results indicate that these organic compounds may contain antioxidant factors or that they are better absorbed at the intestinal level.

Conclusions: The addition of various bioactive additives does not negatively affect the broiler chicken performance thus having similar activity as the growth promoter and their use beneficially would reduce the meat lipid oxidation and prebiotics and probiotics were able to inhibit the development of PSE meat.

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