Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

ISSN 1516-635X Jan - Mar 2008 / v.10 / n.1 / 67 - 71

Studies with Sanguinarine Like Alkaloids as Feed Additive in Broiler Diets[1]

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[1] Presented at Conferência APINCO de Ciência e Tecnologia Avícolas, Santos - SP, p.48-49, 2006.

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■ Keywords

Broilers, feed additive, Sangrovit®, sanguinarine.

ABSTRACT

This research included two studies evaluating the live performance of broilers fed Sangrovit® (minimum of 1.5% sanguinarine, a quaternary benzo[c]phenanthridine alkaloid extracted from *Macleaya cordata*). Both studies were conducted using Ross 308 female broiler chicks. Birds were fed corn-soybean meal all-vegetable diets without growth promoters with 5 treatments and 8 replications in each study. In the first study, treatments were composed of a Negative Control without feed additive and four diets with graded increases of Sangrovit of 12.5, 25, 37.5, and 50 ppm. In the second study, chicks received a similar diet from placement to 21 days of age and subsequently were given feeds with graded reductions in crude protein (CP) as follow: a Negative Control with 19.7% CP without sanguinarine, and then 19.7, 19.2, 18.8 and 18.3% CP supplemented with Sangrovit at 20 ppm. It was demonstrated that body weight was increased when birds were fed 50 ppm of Sangrovit at 21 d when compared to the Negative Control. Also comparatively to the Negative Control, cumulative feed conversion was improved for birds fed with Sangrovit at 37.5 ppm as well as feed intake from placement to 7 days at 12.5 ppm. No differences were observed in feed intake. Birds supplemented with Sangrovit and 18.8% CP had similar body weight gain and feed intake as the Negative Control with 19.7% CP. Mortality in both studies was not correlated with the treatments. Results from both studies indicate benefits of the supplementation of Sangrovit in diets for broilers.

INTRODUCTION

A great deal of controversy remains on the use of antimicrobials added to broiler feeds. Epidemiological events that result in antimicrobial resistance by bacteria are difficult to study, and several factors, including the overuse or misuse of antimicrobials in human and veterinary medicine, may intensify the severity of the problem (Shea, 2004). Growth promoters have been banned in European countries and voluntarily withdrawn by some producers in order to supply specific market demands. There has been an increase in the research on alternatives to growth promoters, including plant extracts. The use of natural antimicrobials produced from herbs and spices lends itself to more favorable acceptance by the general public as well as by countries that restrict the imports of products derived from animals fed antibiotics (Dickens & Ingram, 2001). Active compounds obtained from plants have been used for a variety of human needs for centuries; however, many commercially available additives based on natural extracts lack a definite mode of action in animal feeds.

Natural compounds extracted from plants, such as quaternary benzo[c]phenanthridine alkaloids (QBA) sanguinarine and chelerythrine,

Arrived: December / 2007
Approved: February / 2008

are known to have antimicrobial (Eisenberg *et al*., 1991; Mewton *et al*., 2002; Colombo & Bosisio, 1996), anti-inflammatory (Lenfeld *et al*., 1981; Tanaka *et al*., 1993), and immunomodulatory (Agarwal *et al*., 1991; Chaturvedi *et al*., 1997) effects. Sanguinarine has been incorporated into swine, bovine, and poultry diets to reduce amino acid degradation, increase feed intake, and promote growth (Tschirner, 2004; Susenberth & Wolffran, 2003). Improvements in protein retention by reducing intestinal decarboxylation of aromatic amino acids through the inhibition of L-amino acid decarboxylase (Drsata *et al*., 1996), and enhancement of feed intake by modulating effects on the tryptophan-serotonine pathway have also been suggested as part of their a mode of action (Mellor, 2001).

Our experiments were aimed at evaluating the live performance of broilers fed corn-soybean meal all-vegetable diets supplemented with sanguinarine like alkaloids produced from the extraction of *Macleaya cordata*.

MATERIAL AND METHODS

Two studies were conducted using steel-cage batteries (0.95 X 0.80 m). In Study 1, a titration with graded increases of Sangrovit® was used, whereas in the Study 2, Sangrovit was added at a fixed dosis of 20 ppm in feeds given from 21 to 35 days of age.

Study 1

Four hundred one-day-old Ross X Ross 308 female broiler chicks (average individual weight = 43.9 g), vaccinated for Marek's disease and infectious bronchitis were obtained from a commercial hatchery. Birds were randomly placed in 40 steel-cage batteries, with 10 birds per cage, in a continuous lighting program with feed and water provided *ad libitum*.

Corn-soybean meal all-vegetable mash diets without antibiotic growth promoters or anticoccidials were provided throughout the study. Feeding program consisted of starter feed from placement to 21 d, and grower feed from 21 to 35 d. Feeds had similar energy and nutrients levels after formulation according to Rostagno *et al*. (2005) as shown in Table 1. Treatments consisted of a Negative Control without feed additive and four graded levels of Sangrovit (12.5, 25, 37.5, and 50 ppm). Each treatment had 8 replications.

Birds were weekly weighed, and feed conversion ratio corrected for the weight of dead birds was calculated. Mortality was recorded daily. Data were analyzed using the Anova procedure of SAS (SAS, 2001). Tukey's test was used to assess differences between means, and values of $P \leq 0.05$ were considered statistically significant. Mortality data was analyzed after arcsin transformation.

Study 2

Four hundred one-d-old Ross X Ross 308 female

Table 1 - Composition of experimental diets, %.

Ingredients, %	Study						
	1[1]		2 (21 - 35 d)				
	1 - 21d	21 - 35d	T1	T2	T3	T4	T5
Corn	61.05	60.42	58.83	58.83	61.29	61.61	63.05
Soybean meal	31.14	29.86	32.48	32.48	31.20	29.86	28.58
Soybean oil	3.15	5.33	4.40	4.40	4.17	3.92	3.69
Dicalcium phosphate	1.87	1.72	1.87	1.87	1.88	1.89	1.90
Limestone	1.35	1.23	1.34	1.34	1.35	1.35	1.35
Salt	0.36	0.47	0.47	0.47	0.44	0.40	0.37
Sodium bicarbonate	-	-	0.18	0.18	0.23	0.28	0.33
DL-Methionine	0.31	0.29	0.21	0.21	0.22	0.23	0.24
L-Lysine	0.48	0.44	0.10	0.10	0.11	0.12	0.12
L-Threonine	0.08	0.07	-	-	0.02	0.03	0.05
Choline chloride	0.11	0.09	0.10	0.10	0.11	0.11	0.12
Premixes[2]	0.10	0.08	0.20	0.20	0.20	0.20	0.20
Nutritional levels, % or otherwise noted							
ME, kcal/kg	3,000	3,100	3,100	3,100	3,100	3,100	3,100
CP	23.0	21.5	19.73	19.73	19.26	18.78	18.32
Dig. Lys	1.20	1.10	1.02	1.02	0.99	0.96	0.93
Dig. Met + Cys	0.90	0.82	0.76	0.76	0.76	0.76	0.76
Dig. Thr	0.78	0.71	0.66	0.66	0.66	0.66	0.66
Ca	1.00	0.95	1.00	1.00	1.00	1.00	1.00
Av. P	0.50	0.45	0.48	0.48	0.48	0.48	0.48

1 - Basal diets which had supplementation of Sangrovit. 2 - Vitamin and trace mineral composition per kg of feed: Vit. A: 5,000 IU; Vit. D3: 1,000 IU; Vit. E: 20 mg; Vit. K3: 0.9 mg; Vit. B1: 0.6mg; Vit. B2: 3 mg; Vit. B6: 1 mg; Pantothenic acid: 7 mg; Biotin: 0.04 mg; Folic acid: 0.5 mg; Niacin: 15 mg; Vit. B12: 6 mcg; I: 0.72 mg; Se: 0.28 mg; Cu: 8 mg; Mn: 67.5 mg; Zn: 51 mg; Fe: 64 mg.

broiler chicks vaccinated for Marek's disease and infectious bronchitis were obtained from a commercial hatchery, and randomly placed in 40 steel-cage batteries, with 10 birds per cage. Birds were submitted to a 24-h lighting program with feed and water provided *ad libitum.* Broilers were fed the same feed until 21 d of age, and then individually weighed and randomly distributed with 7 birds per cage (average individual weight = 662 g).

Corn-soybean meal all-vegetable mash diets without antibiotic growth promoters or anticoccidials were provided throughout the study. Diets are shown in Table 1 and had similar energy and nutrient levels after formulation according to Rostagno *et al.* (2005). Treatments included a Negative Control with 19.7% crude protein (CP) without the feed additive, and diets with graded reductions of CP and supplemented with 20 ppm of Sangrovit as follows: 19.7, 19.2, 18.8, and 18.3%. Each treatment had 8 replications. Measurements and statistical analyses were performed as in Study 1.

RESULTS

The effects of treatments on performance parameters of studies 1 and 2 are presented in Tables 2 and 3. In both studies, mortality rate was considered normal for that time of the year and growth rate of the birds, and it was not affected by the treatments (grand means = 4.69 % in Study 1 and 1.50% in Study 2).

In study 1, body weight linearly increased at 21 d in birds fed Sangrovit ($p \leq 0.05$). Effects were not observed in the other periods. Feed conversion ratio corrected for the weight of dead birds improved during the total experimental period when sanguinarine were added at 37.5 ppm to the feed in study 1. This response was quadratically adjusted ($p \leq 0.001$) with the maximum response calculated as (-b/2a) at 32.84 ppm. Feed intake of birds fed Sangrovit improved during the first week,, whereas differences were not observed ($p \leq 0.05$) during the other periods.

In Study 2, body weight and feed intake were not influenced by dietary protein reductions; however, feed conversion ration was negatively affected from 21 to 28 and from 21 to 35 d (Table 3). Mean differences were observed only between treatments with extremes in dietary protein, but the effects of the graded reductions in protein were linear and indicate that loss in feed conversion ratio caused by these reduction were not be recovered with the addition of Sangrovit at 20 ppm.

DISCUSSION

The results from Study 1 indicate a feed conversion ratio benefit with the use of sangrovit in broilers as previously shown in swine (Tschirner *et al.*, 2003). It has been largely accepted that sub-therapeutic doses of antimicrobials are beneficial in intensive animal production systems. However, Niewold (2007) recently suggested that expected growth promoter effects of antimicrobials on animals are in fact mediated by anti-inflammatory mechanisms. Previous research studies on QBAs suggested a combination of antimicrobial and anti-inflammatory effects as an explanation for their benefits on animal performance. QBAs have antimicrobial activity (Lenfeld *et al.*, 1981), and

Table 2 -Growth performance of broilers fed diets supplemented with graded increases of Sangrovit[1] from 1 to 35 d (Study 1).

Sangrovit supplementation, ppm	Body Weight, g					Feed Conversion Ratio, Days						Feed Intake, g					
	7	14	21[2]	28	35	1-7	8-14	15-21	22-28	28-35	1-35[3]	1-7	8-14	15-21	22-28	28-35	1-35
0	153	424	817b	1,340	1,807	1.29	1.34	1.51	1.52	1.79	1.55b	105b	364	589	798	838	2,694
12.5	144	423	841ab	1,360	1,828	1.41	1.32	1.41	1.65	1.79	1.56b	140a	368	591	858	831	2,788
25	148	427	846ab	1,362	1,827	1.34	1.38	1.42	1.56	1.80	1.53ab	141a	371	599	819	828	2,758
37.5	144	417	845ab	1,337	1,824	1.40	1.32	1.43	1.55	1.68	1.51a	141a	360	581	794	821	2,697
50	149	437	862a	1,390	1,863	1.31	1.29	1.44	1.57	1.85	1.55b	138a	371	613	851	852	2,825
Mean	147	426	838	1,358	1,830	1.35	1.33	1.44	1.57	1.78	1.54	133	367	594	824	834	2,752
Probability	0.415	0.140	0.030	0.258	0.342	0.324	0.991	0.404	0.994	0.090	0.004	0.001	0.818	0.138	0.205	0.806	0.141
CV, %	6.88	4.08	3.64	3.61	3.24	8.58	4.78	2.71	4.90	5.04	1.76	11.29	5.85	4.13	8.07	6.01	4.56
Contrasts																	
Without Sangrovit	153	424	817	1,340	1,807	1.29	1.34	1.51	1.52	1.79	1.55	105b	364	589	798	838	2,694
With Sangrovit	146	426	843	1,362	1,836	1.36	1.33	1.42	1.58	1.78	1.54	140a	367	596	830	833	2.767
Probability	0.521	0.934	0.074	0.215	0.245	0.214	0.874	0.235	0.294	0.209	0.895	0.0001	0.673	0.2339	0.348	0.516	0.210

a,b - Mean values without a common superscript are different using Tukey' test ($P \leq 0.05$). 1 - At least 1.5% Sanguinarine. 2 - $Y = 0.00074 X + 0.81784$, $p = 0.0213$, $R^2 = 0.13$. 3 - $Y = -0.00003386 X^2 + 0.00222 X + 0.10876$, $p = 0.0010$, $R^2 = 0.42$.

Table 3 - Growth performance of broilers fed diets with graded reduction of crude protein and supplemented with 20 ppm of Sangrovit[1] from 21 to 35 d (Study 2) .

Treatments	Days								
	Body Weight, g			Feed Conversion			Feed Intake, g		
	21	28	35	21-28[2]	28-35	21-35[3]	21-28	28-35	21-35
19.73% CP, without Sangrovit	664	1,181	1,687	1.55a	1.78	1.66a	804	901	1,706
19.73% CP + 20 ppm Sangrovit	660	1,172	1,664	1.52a	1.84	1.68ab	780	904	1,685
19.26 % CP + 20 ppm Sangrovit	660	1,184	1,683	1.56a	1.82	1.69ab	819	910	1,730
18.78 % CP + 20 ppm Sangrovit	664	1,186	1,688	1.57ab	1.80	1.68ab	816	902	1,718
18.32 % CP + 20 ppm Sangrovit	660	1,164	1,660	1.63 b	1.83	1.73b	821	906	1,726
Mean	662	1,178	1,672	1.56	1.82	1.69	808	901	1,710
Probability	0.731	0.475	0.518	0.003	0.478	0.021	0.155	0.964	0.538
CV, %	2.21	1.41	2.37	2.92	3.60	1.97	4.10	2.81	3.17
Contrasts									
Without Sangovit	664	1,181	1,687	1.55	1.78	1.66	804	901	1,706
With Sangrovit	661	1,177	1,674	1.57	1.82	1.70	809	905	1,715
Probability	0.947	0.944	0.908	0.289	0.519	0.183	0.740	0.764	0.745

a,b - Mean values without a common superscript are different using Tukey's test ($P \leq 0.05$). 1 - At least 1.5% Sanguinarine. 2 - $Y = -0.06339 X + 2.77502$, $p = 0.0002$, $R^2 = 0.37$. 3 - $Y = -0.03164 X + 2.29767$, $p = 0.0099$, $R^2 = 0.20$.

minimum inhibitory concentrations of sanguinarine against several bacteria were found in the oral cavity of humans (Dzink & Socransky, 1985). Many of these bacteria belong to species commonly found in the gastrointestinal tract of chickens. Therefore, sanguinarine effects against them are expected by analogy. In parallel, it is possible to speculate that sanguinarine activity as an anti-inflammatory substance may play a role in the partial improvements obtained in the broilers of Study 1. For instance, anti-inflammatory activity has been largely demonstrated with various QBA´s, including sanguinarine (Lenfeld *et al.*, 1981; Agarwal *et al.*, 1991). In pig intestines, this activity is expected to be caused mostly by contact, since the bulk of these alkaloids are excreted in the feces, and only a very small proportion is absorbed (Kosina *et al.*, 2004).

Sanguinarine has been shown to reduce intestinal decarboxylation of aromatic amino acids through the inhibition of L-amino acid decarboxylase in swine (Drsata *et al.*, 1996). The rationale for reducing dietary protein in Study 2 was to investigate a possible similar effect in broilers. The performance obtained with the graded CP reductions appeared to be sustained to the level of 18.78% CP for feed conversion rate and of 18.32% for body weight and feed intake. However, protein retention measurements should be conducted to conclude that the impact of the reduction in protein was counteracted by the supplementation of the additive.

If Niewold's (2007) theory is correct, the search for alternatives to traditional antibiotic growth promoters does not necessarily mean a search for alternative antimicrobials. Sanguinarine, as some of other QBAs, has several interesting characteristics as a feed additive for broilers. Most of these characteristics support the benefits in body weight and feed conversion ratio observed in this study.

CONCLUSIONS

1. Dietary supplementation with sangrovit significantly improved body weight at 21 days, feed conversion ratio at 35 days, and feed intake at 7 days of age. While body weight was linearly affected by 50 ppm of Sangrovit at 21 days, feed conversion ratio at 35 days was maximized at the supplementation level of 32.8 ppm of Sangrovit.
2. Body weight and feed intake were not affected when broilers were fed diets with graded reductions of crude protein; however, feed conversion ratio was negatively affected when with protein levels of 18.78% and lower.

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