Performance of Broilers Fed Diets Supplemented with Sanguinarine-Like Alkaloids and Organic Acids[1]

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Primary Audience: Nutritionists, Production Personnel, Flock Supervisors

SUMMARY

Considerable controversy still remains on the use of antimicrobials to promote growth in animals raised for human consumption. Therefore, alternative strategies have been actively researched to replace these antimicrobials. This study evaluated the performance of broilers fed Sangrovit (1.5% sanguinarine, a quaternary benzo[*c*]phenanthridine alkaloid extracted from plants), a blend of organic acids (40% lactic, 7% acetic, 5% phosphoric, and 1% butyric) or a combination of Sangrovit and organic acids. One-day-old male broiler chicks (Cobb × Cobb 500) were placed in 44 floor pens, with 35 birds per pen. Birds were fed corn-soybean meal all-vegetable diets without growth promoters or anticoccidials, and were vaccinated for coccidiosis at placement. Four treatments and 11 replications were used. A negative control treatment, Sangrovit 50, 25 ppm (1 to 21 d and 22 to 42 d, respectively) of organic acids (8 kg/ton from 1 to 7 d, 6 kg/ton from 7 to 21 d, 4 kg/ton from 21 to 35 d, 2 kg/ton from 35 to 42 d), or both additives were tested. Body weight, feed intake, feed conversion, and intestinal villus height and crypt depth were evaluated weekly. Birds fed Sangrovit or organic acids had improved BW at 21 d ($P \leq 0.05$), but not thereafter. Cumulative feed conversion was better in birds fed Sangrovit alone or in combination with organic acids ($P \leq 0.05$). No differences were observed in villus height or crypt depth at 7, 14, 21 or 42 d. These results suggest that Sangrovit and the blend of organic acids used in this study were possibly beneficial additives for feeding programs designed without the addition of growth promoters.

Key words: broiler, sanguinarine, organic acid

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DESCRIPTION OF PROBLEM

Considerable controversy still remains on the use of antimicrobials to promote growth in food animal production. Epidemiological events that result in antimicrobial resistance by bacteria are difficult events to study, and several factors, including the overuse or misuse of antimicrobials

[1]The use of trade names or products in this text does not imply approval of that product over similar products that may be available.

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Table 1. Ingredient composition of the experimental diets

Item	Starter, 1–21 d	Grower, 21–35 d	Finisher, 35–42 d
Ingredient			
Corn	57.55	59.34	60.64
Soybean meal, 45% CP	36.35	32.80	30.45
Soybean oil	2.36	4.37	5.64
Dicalcium phosphate	1.74	1.61	1.47
Limestone	0.90	0.83	0.81
Salt	0.33	0.35	0.35
DL-Met	0.27	0.24	0.20
L-Lys	0.20	0.17	0.17
L-Thr	0.07	0.05	0.05
Choline chloride 60%	0.06	0.07	0.05
Premix[1]	0.17	0.17	0.17
Energy and nutrients, % or otherwise stated			
ME, kcal/kg	3,000	3,150	3,200
CP	21.60	20.10	19.10
Digestible Lys	1.15	1.08	1.03
Digestible Met + Cys	0.85	0.78	0.73
Digestible Thr	0.74	0.71	0.67
Ca	0.90	0.83	0.78
Average P	0.44	0.41	0.38
Choline, ppm	1,600	1,600	1,450

[1]Vitamin and micromineral composition per kilogram of feed: vitamin A, 5,000 IU; vitamin D_3, 1,000 IU; vitamin E, 20 mg; vitamin K_3, 0.9 mg; vitamin B_1, 0.6 mg; vitamin B_2, 3 mg; vitamin B_6, 1 mg; pantothenic acid, 7 mg; biotin, 0.04 mg; folic acid, 0.5 mg; niacin, 15 mg; vitamin B_{12}, 6 μg; I, 0.72 mg; Se, 0.28 mg; Cu, 8 mg; Mn, 67.5 mg; Zn, 51 mg; Fe, 64 mg.

in human and veterinary medicine, may intensify the severity of the problem [1]. Interestingly, a recent economic analysis based on a large data set available in the United States suggested that production costs may not increase if growth promoters are not included in the feed [2]. In recent years, growth promoters have been banned in some European countries and have undergone voluntary withdrawal by some producers to attend to specific market demands.

There has been an increase in investigations of alternatives to growth promoters, including plant extracts and organic acids. A frequent difficulty in accepting plant extracts as feed additives is the identification and standardization of their active substances. The modes of action of herbal extracts available on the market are not well understood. Limitations are more apparent for compounds that are mixtures of several extracts, each with a different claimed activity. However, active compounds obtained from plants have been used for a variety of human needs for centuries. Natural compounds extracted from plants, such as the quaternary benzo[*c*]phenanthridine alkaloids (**QBA**) sanguinarine and chelerythrine, are known to have antimicrobial [3–5], antiinflammatory [6, 7], and immune-modulatory [8, 9] effects. Sanguinarine has been incorporated into swine, bovine, and poultry diets to decrease amino acid degradation, increase feed intake, and promote growth [10, 11]. Improvements in protein retention by reducing the intestinal decarboxylation of aromatic amino acids through the inhibition of L-aminoacid decarboxylase [12] and enhancement of feed intake by modulating effects on the Trp-serotonin pathway have also been suggested as part of their a mode of action [13].

Short-chain organic acids have also been added in animal feeds as antimicrobial agents [14, 15]. Their antimicrobial properties are accentuated at low pH, where their dissociated carboxyl groups penetrate the microbial cells and lead to the eventual death of the cells [14–17]. Blends of organic acids having different pKa values have a broader spectrum of action throughout the intestine, where different pH values are encountered when the feed moves toward the large intestine. Organic acids also have energy values that may

Table 2. Outline of the experimental treatments

Treatment	Diet
Control	No additive
Sanguinarine	Sangrovit[1]: 50 ppm from 1 to 21 d and 25 ppm from 22 to 42 d
Organic acid blend[2]	8 kg/ton from 1 to 7 d, 6 kg/ton from 7 to 21 d, 4 kg/ton from 21 to 35 d, 2 kg/ton from 35 to 42 d
Sanguinarine + organic acid blend	Sangrovit as above and organic acid blend at 6 kg/ton from 1 to 7 d, 4.5 kg/ton from 7 to 21 d, 2 kg/ton from 21 to 35 d, 1 kg/ton from 35 to 42 d

[1]At least 1.5% of sanguinarine.
[2]40% lactic acid + 7% acetic acid + 5% phosphoric acid + 1% butyric acid; estimated ME = 3,200 kcal/kg. Product was added over the top on control feeds to a complete batch of 1,000 kg, as recommended by provider.

represent an immediate supply to the enterocyte [18]. Because of their antimicrobial properties, organic acids are also viewed as alternatives to growth promoters. Their supplementation in broiler feeds, however, has shown conflicting results, in part because of the different organic acids, doses, microbial challenges, or evaluated responses that have been used in the published experiments [19–25].

Our experiment was aimed at determining the live performance of broilers fed corn-soybean meal all-vegetable diets supplemented with sanguinarine, organic acid blends, or both. Body weight, feed intake, feed conversion, and the evaluation of villus height and crypt depth were measured in experimental broiler chickens raised to 42 d.

MATERIALS AND METHODS

General Husbandry Practices

One-day-old Cobb × Cobb 500 male chicks [26] (1,540 total; average BW = 49 g), were obtained from a commercial hatchery. Birds were randomly placed in 44 floor pens (35 per pen) measuring 1.50 × 1.75 m each, in a broiler house with a concrete floor. Recycled litter after 3 batches of chickens was used. A 24-h lighting program was used in the first week and an 18-h program was used thereafter. Birds were vaccinated for Marek's disease and infectious bronchitis at the hatchery, and for coccidiosis (Paracox-5) [27] at placement. Management practices were similar to those in commercial broiler rearing.

Diets

Corn-soybean meal all-vegetable mash diets without growth promoters were provided throughout the experiment. Feed and water were offered ad libitum by 1 tubular feeder and 1 bell drinker per pen. Feeds were provided as starter diet from placement to 21 d, grower diet to 35 d, and finisher diet to 42 d. Control diets were formulated according to Rostagno [28] and are presented in Table 1. Treatments included the following 4 feeding programs: feeds without additive, feeds supplemented with sanguinarine, feeds supplemented with a blend of organic acids, and feeds supplemented with both additives. Sanguinarine was provided in its commercially available form in Brazil as Sangrovit (1.5% sanguinarine) [29], as was the organic acid blend (Premium Lac [30]; 40% lactic acid + 7% acetic acid + 5% phosphoric acid + 1% butyric acid; ME = 3,200 kcal/kg). The organic acid blend was added on top of the other ingredients; therefore, its energy value was not considered in feed formulation. A description of the treatments is presented in Table 2. Each treatment had 11 replications.

Attributes Measured

Body weight per pen was evaluated at 0 (placement), 7, 14, 21, 35, and 42 d. Feed intake and feed conversion were determined and corrected by using the weight of dead birds. Mortality was recorded daily. At 7, 14, and 21 d, 1 bird was randomly selected from each pen, euthanized, and sampled for villus height and crypt depth by removing the duodenum and jejunum. At 42 d, 6 birds per pen, with BW close to the pen average, were selected, fasted for 10 h, tagged, and euthanized. Each carcass was bled for 3 min, defeathered, and the viscera manually removed. Carcasses were chilled in slush ice for 3 h. A team of deboners from a commercial integrator processed the carcasses to collect the breast fillets (pectoralis major plus pectoralis minor muscles), wings, thighs, drumsticks, cage, and abdominal fat. Cuts were expressed as percentage of carcass weight.

Statistical Analysis

Data were analyzed by using the ANOVA procedure of SAS [31]. Tukey's test was used to assess differences between means, and values of $P < 0.05$ were considered statistically significant. Mortality data were analyzed after arc sin transformation.

RESULTS AND DISCUSSION

The effects of dietary treatments on BW and feed conversion are presented in Table 3. Comparisons of the control treatment with sanguinarine- and organic acid-supplemented treatments show improvements in BW at 7 and 14 d when they were added together. However, improvements in BW at 21 d were observed only when the additives were added individually. Differences in BW between treatments disappeared after 21 d. Feed conversion corrected for the weight of dead birds was also affected by the treatments, with weekly improvements attributable to organic acids from 1 to 3 wk and to sanguinarine in the second week. Benefits on cumulative feed conversion from placement to 42 d were observed when sanguinarine was added to the feeds, individually or in combination with organic acids. Cumulative feed conversion was not significantly improved when organic acids were supplemented.

Feed intake was affected only from 22 to 35 d (Table 4). In that period, adding both additives in the same feed resulted in a reduction of feed intake. Because BW gain was not affected in that period, it was possibly due to the observed improvement in feed conversion for that treatment.

Mortality rate was considered normal for the time of year and growth rate of the birds, and it was not affected by the treatments (average = 5.3%). Intestinal morphology measurements as well as the yield of carcass and commercial cuts were not affected by the treatments.

Research with sanguinarine or other QBA have been conducted in many scientific areas. However, to our knowledge, investigations of

Table 3. Growth performance parameters of broilers fed diets supplemented with sanguinarine, organic acids, or both from 1 to 42 d

	Day										
	BW, g					Feed conversion					
Treatment	7	14	21	35	42	1–7	8–14	15–21	22–35	36–42	1–42
Control	190^{b}	520^{b}	907^{b}	2,258	3,092	1.165^{a}	1.299^{a}	1.433^{a}	1.670^{ab}	1.914	1.635^{a}
Sanguinarine	193^{ab}	529^{ab}	929^{a}	2,258	3,133	1.133^{ab}	1.259^{b}	1.408^{ab}	1.680^{a}	1.803	1.597^{b}
Organic acids	193^{ab}	526^{ab}	929^{a}	2,245	3,109	1.113^{b}	1.274^{b}	1.403^{b}	1.678^{ab}	1.839	1.606^{ab}
Sanguinarine + organic acids	196^{a}	530^{a}	926^{ab}	2,275	3,141	1.114^{b}	1.273^{ab}	1.419^{ab}	1.612^{b}	1.834	1.577^{b}
Probability	0.050	0.033	0.017	0.608	0.162	0.003	0.015	0.012	0.050	0.071	0.001
Mean	193	526	922	2,259	3,119	1.131	1.277	1.416	1.660	1.848	1.604
CV, %	2.51	1.63	1.95	2.30	1.76	3.06	2.16	1.53	3.90	5.32	1.98

[a,b]Mean values in a column without a common superscript are different ($P \leq 0.05$).

Table 4. Feed intake of broilers fed diets supplemented with sanguinarine, organic acids, or both from 1 to 42 d

	Feed intake, g					
Treatment	1–7 d	8–14 d	15–21 d	22–35 d	36–42 d	1–42 d
Control	163	429	567	2,253[a]	1,593	4,975
Sanguinarine	163	422	564	2,233[ab]	1,575	4,926
Organic acids	160	423	565	2,209[ab]	1,586	4,916
Sanguinarine + organic acids	163	425	562	2,172[b]	1,584	4,877
Probability	0.180	0.504	0.913	0.033	0.941	0.274
Mean	162	425	565	2,217	1,585	4,923
CV, %	2.2	2.8	3.5	2.9	4.1	2.4

[a,b]Mean values within a column without a common superscript are different ($P \leq 0.05$).

these compounds as feed additives for broiler chickens have never been conducted. Organic acids, on the contrary, have been used in broiler feeds in many ways and with several objectives. Consistency in the results and description on the mode of action of organic acids with broilers has been shadowed by the great variety of acids used and possible combinations in the existing blends.

It has been largely accepted that subtherapeutic doses of antimicrobials are beneficial in intensive systems of farm animals. Antimicrobial effects are usually accepted as the only function of these additives. However, Niewold [32] recently suggested that the effects of growth promoters may be mediated through antiinflammatory mechanisms. This opens a new window of opportunity in the search for additives to promote animal growth. If this is correct, the search for alternatives to growth promoters does not necessarily mean a search for substances with the same mode of action as antimicrobials. Sanguinarine, like some other QBA, has several interesting characteristics as feed additives for broilers. Most of these characteristics serve as support for the benefits in BW and feed conversion observed in this study. For instance, antiinflammatory activity has been demonstrated with various QBA, including sanguinarine [6, 8]. In pig intestines, this activity is mostly expected to be caused by contact because the bulk of these alkaloids are excreted in the feces, with a very small proportion being absorbed [33]. Quaternary benzo[*c*]phenanthridine alkaloids have antimicrobial activity [6], and minimum inhibitory concentrations of sanguinarine have been found for several bacteria present in the oral cavity of humans [34]. Many of these bacteria belong to the genus commonly found in the gastrointestinal tract of chickens. Therefore, sanguinarine effects against them could, by analogy, be expected.

The organic acid blend used in this study was effective in improving BW to 3 wk, with no effect afterward. However, its positive benefits on feed conversion toward the end of the study were dependent on the concomitant presence of sanguinarine. We cannot determine from the current results whether sanguinarine and the organic acid blend have similar modes of action. However, results apparently indicate that no synergism occurred when both additives were supplemented to the same feed. The gross energy of the organic acid blend in this study was 3,200 kcal/kg [18]. Because it was added on top of the other ingredients to make up a fixed amount of feed, the final ME of diets was very similar and not expected to influence energy intake by broilers fed diets supplemented with organic acids. On the basis of previous research, it should therefore be expected that their antimicrobial effects were responsible for the observed benefits.

None of the additives was effective in modifying the measured parameters of intestinal morphology. Villus condition has become a common measurement in supporting the effects of nutrition on gastrointestinal physiology. However, relationships between live performance improvements and villus height or crypt depth measurements many times have failed to show significant correlations, as was the case in the present study.

CONCLUSIONS AND APPLICATIONS

1. Dietary supplementation with sanguinarine or organic acids improved BW at 3 wk of age. However, no benefits were observed thereafter.

2. Cumulative feed conversion from placement to 42 d was improved by the supplementation of sanguinarine and organic acids.
3. Gut morphology and the yield of carcass and commercial cuts were not improved when sanguinarine or organic acids were supplemented to the feeds.

REFERENCES AND NOTES

1. Shea, K. M. 2004. Nontherapeutic use of antimicrobial agents in animal agriculture: Implications for pediatrics. Pediatrics 114:862–868.

2. Graham, J. P., J. J. Boland, and E. Silbergeld. 2007. Growth promoting antibiotics in food animal production: An economic analysis. Public Health Rep. 22:79–87.

3. Eisenberg, A. D., D. A. Young, J. Fan-Hsu, and L. M. Spitz. 1991. Interaction of sanguinarine and zinc on oral streptococci and *Actinomyces* species. Caries Res. 25:185–190.

4. Newton, S. M., C. Lau, S. S. Gurcha, G. S. Besra, and C. W. Wright. 2002. The evaluation of forty-three plant species for in vitro antimycobacterial activities: Isolation of active constituents from *Psoralea corylifolia* and *Sanguinaria canadensis*. J. Ethnopharmacol. 79:57–67.

5. Colombo, M. L., and E. Bosisio. 1996. Pharmacological activities of *Chelidonium majus* L. (*Papaveraceae*). Pharmacol. Res. 33:127–134.

6. Lenfeld, J., M. Kroutil, E. Maršálek, J. Slavik, V. Preininger, and V. Šimánek. 1981. Antiinflammatory activity of quaternary benzophenanthridine alkaloids from *Chelidonium majus*. Planta Med. 43:161–165.

7. Tanaka, T., K. Metori, S. Mineo, M. Hirotani, T. Furuya, and S. Kobayshi. 1993. Inhibitory effects of berberine-type alkaloids on elastase. Planta Med. 59:200–202.

8. Agarwal, S., M. A. Reynolds, S. Pou, D. E. Peterson, J. A. Charon, and J. B. Suzuki. 1991. The effect of sanguinarine on human peripheral blood neutrophil viability and functions. Oral Microbiol. Immunol. 6:51–61.

9. Chaturvedi, M. M., A. Kumar, B. G. Darnay, G. B. N. Chainy, S. Agarwal, and B. B. Aggarwal. 1997. Sanguinarine (pseudochelerythrine) is a potent inhibitor of NF-κB activation, IκBα phosphorylation, and degradation. J. Biol. Chem. 272:30129–30134.

10. Tschirner, K. 2004. Untersuchungen zur Wirksamkeit und zum Nachweis des Pflanzlichen Alkaloids Sanguinarin beim Schwein [Effects and analysis of the phytogenic alkaloid Sanguinarin in pigs]. PhD Thesis. Christian Albrechts Univ. Kiel, Germany.

11. Tschirner, K., Susenbeth, A., and S. Wolffram. 2003. Influence of Sangrovit supplementation on nitrogen balance and feed intake in growing pigs. Page 45 in 9th Symp. Vitamins and Additives in Nutrition of Man and Animal, Jena/Thuringia, Germany. (Abstr.) Friedrich Schiller Univ. Jena, Jena, Germany.

12. Drsata, J., J. Ulrichová, and D. Walterová. 1996. Sanguinarine and chelerythrine as inhibitors of aromatic amino acid decarboxylase. J. Enzyme Inhibit. 10:231–237.

13. Mellor, S. 2001. Natural appetizers from plants. Feed Mix 9:29–31.

14. Cherrington, C. A., M. Hinton, and I. Chopra. 1991. Organic acids: Chemistry, antibacterial activity and practical applications. Adv. Microb. Physiol. 32:87–108.

15. Dibner, J. J., and P. Buttin. 2002. Use of organic acids as a model to study the impact of gut microflora on nutrition and metabolism. J. Appl. Poult. Res. 11:453–463.

16. Russell, J. B. 1992. Another explanation for the toxicity of fermentation acids at low pH: Anion accumulation versus uncoupling. J. Appl. Bacteriol. 73:363–370.

17. Roth, F. X. 2000. Ácidos orgánicos en nutrición porcina: Eficacia y modo de acción. Pages 169–181 in XVI Curso de Especialización FEDNA: Avances in Nutrición y Alimentación Animal. Fund. Esp. Desarro. Nutr. Anim., Barcelona, Spain.

18. Eidelsburguer, U. 2001. Feeding short-chain fatty acids to pigs. Pages 107–121 in Recent Developments in Pig Nutrition 3. P. C. Garnsworthy and J. Wiseman, ed. Nottingham Univ. Press, Nottingham, UK.

19. Patten, J. D., and P. W. Waldroup. 1988. Use of organic acids in broiler diets. Poult. Sci. 67:1178–1182.

20. Snow, J. L., D. H. Baker, and C. M. Parsons. 2004. Phytase, citric acid, and 1-α-hydroxycholecalciferol improve phytate phosphorus utilization in chicks fed a corn-soybean meal diet. Poult. Sci. 83:1187–1192.

21. Rafacz-Livingston, K. A., C. M. Parsons, and R. A. Jungkt. 2005. The effects of various organic acids on phytate phosphorus utilization in chicks. Poult. Sci. 84:1356–1362.

22. Rafacz-Livingston, K. A., C. Martinez-Amezcua, C. M. Parsons, D. H. Baker, and J. Snow. 2005. Citric acid improves phytate phosphorus utilization in crossbred and commercial broiler chickens. Poult. Sci. 84:1370–1375.

23. Izat, A. L., N. M. Tidwell, R. A. Thomas, M. A. Reiber, M. H. Adams, M. Colberg, and P. W. Waldroup. 1990. Effects of buffered propionic acid in diets on the performance of broiler chickens and on microflora of the intestine and carcass. Poult. Sci. 69:818–826.

24. Izat, A. L., M. H. Adams, M. C. Cabel, M. Colberg, M. A. Reiber, J. T. Skinner, and P. W. Waldroup. 1990. Effects of formic acid or calcium formate in feed on performance and microbial characteristics of broilers. Poult. Sci. 69:1876–1882.

25. Leeson, S., H. Namkung, M. Antongiovanni, and E. H. Lee. 2005. Effect of butyric acid on the performance and carcass yield of broiler chickens. Poult. Sci. 84:1418–1422.

26. Cooperativa Languiru, Teutonia, Rio Grande do Sul, Brazil.

27. Schering-Plough Produtos Farmacêuticos Ltda, São Paulo, São Paulo, Brazil.

28. Rostagno, H. S. 2005. Tabelas Brasileiras para Aves e Suínos. Composição de Alimentos e Exigências Nutricionais. 2nd ed. UFV, Viçosa, Minas Gerais, Brazil.

29. Phytobiotics Futterzusatzstoffe GmbH, Eltville, Germany.

30. Nutriacid, Paulínia, São Paulo, Brazil.

31. SAS Institute. 2001. SAS/STAT User's Guide: Release 8.0. SAS Inst. Inc., Cary, NC.

32. Niewold, T. A. 2007. The nonantibiotic anti-inflammatory effect of antimicrobial growth promoters, the real mode of action? A hypothesis. Poult. Sci. 86:605–609.

33. Kosina, P., D. Walterová, J. Ulrichová, V. Lichnovsky, M. Stiborova, H. Rýdlová, J. Vicar, V. Krecman, M. J. Brabec, and V. Simánek. 2004. Sanguinarine and chelerythrine: Assessment of safety on pigs in ninety days feeding experiment. Food Chem. Toxicol. 42:85–91.

34. Dzink, J. L., and S. S. Socransky. 1985. Comparative in vitro activity of sanguinarine against oral microbial isolates. Antimicrob. Agents Chemother. 27:663–665.