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

Pelícia, VC; Stradiotti, AC; de Araujo, PC; Maruno, MK; Carvalho, FB; Pezzato AC; Sartori JR
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Revista Brasileira de Ciência Avícola, vol. 15, núm. 4, octubre-diciembre, 2013, pp. 295-300
Fundação APINCO de Ciência e Tecnologia Avícolas
Campinas, SP, Brasil

Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola
ISSN 1516-635X Oct - Dec 2013 / v.15 / n.4 / 295-300

Phytogenic Additives and Glutamine Plus Glutamic Acid in Broiler Diets

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■Keywords

Alternative additives, plant extracts, essential oils.

Submitted: July/2012
Approved: June/2013

ABSTRACT

The objective of this study was to evaluate the effect of the dietary supplementation of phytogenic additives (PAs) and glutamine plus glutamic acid (Gln/Glu), associated or not, in replacement of antibiotic growth promoters and anticoccidials (AGP/AC) on the performance and carcass yield of broilers. Five hundred male Cobb broilers were housed in an experimental house and randomly distributed into five treatments, with four replicates of 25 birds each. Treatments consisted of a control diet (CD); CD+AGP/AC; CD+Gln/Glu; CD+PAs; CD+Gln/Glu+PAs. Diets were formulated only with plant feedstuffs, i.e., they did not contain any animal byproducts. Performance data were collected for the accumulated periods of 1-7, 1-21, and 1-42 days of age. Carcass yield and parts yield were determined at 42 days of age. Treatments did not influence performance during none of the evaluated periods. The greatest carcass yield ($p<0.05$) was obtained in birds in the treatments CD+Gln/Glu and CD+Gln/Glu+PAs relative to CD, but not different from birds in the AGP+AC and PAs treatments, which were not different from the CD treatment. Birds fed the CD+Gln/Glu diet presented greater breast yield ($p<0.05$) compared with those in the CD and AGP/AC treatments, but there was no difference in comparison with the other treatments. Under the conditions of the present experiment, the dietary supplementation with phytogenic additives and with glutamine plus glutamic acid does not affect the performance, but improves carcass yield and breast yield of broilers.

INTRODUCTION

The public is increasingly concerned with animal welfare and food safety and quality. Also, there is a growing public pressure at domestic and international level against the addition of low doses of antibiotics and chemotherapeutic drugs in animal feeds, with a consequent increasing demand for the so-called alternative animal products, particularly for alternative poultry products. Alternative chicken products are those derived from industrial broilers that were not fed antibiotic growth promoters, anticoccidials, or animal feedstuffs and that were reared at lower stocking density, i.e., at a maximum of 12 birds/m^2 (Demattê Filho & Mendes, 2001). Therefore, there is a need to search for alternative natural additives to replace antibiotics and chemotherapic drugs in broiler diets with beneficial effects on broiler intestinal flora, thereby protecting the birds against pathogens, controlling or preventing diseases, and improving the efficiency of nutrient utilization.

Several researchers (Bartel & Batal, 2007; Murakami *et al.*, 2007; Lora *et al.*, 2006; Mcreynolds *et al.*, 2009) observed the beneficial effects of glutamine and glutamic acid, as well as phytogenic additives (essential oils and plant extracts) on broiler immune response and

intestinal microflora and structure, and consequently, on broiler performance, particularly in the presence of health challenges.

Glutamine (Gln) and glutamic acid (Glu) are important energy substrates for high turnover cells, such as those of the immune system and of the intestinal mucosa (Windmueller & Spaeth, 1974; Newsholme *et al.*, 1985; Newsholme, 2001; Piva *et al.*, 2001; Newsholme *et al.*, 2003a and b), in addition of being part of proteins and peptides. Glutamine is also used for the synthesis of other amino acids and nucleotides (Wu, 1998).

Bartel & Batal (2007) observed higher antibody levels in broilers fed a Gln-supplemented diet compared to those not fed Gln. When supplementing 1% Gln to broiler diets, Maiorka *et al.* (2000), Sakamoto *et al.* (2005), and Murakami *et al.* (2007) observed beneficial effects on the development of the intestinal mucosa in one-week-old broilers, with better nutrient digestion and absorption capacities, which may improve their performance.

Lora *et al.* (2006) studied the effect of the inclusion of different levels of a product containing a mixture of glutamine and glutamic acid (10% each) on the performance of 1- to 42-d-old broilers reared on reused litter. Broilers fed the mixture at 0.5, 1.0, and 1.5% presented higher weight gain relative to the control birds during the entire experimental period. The best production efficiency index was obtained with the 1.0% inclusion level.

Phytogenic additives (PAs) are products derived from plants and consist mostly of plant extracts (PEs) and essential oils (EOs). The reported therapeutic properties of these additives include antimicrobial activity (McReynolds *et al.*, 2009), anticoccidial activity (Miguel *et al.*, 2009), and better nutrient digestibility and broiler performance (García *et al.*, 2007; Fascina *et al.*, 2010 e 2012).

Considering the reported beneficial effects of these natural additives on the intestinal structure, nutrient digestibility, and immune system of broilers, it may be possible to use them to replace antibiotic growth promoters and chemotherapeutic drugs.

The objective of this study was to evaluate the effect of the dietary supplementation with phytogenic additives and glutamine plus glutamic acid, associated or not, as an alternative to the inclusion of antibiotic growth promoter and anticoccidials on the performance and carcass yield and parts yield of broilers.

MATERIALS AND METHODS

The study was carried out at the facilities of the Poultry Nutrition Laboratory, School of Veterinary Medicine and Animal Science, Universidade Estadual Paulista (UNESP), Botucatu campus. The trial was approved by the Committee of Ethics of Animal Experimentation (protocol n. 07/2009, CEEA).

Five hundred one-d-old male Cobb broilers were housed on a twice-reused litter at a density of 10 birds/m^2, as recommended for alternative broiler production (Demattê Filho & Mendes, 2001). Chicks were vaccinated in the hatchery against Marek's disease, infectious bursal disease (IBD), and fowl pox. Birds received booster vaccine against IBD at eight and 14 days of age.

A completely randomized experimental design, of five treatments with four replicates of 25 birds each, was applied. Treatments consisted of a control diet with no antibiotic growth promoters or anticoccidials (CD); CD+antibiotic growth promoter[1] and anticoccidial[2] (AGP/AC); CD+glutamine+glutamic acid[3] (Gln/Glu); CD+phytogenic additive[4] (PAs), and; CD+Gln/Glu+PAs.

Glutamine and glutamic acid were added to the diets at the expense of corn starch as they have similar energy values, while the other additives were added at the expense of inter material (kaolin), according to the recommendations of the manufacturer.

The rearing period was divided in four phases: pre-starter, starter, grower, and finisher. Feeds were formulated according to the nutritional requirement tables of Rostagno *et al.* (2005), as shown in Table 1.

Performance data were collected and analyzed for the accumulated periods of 1-7, 1-21, and 1-42 days of age. The following parameters were calculated: weight gain (difference between body weight at the end of each period and body weight at housing; feed

[1] Surmax® 10%, Elanco (avilamycin): 10ppm inclusion level.

[2] Monenpac®, M Cassab (monensin): 100ppm inclusion level.

[3] AminoGut®, Ajinomoto (guaranteed minimal levels: 10% glutamine and 10% de glutamic acid): 1.0% inclusion days 1-21 and 0.5 inclusion days 22-42.

[4] Imunostart®, M Cassab (composed of turmeric extract, citrus extract, and grape seed extract): inclusion of 700g/ton feed days 1-10; 500g/ton days 11-21; Enterocox®, M Cassab (composed of eucalyptus oil, cinnamon essential oil, boldo leaves, fenugreek seeds): inclusion of 300g/ton days 1-10, 1000g/ton days 11-35, and 500g/ton days 36-42.

Table 1 – Composition of the control diets according to rearing phase.

Ingredients (%)	Pre-starter 1-7 days	Starter 8-21 days	Grower 22-35 days	Finisher 36-42 days
Corn	55.174	58.107	61.661	65.929
Corn starch	1.000	1.000	0.500	0.500
Soybean meal	37.365	34.600	30.870	26.940
Soybean oil	1.974	2.272	3.197	3.110
Salt	0.520	0.500	0.480	0.450
Vitamin suppl.[1]	0.100	0.100	0.100	0.050
Mineral suppl.[2]	0.050	0.050	0.050	0.050
Calcitic limestone	0.910	0.880	0.830	0.790
Dicalcium phosphate	1.950	1.809	1.670	1.520
DL-methionine	0.240	0.175	0.175	0.170
L-lysine HCl	0.375	0.210	0.230	0.275
L-threonine	0.155	0.060	0.060	0.075
Choline chloride[3]	0.060	0.060	0.050	0.040
Kaolin[4]	0.125	0.175	0.125	0.100
Total	100.00	100.00	100.00	100.00
Calculated values				
Metab. energy (kcal/kg)	2950	3000	3100	3150
Crude protein (%)	22.04	20.79	19.41	18.03
Calcium (%)	0.94	0.89	0.83	0.76
Avail. phosphorus (%)	0.47	0.44	0.41	0.38
Methionine (%)	0.57	0.49	0.48	0.45
Met+Cys (%)	0.91	0.82	0.79	0.75
Lysine (%)	1.46	1.26	1.18	1.12
Threonine (%)	0.99	0.86	0.81	0.76
Potassium (%)	0.84	0.80	0.74	0.68
Sodium (%)	0.22	0.22	0.21	0.20
Chlorine (%)	0.36	0.34	0.33	0.31
Linoleic acid (%)	2.32	2.52	3.06	3.06

[1]Vitamin supplement (per kg feed) vit. A, 11,000 IU; vit. D_3, 2,000 IU; vit. E, 16 mg; vit. K_3, 1.5 mg; B_1, 1.2 mg; vit. B_2, 4.5 mg; vit. B_6, 2 mg; vit. B_{12}, 16 mcg; niacin, 35 mg; folic acid, 0.4 mg; pantothenic acid, 10 mg; biotin, 60 μg; selenium, 250 μg. (pre-starter, starter, and grower phases); vit. A, 5,500 IU; vit. D_3, 1,000 IU; vit. E, 8 mg; vit. K_3, 0.75 mg; B_1, 0.6 mg; vit. B_2, 2.25 mg; vit. B_6, 1 mg; vit. B_{12}, 8 μg; niacin, 17.5 mg; folic acid, 0.2 mg; pantothenic acid, 5 mg; biotin, 30 μg; selenium, 125 μg. (finisher phase). [2] Mineral supplement (per kg feed); iodine, 1.000 μg; iron, 30 mg; copper, 9 mg; manganese, 60 mg; zinc, 60 mg. [3]Choline chloride (70). [4]Inert material.

intake (difference between total feed offer and feed residues at the end of each period); feed conversion ratio (ratio between total feed offer and weight gain in the period corrected for weight of birds that died during the period), livability (percentage of live birds in each period), and production factor (average daily weight gain multiplied by livability and feed conversion ratio, and dividing the result by 100).

On day 42, 20 birds per treatment were randomly selected, submitted to fasting for eight hours, electrically stunned, and bled. Carcass yield was then calculated as the difference between live weight and the weight of the eviscerated carcass with no head, neck, or feet. Breast and leg (thighs and drumstick) yields were calculated relative to empty carcass weight.

Data were analyzed using the GLM procedure of the SPSS® version 13.0 (2004) statistical package, and means were compared by the test of Tukey at 5% probability level.

RESULTS

Variance homogeneity was confirmed by the test of Levene. All data presented normal distribution according to the KS test.

There was no effect ($p>0.05$) of treatment on broiler performance in the cumulative periods of 1 to 7, 1 to 21 and 1 to 42 days of age (Table 2).

However, treatments influenced carcass yield and breast yield (Table 3). Broilers in the CD+Gln/Glu treatment presented higher ($p<0.05$) breast yield when compared with those in the CD and AGP/AC treatment, which were not different from each other, but similar breast yield compared with the remaining treatments.

Table 2 – Average weight gain (WG), feed intake (FI), feed conversion ratio (FCR), livability (L), and production factor (PF) of broilers during the periods of 1-7, 1-21, and 1-42 days of age as a function of treatment.

Parameters	CD[1]	CD+ AGP/AC[2]	CD+ Gln/Glu[3]	CD+ PAs[4]	CD+Gln/ Glu+PAs	CV (%)	p-value
1-7 days of age							
WG, g	111.05	109.15	109.90	109.06	111.90	3.65	0.895
FI, g	133.82	126.99	132.74	126.38	129.41	4.28	0.363
FCR	1.208	1.173	1.205	1.163	1.160	2.68	0.208
L, %	100.00	98.00	100.00	97.00	99.00	2.22	0.341
1-21 days of age							
WG, g	899.49	892.32	896.70	901.13	902.62	3.33	0.987
FI, g	1249.07	1234.94	1249.07	1237.41	1238.95	2.92	0.850
FCR	1.405	1.390	1.395	1.385	1.390	2.27	0.939
L, %	99.00	98.00	99.00	96.00	97.00	2.84	0.563
1-42 days of age							
WG, g	2887.64	2875.58	2916.54	2906.53	2898.77	3.41	0.991
FI, g	4920.67	4835.65	4937.24	4877.28	4856.67	3.31	0.983
FCR	1.710	1.692	1.695	1.693	1.705	1.86	0.909
L, %	98.00	95.00	97.00	95.00	93.00	3.74	0.323
PF[5]	393.80	384.38	397.62	388.75	376.37	4.49	0.450

[1] CD = control diet with no antibiotic growth promoter (AGP) or anticoccidial (AC). [2]CD+ AGP addition (avilamycin) and AC (monensin) [3]CD with addition of glutamine and glutamic acid. [4]CD with addition of phytogenic additives. [5] Production factor = [(ADG x livability)/FCR]*100. Means followed by the same letters in the same row are statistically different by the test of Tukey ($p<0.05$).

DISCUSSION

The performance results obtained in the present study are consistent with those of other authors, who did not report any effect on performance during the periods of 1 to 7 days (Miguel *et al.*, 2009), 1 to 21 days (Hernández *et al.*, 2004; Toledo *et al.*, 2007; Barreto *et al.*, 2008) and 1 to 42 days (Fukayama *et al.*, 2005; Toledo *et al.*, 2007; Barreto *et al.*, 2008) when supplementing broilers diets with phytotherapic products. However, working with 1- to 42-d-old broilers challenged with *Eimeria tenella*, Christaki *et al.* (2004) observed better performance when birds were fed a diet supplemented with a phytogenic additive (mixture of essential oils) compared than those fed a negative-control diet, but worse performance compared with broilers fed a diet containing an anticoccidial agent. On the other hand, Miguel *et al.* (2009) obtained better performance results in broilers between one and 42 days of age challenged with *E. tenella, E. acervulina* and *E. maxima* and fed the same PA as that utilized in the present study, when compared both with birds fed a negative-control diet and those fed antibiotic growth promoters (bacitracin and salinomycin). Fascina *et al.* (2012) also found better performance in non-challenged broilers also fed the same PAs relative to the negative-control birds during the periods of 1-21 and 1-42 days of age.

Alves *et al.* (2008) reported that the supplementation of broiler diets only with glutamine or glutamine associated with glutamic acid improved live performance, and recommended supplementation levels of 1.5% L-Gln and 3.0% Gln/Glu. Sakamoto *et al.* (2010) also obtained better performance when broilers were fed a diet supplemented with 3% Gln/Glu. In the pres-

Table 3 – Average carcass yield and parts yield of 42-d-old broilers as a function of treatment.

Parameters (%)	CD[1]	CD+ AGP/AC[2]	CD+Gln/Glu[3]	CD+ PAs[4]	CD+Gln/Glu+PAs	CV (%)	p-value
Carcass[5]	73.35b	74.44ab	74.66a	74.10ab	74.73a	2.11	0.045
Wings[6]	10.20	10.20	9.93	10.05	10.17	4.85	0.333
Breast[6]	39.01b	38.95b	40.33a	39.34ab	39.54ab	3.90	0.026
Legs[6]	29.00	29.01	28.38	28.94	28.53	3.76	0.292
Back[6]	21.37	21.53	21.00	21.22	21.35	5.60	0.855

[1] CD = control diet with no antibiotic growth promoter (AGP) or anticoccidial (AC). [2]CD+ addition of AGP (avilamycin) and AC (monensin). [3]CD with addition of glutamine and glutamic acid. [4]CD with addition of phytogenic additives. [5]Percentage relative to live weight. [6]Percentage relative to empty carcass.

Means followed by the same letters in the same row are statistically different by the test of Tukey ($p<0.05$).

ent study, although the supplemented Gln/Glu levels (1.0% in the pre-starter and starter, and 0.5% in the grower and finisher diets) were lower than those recommended by Alves *et al.* (2008), the performance results obtained may be attributed to the lack of health challenge, as they were similar to those observed in broilers fed the control diet and the diet containing antibiotic growth promoter and anticoccidial agent.

The best carcass yield and breast yield results were obtained in broilers fed the diets with glutamine associated with glutamic acid. This may be explained by the fact that glutamine is part of the structure of proteins and peptides, and it is used for synthesis of other amino acids, purines, and pyrimidines, which are the bases of the nucleotides that make up the DNA and RNA molecules (Newsholme *et al.*, 2003a and b), which determine protein synthesis. Glutamine, in addition of stimulating muscle protein synthesis, inhibits muscle protein breakdown (Maclennan *et al.*, 1988), and therefore may function as a metabolic regulator, increasing protein synthesis and reducing protein catabolism when supplemented in the diet (Lobley *et al.*, 2001). Studies have shown that there is a positive correlation between free glutamine concentration and protein synthesis rate in the skeletal muscle (Souba *et al.*, 1990), suggesting that higher circulating glutamine levels would promote higher protein synthesis, which could explain the results of the present study.

As reported above, the main actions of PAs are their antimicrobial and anticoccidial activities, and enhancement of nutrient digestibility and broiler performance. The results obtained with PAs relative to carcass and parts yields in the present study are in agreement with other experiments (García *et al.*, 2007; Sheuermann *et al.*, 2009) that did not find any influence of the dietary addition of PAs on these parameters.

CONCLUSIONS

Under the conditions of the present experiment, the dietary supplementation with phytogenic additives and with glutamine associated with glutamic acid does not affect the performance, but improves carcass yield and breast yield of broilers.

ACKNOWLEDGEMENTS

The author thank Fundação de Apoio à Pesquisa do Estado de São Paulo (FAPESP) for the scholarship and the companies Ajinomoto Animal Nutrition, Phytosynthese La Phytothérapie Animale Titrée, and M.Cassab Tecnologia Animal for donating the products tested in the experiment.

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