Effect of hops β-acids (*Humulus lupulus*) on performance and intestinal health of broiler chickens

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Primary Audience: Feed Manufacturers, Nutritionists, Researchers, Veterinarians

SUMMARY

To evaluate a novel feed additive, hops β-acids, a pen trial using 1,440 one-day-old broiler chickens, in a randomized design with 6 treatments and 6 replicates of 40 birds/pen, was conducted. The experimental treatments were a negative control (NC) basal diet, a positive control (PC) basal diet supplemented with zinc 30 mg/kg of bacitracin, and 4 additional treatments consisting of the basal diet supplemented with 30, 60, 120, and 240 mg/kg of hops β-acids in a microencapsulated form. All birds were vaccinated against coccidiosis by eye drops. Productive performance, jejunum morphology, and *Clostridium* spp. count in jejunum and cecum contents were evaluated. At 21 d of age, the birds fed the PC, 30, or 60 mg/kg of β-acids had the same weight gain of those fed the NC, whereas the highest level of β-acids decreased the gain. In addition, feed intake was decreased and FCR was improved in all treatments when compared with the NC. At 42 d, compared with the NC, the treatments containing 30 or 240 mg/kg of β-acids and the PC resulted in improved FCR. No differences were found for other performance variables. No difference was observed among the treatments on villus length, crypt depth, and villus-to-crypt ratio. Except for one sample, all others had negative results for *Clostridium* spp. based on the methodology of sulphite reduction. In this study, we demonstrated positive effects of supplementation of hops β-acids on productive performance of broilers, and the best results were obtained with addition of 30 mg/kg.

Key words: β-acids, hops, *Humulus lupulus*, phytogenics

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DESCRIPTION OF PROBLEM

Phytogenic additives are volatile substances, generally lipophilic [1], mainly terpenes hydrocarbons, simple alcohols, ketones, phenols, esters, and organic acids, in which a pharmacologically active compound is predominant. They have considerable variation in chemical composition, depending on weather conditions, harvesting stage, location, or storage conditions [2].

Some compounds of hops (*Humulus lupulus*) have an antimicrobial effect, mainly against gram-positive and -negative bacteria, fungi, and yeast [3], and they have potential to be used as feed additives to broilers. The hops and the extract of its flowers are used in beer manufacturing to give flavor and taste, but these products were initially used due their effects against lactic acid bacteria during the production of beer [4].

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The main substances found in the hops are α- and β-acids. Other associated compounds are present naturally or are formed during the drying and storage of the plant [5]. The main components of α-acids are humulon and isohumulon, responsible for the bitter taste of beer. The β-acids have less bitter taste and provide greater antimicrobial activity, due to their hydrophobic nature, and lupulon is the most important component of this fraction [6].

Little information exists about the efficacy of hops β-acids as a dietary supplement on animal performance, but some references in the literature indicate that some compounds of hops can replace antimicrobial performance enhancers in the diets of broilers [7, 8]. Cornelison et al. [7] compared the effect of penicillin and hops (equivalent to 23 mg/kg of β-acids) and found that BW was similar for both treatments at 42 d of age. Later, Bozkurt et al. [8] obtained a higher BW at 21 d of age when broilers were fed hops extract compared with birds fed diet containing avilamycin. The objective of the current work was to evaluate the effect of increasing levels of hops β-acids in the diet of broilers on performance, intestinal morphology, and counts of *Clostridium* spp. in the jejunum and ceca of the birds.

MATERIALS AND METHODS

Birds, Housing, and Feeding

All the procedures used in this experiment were approved by the institutional animal care and use committee of the Luiz de Queiroz College of Agriculture , University of Sao Paulo. One-day-old male Cobb 500 broiler chicks (1,440 birds, initial BW of 42.3 ± 0.4 g) were used in the experiment. Chicks were weighed by pen for equal weight distribution and placed into 36 pens (40 birds/pen and 6 replicates/treatment), from 1 to 42 d, in a completely randomized design. The nutritional program consisted of 4 diets: prestarter (1–7 d), starter (7–21 d), grower (21–35 d), and finisher (35–42 d). For each phase a basal diet was formulated with corn, soybean meal, and the inclusion of 5% poultry by-products meal and 5% wheat bran. The latter 2 ingredients were used with the purpose of imposing a challenge to the intestinal health, because the poultry by-products meal may serve as a source of microorganisms and the wheat bran is known to increase the viscosity inside the gastrointestinal tract [9]. Ingredient composition and nutritional specifications followed the Brazilian Tables [10] (Table 1). Chickens had *ad libitum* access to water and feed in mash form during the entire experimental period.

Experimental Treatments and Vaccination

The experimental treatments were a negative control (**NC**) basal diet; a positive control (**PC**) basal diet supplemented with zinc bacitracin at 30 mg/kg; and a basal diet supplemented with 30, 60, 120, and 240 mg/kg of hops β-acids in a microencapsulated form. The microencapsulated product used was prepared to contain 30% of hops β-acids [11]. The concentrations of β-acids in the mixed feeds were determined in samples collected in the chicken feeders at the moment they were distributed (4 d after feed mixture); the samples were frozen until analyses, and the concentrations of β-acids are shown in Table 2. The antimicrobial used as a positive control was a commercial product containing 15% zinc bacitracin. Both additives were used to replace the Kaolin present in the basal diet.

As an additional challenge to the intestinal health, at 7 d of age, all birds were vaccinated against coccidiosis [9, 12] using a commercial product [13] with sporulated oocysts of *Eimeria acervulina*, *Eimeria brunetti*, *Eimeria maxima*, *Eimeria necatrix*, *Eimeria praecox*, *Eimeria tenella*, and *Eimeria mitis* by eye drops according to the manufacturer's instruction. This vaccination was applied to promote immune stress to the chickens, cause damage to the intestinal epithelium, and facilitate the proliferation of *Clostridium* spp. in the gastrointestinal tract.

Live Performance, Intestinal Morphology, and Clostridium Count

At 7, 21, 35, and 42 d of age, the chickens were weighted by pen to calculate BW, BW gain (BWG), feed intake, and FCR. At 42 d, the productive efficiency index (PEI), using the equation [14]

$$\text{PEI} = [\text{daily BWG (g)} \times \text{variability (\%)}] \div \text{FCR} \times 10.$$

Table 1. Composition and calculated contents of the experimental diets (as-fed basis)

Item (%, unless otherwise noted)	Prestarter	Starter	Grower	Finisher
Ingredient				
Corn	50.90	56.50	60.00	62.95
Soybean meal	32.87	28.00	24.12	21.42
Wheat bran	5.0	5.0	5.0	5.0
Poultry by-product meal	5.0	5.0	5.0	5.0
Soybean oil	2.29	2.01	2.84	2.98
Dicalcium phosphate	1.61	1.23	1.00	0.78
Limestone	0.79	0.80	0.74	0.64
Salt	0.47	0.44	0.42	0.40
MHA[1]	0.450	0.365	0.330	0.290
L-Lys HCl	0.322	0.294	0.290	0.285
L-Thr	0.071	0.042	0.031	0.021
Vitamin premix[2]	0.100	0.100	0.080	0.060
Mineral premix[3]	0.05	0.05	0.05	0.05
Choline chloride (60%)	0.08	0.08	0.06	0.04
Kaolin	0.08	0.08	0.08	0.08
Total (kg)	100	100	100	100
Calculated composition				
ME (kcal/kg)	2,950	3,000	3,100	3,150
CP	23.30	21.50	20.00	19.00
Digestible Lys	1.310	1.174	1.078	1.010
Digestible Met + Cys	0.944	0.846	0.787	0.737
Digestible Met	0.620	0.542	0.501	0.463
Digestible Thr	0.852	0.763	0.701	0.656
Ca	0.920	0.819	0.732	0.638
Available P	0.470	0.391	0.342	0.298
Na	0.220	0.210	0.200	0.195

[1]Methionine hydroxy analogue.

[2]Provided per kilogram of feed: vitamin A, 9,000,000 IU; vitamin D_3, 2,500,000 IU; vitamin E, 20,000 IU; vitamin K_3, 2,500 mg; vitamin B_1, 2,000 mg; vitamin B_2, 6,000 mg; vitamin B_6, 3,000 mg; vitamin B_{12}, 15,000 µg; niacin, 35 g; pantothenate, 12 g; biotin, 100 mg; folic acid, 1,500 mg; selenium, 250 mg.

[3]Provided per kilogram of feed: Mn, 160,000 mg; Fe, 100,000 mg; Zn, 100,000 mg; Cu, 20,000 mg; Co, 2,000 mg; I, 2,000 mg.

At 42 d of age, 1 broiler per replicate was euthanized by cervical dislocation and the jejunum was collected to determine villus height and crypt depth and calculate villus-to-crypt ratio. Measurements of villus and crypt were performed in 20 intact villi and crypts from 6 chickens per treatment. In addition, the digesta from jejunum and ceca from the birds of each treatment were collected to obtain one pool for each segment. The *Clostridium* spp. counts in these pools were obtained using the methodology proposed by Da Silva et al. [15]. This methodology allows the counts of *Clostridium* spp. that first proliferate in a medium incubated in anaerobic conditions and cause reduction of sulphite to sulfide. Further tests are used to confirm the presence of *Clostridium perfringens*.

Statistical Analysis

Data were analyzed by ANOVA with procedures appropriate for a completely randomized design using the GLM procedure of SAS [16].

Table 2. Concentration of hops β-acids in the diets

Treatment (mg/kg)	Experimental phase		
	Prestarter	Starter	Grower
Negative control	ND[1]	ND	ND
Positive control	NA[2]	NA	NA
30 mg/kg of β-acids	22.8	23.4	22.2
60 mg/kg of β-acids	63.1	51.3	54.5
120 mg/kg of β-acids	114	122	111
240 mg/kg of β-acids	212	224	216

[1]ND = not detected.

[2]NA = not analyzed.

The means for treatments showing significant differences ($P \leq 0.05$) were compared using Duncan's test.

RESULTS AND DISCUSSION

Productive Performance

No effect of treatments from 1 to 7 d of age was observed for any variable (Table 3). However, a trend was observed ($P = 0.06$) for increased feed intake in the treatment with 30 mg/kg of β-acids and lower feed intake with the higher levels (120 and 240 mg/kg) of hops β-acids. The results of performance at 21 d of age are shown in Table 3. We observed an effect of treatments in all variables analyzed ($P < 0.05$), except for viability. The PC, 30, and 60 mg/kg of hops β-acids treatments did not affect the BW and BW gain, but 240 mg/kg of hops β-acids caused a reduction in these variables compared with the NC. The feed intake was reduced and the FCR was improved for all treatments when compared with the NC. In addition, it was observed that the highest level of hops β-acids (240 mg/kg) resulted in the greatest reduction of feed intake; this trend was noticed as early as the first week of the trial. The best FCR was obtained with zinc bacitracin (1.472), whereas the β-acid treatments resulted in intermediate values and the NC had the worst FCR (1.580).

In contrast with our results, Barreto et al. [17] did not find differences in the performance of broilers at 21 d of age fed diets supplemented with different plant extracts (oregano extract, garlic, cinnamon, and red pepper). Conversely, Bozkurt et al. [8] obtained better BW and FCR in broilers fed diets containing hops when compared with those fed avilamycin. Furthermore, Rizzo et al. [18] observed better FCR at 21 d of age in broilers fed plant extracts (thyme, cinnamon, and pepper) when compared with broilers fed an antibiotic growth promoter (avilamycin).

In studies designed to evaluate potential substitutes for antimicrobials in the feed, it is accepted that the proliferation of enteric pathogens must be stimulated. It has been recognized that *Eimeria* infection, withdrawal of coccidiostat, immunological stress, and the nature and form of diet could misbalance the microbiota, mainly in the initial phase [9]. For this reason, we included 5% of an animal-derived ingredient and 5% of wheat bran in the diets and vaccinated the chicks against *Eimeria* spp. as measures to induce greater sanitary challenge to the birds. Our results indicate that the challenge imposed was successful because, among all treatments, the birds on the NC treatment showed the worst FCR, which can be a consequence of misbalance in the intestinal microbiota. It is known that diets with low digestibility increase the substrate available to bacteria in the gastrointestinal tract [19].

The results of performance of the chickens at 42 d of age are presented on Table 3. Compared with the NC, the treatments containing 30 or 240 mg/kg of β-acids resulted in improved FCR; this improvement was similar to that obtained with the PC treatment. Although the FCR was improved, the productive efficiency index at 42 d of age was not affected by treatments.

It has been recognized that a higher standard of poultry production has a strict relationship with the intestinal microbiota and its association with the host. These interactions improve health, mainly the integrity of the gut [20]. The first line of defense against pathogens is the normal intestinal microbiota. Many bacteria produce organic acids, such as lactic, propionic, and butyric acid, as well as bacteriocins that have effect against gram-positive and -negative bacteria [21]. The manipulation of the microbiota can be obtained by the inclusion of antibiotics or other additives because the main objective is to alter the intestinal ecology to improve broiler performance, especially in the initial phase, when the birds are more susceptible to infection [20].

As we observed, both feed additives, zinc bacitracin and β-acids, were able to improve FCR at 21 and 42 d of age in the chickens when compared with unsupplemented birds. In recent literature [22] it has been reported that common in-feed performance enhancers, including essential oils of medicinal herbs, can potentially replace antimicrobials in broiler nutrition. According to those authors, scientific evidence has shown that these additives can be used to improve the intestinal health of broilers, mainly when different types of challenge are present, to avoid subclinical diseases. When enteric disease occurs in broilers, significant reductions in performance are observed and, with the restric-

Table 3. Productive performance of broilers at 7, 21, and 42 d and jejunum morphology at 42 d of age

Item[1]	Treatment						*P*-value	SEM
	Negative control	Positive control	30 mg/kg of β-acid	60 mg/kg of β-acid	120 mg/kg of β-acid	240 mg/kg of β-acid		
1–7 d								
BW (g)	191	192	191	192	189	189	NS	1.49
BWG (g)	149	150	148	150	146	150	NS	1.48
FI (g)	169	169	171	167	165	165	0.06	1.78
FCR	1.133	1.126	1.153	1.113	1.126	1.124	NS	0.02
Viability (%)	99.6	100	99	100	100	99.6	NS	0.38
1–21 d								
BW (g)	944[a]	958[a]	950[a]	959[a]	928[ab]	904[b]	0.01	9.41
BWG (g)	905[a]	916[a]	910[a]	922[a]	893[ab]	866[b]	0.02	9.69
FI (g)	1,430[a]	1,348[bc]	1,364[b]	1,373[b]	1,353[bc]	1,312[c]	0.001	12.88
FCR	1.580[a]	1.472[c]	1.498[bc]	1.490[bc]	1.514[b]	1.514[b]	<0.0001	8.74
Viability (%)	96.2	97.0	97.5	97.5	96.6	95.0	NS	0.95
1–42 d								
BW (g)	3,027	3,045	3,087	3,053	3,000	3,063	NS	20.51
BWG (g)	2,988	3,002	3,047	3,016	2,966	3,026	NS	20.89
FI (g)	5,344	5,236	5,296	5,310	5,244	5,273	NS	35.83
FCR	1.788[a]	1.745[b]	1.738[b]	1.761[ab]	1.768[ab]	1.742[b]	0.03	0.009
Viability (%)	89.1	91.0	92.0	89.6	90.0	86.0	NS	1.64
PEI	354	373	384	366	359	357	NS	7.71
Jejunum morphology at 42 d of age								
Villus height (μm)	904	952	1,078	981	955	961	NS	37.47
Crypt depth (μm)	82.8	84.7	77.9	97.9	91.3	87.0	NS	5.68
Villus:crypt	11.3	11.4	14.1	10.3	10.5	11.4	NS	0.84

[a–c]Means within a rows with no common superscripts are significantly different ($P \leq 0.05$).

[1]BWG = BW gain; FI = feed intake; PEI = productive efficiency index.

tion of use of antibiotic growth promoters, it has been more pronounced [23].

Cornelison et al. [7] found that, at 35 d of age, the performance of broilers decreased with the inclusion of increasing levels of hops (equivalent to approximately 23, 46, 69, and 93 mg/kg of β-acids) when compared with penicillin-fed birds. However, at 42 d, the lower level of hops (equivalent to 23 mg/kg of β-acids) resulted in BW similar to that obtained with the antibiotic. In a recent study with rabbits [4] the feed intake was lower and the FE was improved when the animals were fed hops extract. Although the potential of this additive has been shown in other studies, our study was the first to evaluate the increasing levels of β-acids in microencapsulated form in the diets to broilers. Different and sometimes conflicting results found in published work involving β-acids may have occurred because the previous studies used hops extracts that contain more substances than the purified β-acids used in the present trial; furthermore, the purified β-acids used in our study were microencapsulated to protect them from volatilization.

Intestinal Morphology and Clostridium Count

Jejunum morphology was not affected by treatments (Table 3). Interestingly, the greatest villus-to-crypt ratio was observed in the 30 mg/kg of hops β-acids treatment, which also had the best performance. Fernandes et al. [12] found lower villus height at 7 d of age in chickens followed vaccination against coccidiosis at 1 d of age. Therefore, the injured mucosa influences the metabolism and the production of broilers, causing decreases in growth, mainly of skeletal muscle [24].

After a European Union ban on the use of antibiotics as growth promoters to broilers, an increase in the use of ionophores to control the proliferation of *Eimeria* and some strains of gram-positive bacteria was observed [25]. However, as a feed additive, the use of these compounds has been questioned and, for this reason, identifying alternatives to inhibit the growth of some bacteria, mainly *Clostridium perfringens*, and the production of its toxins is a concern. In our study, except for one sample, we found negative results for *Clostridium* spp. based on the methodology of sulphite reduction. The positive sample, from the ceca of chickens receiving 120 mg/kg of hops β-acids, was further identified as *Clostridium ramosum* with 4×10^1 cfu/g. Siragusa et al. [6] found that hops extract in water was able to decrease *Clostridium perfringens* in the jejunum and ceca of broilers. In addition, Tillman et al. [26], using real-time PCR, observed lower counts of *Clostridium perfringens* in the small intestine and ceca of broilers fed lupulon, showing the potential for use of this additive.

CONCLUSIONS AND APPLICATIONS

1. In our initial study, based on supplementation of hops β-acids in a microencapsulated form in broiler feed, it was demonstrated that it is possible to use this product to replace antibiotics in the diets because the FCR was improved relative to the unsupplemented chickens and was similar to that achieved with antibiotic in the diet.
2. The best results in performance improvement were observed at 21 and 42 d of age and were obtained with the lower level of β-acids supplementation, 30 mg/kg. Higher levels must be studied in cases of high sanitary challenge to the birds, when this additive could have more pronounced effects.

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