CUPRIC CITRATE AS GROWTH PROMOTER FOR BROILER CHICKENS IN DIFFERENT REARING STAGES

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ABSTRACT: Feeding cupric citrate as alternative to cupric sulfate to broilers has been suggested in the literature. Day-old male broiler chicks (1,200) were used in an experiment to evaluate the efficacy of cupric citrate supplementation (75 mg Cu kg^{-1}) during the 1-21, 22-42 or 1-42 d periods in comparison to an unsupplemented diet and a diet supplemented with cupric sulfate (200 mg Cu kg^{-1}, 1-42 d). A randomized block design was used, with five treatments, six replicates and 40 birds per pen. The diets, based on corn and soybean meal, and water were offered *ad libitum* during the 42-day experimental period. Over the entire period, there was no effect of copper supplementation ($P > 0.05$) on bird live weight, weight gain, feed intake, feed conversion and mortality. Cupric citrate supplementation on the 22-42 d period resulted in worse feed conversion as compared to broilers receiving cupric sulfate (2.014 vs. 1.967, $P < 0.05$). Copper residues in the litter were reduced when broilers were fed cupric citrate, as compared to cupric sulfate ($P < 0.01$). The absence of response to copper supplementation can be attributed to the environmental and sanitary rearing conditions.
Key words: broiler litter, poultry, copper, performance

CITRATO CÚPRICO COMO PROMOTOR DE CRESCIMENTO DE FRANGOS DE CORTE DIFERENTES EM FASES DE CRIAÇÃO

RESUMO: Citrato cúprico foi apontado como alternativa ao sulfato cúprico como promotor de crescimento na dieta de frangos. Este trabalho avaliou a eficácia do citrato cúprico em diferentes fases da criação de frangos de corte. Foram utilizados 1200 pintos machos, em um experimento em blocos casualizados, com cinco tratamentos, seis repetições e 40 aves por parcela. Os tratamentos consistiram de uma dieta não suplementada ou suplementada com citrato cúprico anidro (75 mg Cu kg^{-1}) de 1 a 21 dias, de 22 a 42 dias ou de 1 a 42 dias, ou com sulfato cúprico pentahidratado (200 mg Cu kg^{-1}) de 1 a 42 dias. Foram avaliados o desempenho das aves e o resíduo de cobre na cama. Dietas, à base de milho e farelo de soja, e água foram fornecidas à vontade durante todo o período experimental. Não houve efeito da suplementação de cobre ($P > 0,05$) sobre o peso vivo, ganho de peso, consumo de ração, conversão alimentar e mortalidade mais refugagem. Os frangos que receberam citrato cúprico na ração a partir dos 22 dias tiveram, no período 22-42 dias, conversão alimentar pior que aqueles que receberam sulfato cúprico (2,014 vs. 1,967, $P < 0,05$). O resíduo de cobre na cama das aves que receberam citrato cúprico na dieta foi reduzido em comparação às que receberam sulfato cúprico ($P < 0,01$). O cobre não resultou em efeito promotor de crescimento em função das condições ambientais e sanitárias do experimento.
Palavras-chave: aves, cobre, cama de frango, desempenho

INTRODUCTION

Copper (Cu) is an essential micromineral that can be added to broiler chicken diet at high levels, acting as a growth promoter. Several authors have observed that the addition of levels ranging from 125 to 250 mg Cu kg^{-1}, as copper sulfate or carbonate, resulted in a positive response in body weight and feed conversion efficiency (Fisher et al., 1973; Hoda & Maha, 1995). Cu can be added to the diet under several forms as growth stimulant; however, the most utilized is the sulfate form (Rostagno et al., 1994).

The mechanisms by which Cu promotes performance improvements in poultry and swine are not completely cleared. One of the mechanisms pointed out as responsible for the effects of Cu in relation to performance improvement would be its action over disease-causing microorganisms that, even without displaying clinical signs, might be detrimental to animal growth (Cromwell, 1991). In this respect, there is evidence that antimicrobial agents are usually more efficient as growth promoters in old facilities or buildings with poor sanitary conditions than in new, isolated environments (Hill et al., 1953; Visek, 1978).

Stimulation of the immune system results in metabolic alterations known as immunological stress, which decrease the efficiency of nutrient utilization toward weight gain, possibly because of alterations in the absorption of nutrients or in the metabolic rate. The main mediator of these metabolic modifications is interleukin-1 (Klasing et al., 1987). The degree of sanitation in the environment affects the efficacy of antibiotics in promoting growth, since the ability of these additives in improving poultry performance depends on the extent of the microbial challenge (Roura et al., 1992). It has been demonstrated by those authors that, in a clean environment, weight gain and feed conversion in birds supplemented with antibiotics were similar to those that did not receive supplementation in their diet. However, in a poorly sanitized environment, the increase in growth and feed conversion rates attributed to antibiotics was accompanied by a reduction in circulating interleukin levels, which suggests that antibiotics reduce the immunological stress.

Studies carried out by Pesti & Bakalli (1996) and Ewing et al. (1998) lead to positive results on performance improvement of broiler chicken either with the use of pentahydrate cupric sulfate or anhydrous cupric citrate. However, the latter resulted in bird performance improvement at lower levels (63 or 75 mg Cu kg^{-1}) than cupric sulfate (125 or 250 mg Cu kg^{-1}). Lower levels of Cu residues in the litter were also observed when cupric citrate was utilized, in addition to a more pronounced growth promoting effect at growing-finishing rearing stages as compared to the initial stages.

There is currently great concern with respect to the use of high Cu levels in broiler chicken feed, because of the residues of this mineral in the litter, which might compromise its commercial value. The value of litter as a source of nutrients for animal feeding and for fertilization has been long acknowledged, and several authors observed that the addition of Cu sources to broiler chicken feed yields excreta that are potentially toxic to cattle, and to sheep in particular (Ryssen et al., 1977; Banton et al., 1987).

The present study was motivated by the fact that in experiments by Pesti & Bakalli (1996), an effect of cupric citrate on the performance of broiler chicken was found for the period from 1-42 days, but not in the period from 1-21 days. Therefore, the growth promoter effect was manifested in the age period between 22-42 days. These results are contrary to those observed by other authors who have studied the action of antimicrobial agents at different rearing stages and verified greater growth stimulation in birds fed diets containing antibiotics during the first four weeks of life, with differences disappearing by the end of the experimental period (Fernandez et al., 1973; Marusich et al., 1973; Zuanon, 1995).

The objective of this study was to evaluate cupric citrate effects on the performance of broiler chicken when supplemented at rearing stages from 1 to 21 days, 22 to 42 days and from 1 to 42 days, as compared to cupric sulfate supplementation, and to determine Cu concentration in broiler chicken litter under these treatments.

MATERIAL AND METHODS

The experiment was carried out utilizing 1,200 day-old male chicks, line AgRoss, during the period from 1 to 42 days of age. The chicks were divided into homogeneous, 40-bird groups, arranged in thirty, 5.0-m^2 pens with concrete floor over a 4.0-cm rice hulls bedding. Birds were maintained under constant lighting and received water and feed without restriction.

The base diets utilized during the experimental period (Table 1) were divided into three types, according to the nutritional requirements of the rearing stages: starter (1 to 21 days), grower (22 to 35 days) and finisher (36 to 42 days), and were formulated based on corn and soybean meal, following recommendations by Rostagno et al. (1994).

Table 1 - Percentage composition and calculated values for base diets.

	Feed		
Ingredient	Starter	Grower	Finisher
Corn	57.32	62.45	64.82
Soybean meal	36.53	32.05	29.43
Soybean Oil	2.30	2.05	2.39
Dicalcium Phosphate	1.95	1.74	1.75
Limestone	1.12	1.02	1.04
DL-Methionine 99%	0.16	0.12	0.11
Common salt	0.30	0.30	0.30
Choline Chloride 60%	0.08	0.04	0.02
Vitamin Supplement[a]	0.12	0.10	0.08
Mineral Supplement[b]	0.06	0.06	0.06
Anticoccidial Agent	0.05[c]	0.06[d]	-
Calculated Composition			
Crude Protein, %	21.6	20.0	19.0
Methionine, %	0.49	0.44	0.41
Meth + Cystine, %	0.85	0.77	0.73
Lysine, %	1.10	0.99	0.93
Calcium, %	1.00	0.90	0.90
Available Phosphorus, %	0.45	0.41	0.41
Metabolizable Energy, kcal kg^{-1}	3,000	3,050	3,100

[a]Each kg of the vitamin premix contains: vit. A 10,000,000 UI; vit. D_3 2,000,000 UI; vit. E 30,000 mg; vit. K_3 3,000 mg; vit. B_1 2,000 mg; vit. B_2 6,000 mg; vit. B_6 4,000 mg; vit. B_{12} 15,000 mcg; folic acid 1,000 mg; pantothenic acid 12,000 mg; nicotinic acid 50,000 mg:
[b]Each kg of the mineral premix contains: Mn 16,000 mg; Cu 20,000 mg; Zn 100,000 mg; Fe 100,000 mg; I 2,000 mg; Se 250 mg.
[c]Anticoccidial utilized: maduramicin 0.75% + nicarbazin 8.0%.
[d]Anticoccidial utilized: lasalocid sodium 15%.

At the end of the experiment, litter samples were collected from all pens for Cu residue analysis by atomic absorption spectrophotometry; a sample of the rice hulls utilized as bedding substrate was also analyzed. During the experimental phase, no moist portions of the litter were removed, and a fine layer of dry bedding was added under and around the water troughs as needed. Water samples were collected from the troughs for Cu residue analysis, and only traces of Cu were detected (less than 0.05 mg kg^{-1}).

The experimental design was set up as randomized blocks, with five treatments, six replicates and 40 birds per pen. The replicates corresponded to blocks, which were created based on insolation and temperature differences in the facilities.

Supplementations of 75 mg Cu kg^{-1} in the form of anhydrous cupric citrate ($C_6H_4Cu_2O_7$) in the diet were tested as a growth promoter, at different rearing stages. Therefore, treatments corresponded to a base diet not supplemented with any antimicrobial agent (Control), base diet + cupric citrate from 1 to 21 days, followed by the base diet (Citrate 1-21), base diet + cupric citrate from 22 to 42 days, preceded by the base diet (Citrate 22-42), base diet + cupric citrate from 1 to 42 days (Citrate 1-42) and base diet + 200 mg Cu kg^{-1} in the form of cupric sulfate pentahydrate from 1 to 42 days (Sulfate). Cu concentrations in the products were 37.4% for the cupric citrate and 25.0% for the cupric sulfate.

The variables analyzed over 1 to 21 days, 22 to 42 days and from 1 to 42 days were: weight gain (WG), feed intake (FI), feed conversion efficiency (kg of feed consumed per kg weight gain, FC), mortality + culling (MORT) and Cu residues in litter (RES). Live weight (LW) at 21 and 42 days was also analyzed. In all periods, MORT data were transformed according to arc sin $(\%/100)^{1/2}$. Data were analyzed with the GLM SAS (1990) procedure and means compared by the Tukey test (P = 0.05).

RESULTS AND DISCUSSION

There was no difference ($P > 0.05$) between treatment means for LW at 21 days and WG, FI, FC and MORT during the period of 1 to 21 days (Table 2). There was no difference ($P > 0.05$) between treatment means for WG, FI, and MORT during the period of 22 to 42 days (Table 3). However, when anhydrous cupric citrate was utilized during the period from 22 to 42 days in the diet of birds that had not received any supplementation previously (Citrate 22-42 treatment), FC was poorer than for birds of the Sulfate treatment ($P < 0.05$). There was no difference ($P > 0.05$) between treatments for LW at 42 days and for WG, FI, FC and MORT from 1 to 42 days, i.e., none of the Cu sources provided a growth promoting effect (Table 4).

Even though some authors (Fisher et al., 1973; Hoda & Maha, 1995) verified that adding high levels of several Cu sources to the diet (from 125 to 160 mg kg^{-1}) of broiler chicken improved performance, in a different paper (Poupoulis & Jensen, 1976) the utilization of 250 mg Cu kg^{-1} in the broiler chicken diet did not improve growth and feed conversion, and there was no difference in the response to Cu supplementation in the initial or final rearing periods.

Table 2 - Effects of dietary cupric citrate and cupric sulfate pentahydrate on performance of broiler chicken at age between 1 and 21 days.

Treatment	LW 21 d	WG	FI	FC	MORT
	----------- kg	-----------		kg kg^{-1}	%
Control	0.77	0.73	1.16	1.591	1.30
Citrate 1-21	0.79	0.75	1.16	1.552	0
Citrate 22-42	0.78	0.74	1.13	1.530	0.40
Citrate 1-42	0.76	0.72	1.12	1.570	0.40
Sulfate	0.77	0.72	1.14	1.579	0.80
CV (%)	2.30	2.40	2.00	3.60	1.60

LW = live weight; WG = weight gain; FI= feed intake; FC = feed conversion; MORT = mortality + culling

Table 3 - Effects of cupric dietary citrate and cupric sulfate pentahydrate on performance of broiler chicken at age between 22 and 42 days.

Treatment	WG	FI	FC	MORT
	------- kg	-------	kg kg^{-1}	%
Control	1.65	3.25	1.969 ab	1.60
Citrate 1-21	1.65	3.32	2.011 ab	0.80
Citrate 22-42	1.63	3.29	2.014 a	0.40
Citrate 1-42	1.68	3.31	1.973 ab	0.80
Sulfate	1.65	3.25	1.967 b	1.70
CV (%)	2.00	2.00	1.30	2.40

[a, b]Values in the same column, followed by different letters, differ by Tukey test ($P < 0.05$).
LW = live weight; WG = weight gain; FI= feed intake; FC = feed conversion; MORT = mortality + culling

Table 4 - Effects of dietary cupric citrate and cupric sulfate pentahydrate on performance of broiler chicken at age between 1 and 42 days.

Treatment	LW 42 d	WG	FI	FC	MORT
	---------- kg	----------		kg kg^{-1}	%
Control	2.43	2.38	4.42	1.852	2.90
Citrate 1-21	2.44	2.40	4.48	1.868	0.80
Citrate 22-42	2.42	2.38	4.43	1.863	0.80
Citrate 1-42	2.44	2.39	4.44	1.853	1.20
Sulfate	2.42	2.38	4.39	1.849	2.50
C.V. (%)	1.80	1.80	1.40	1.40	2.70

LW = live weight; WG = weight gain; FI= feed intake; FC = feed conversion; MORT = mortality + culling

Pesti & Bakalli (1996) confirmed the growth promoting effect of Cu in broiler chicken feed, and observed that cupric citrate was more efficient, at lower Cu levels, than cupric sulfate. The present work utilized the same cupric citrate (75 mg Cu kg^{-1}) and cupric sulfate (200 mg Cu kg^{-1}) levels for which the above authors obtained the best growth responses in their studies. However, no difference in response was observed between the sulfate and citrate forms. In two experiments, Ewing et al. (1998) observed increases in weight gain of 4.9% and 9.1% with cupric sulfate and citrate supplementation, respectively, and feed conversion was 5.2% and 7.6% better, when compared to the base diets.

The WG and FC results at 42 days for birds supplemented with cupric citrate in the present study (2.395 and 1.853 kg, respectively) are similar to the results obtained by Pesti & Bakalli (1996), with the same treatment (2.31 and 1.98 kg, respectively). However, differences in WG and FC results of birds submitted to the Control treatment were observed in the present study (2.384 and 1.852 kg, respectively), relative to the work by the previously mentioned authors (2.15 kg and 2.05, respectively). The Control birds in the present study, even though fed a base diet, without any growth promoter supplementation, attained a LW of 2.427 kg at 42 days, considered excellent for males of the utilized line. The lack of positive results for Cu action as a growth promoter in this study was, for the most part, attributed to the excellent performance attained by birds submitted to the Control treatment, which might be related to the sanitary conditions of the facilities during the conduction of the experiment.

Progressive weight gain and feed conversion rate increases can be observed when Cu is utilized in broiler chicken diet in successive trials. Johnson et al. (1985) tested the effect of 125 mg Cu kg^{-1} (cupric sulfate) in broiler chicken diet on the performance and the concentration of this mineral in the litter, and its effects on the microflora in four successive trials carried out during a 12-month period, without changing the bedding. The authors did not find differences in bird growth and feed conversion efficiency for the first and second trials. However, in the third and fourth trials of the series, an increase in weight gain and feed conversion efficiency between 2 and 3% was observed. The Cu concentrations in the litter of supplemented birds significantly increased to above 600 mg Cu kg^{-1} in the last trial. The concentration of fungi in the litter of supplemented birds progressively decreased as the trials were repeated. However, the bacterial counts in the litter was not affected by Cu supplementation. According to the authors, the lack of response of broiler chicken to supplementation with cupric sulfate in the first two trials of the series suggests that the performance improvement in the last trials was caused by other nutritional or environmental factors, not by the antimicrobial activity of Cu in the diet. It is possible that the growth responses in the last trials of the study are related to gradual alterations in the condition of the litter, resulting from increasing Cu concentration and simultaneous reduction in the concentration of fungi. Further studies are needed to support this hypothesis.

It seems that conducting a single trial in which new bedding is utilized, under a controlled environment, to test the growth promoting effect of Cu is just not sufficient to obtain performance improvement results with supplemented birds. Since the present experiment consisted of a single assay, it is recommended that it should be repeated, given that the study by Pesti & Bakalli (1996) consisted of a series of seven assays, to ensure a more reliable verification of the growth promoting action of Cu in broiler chicken diets.

There were differences ($P < 0.01$) between the Cu residue means in the litter of broiler chicken submitted to different treatments (Table 5). The Cu residues in treatments Control and Citrate 1-21 did not differ between themselves (43.2 and 54.7 mg Cu kg^{-1}, respectively). The same occurred with treatments Citrate 22-42 and Citrate 1-42 (97.7 and 115.2 mg Cu kg^{-1}, respectively). The highest Cu residue level (197.2 mg Cu kg^{-1}) was obtained with the utilization of cupric sulfate in the diet ($P < 0.01$). The rice hulls sample collected before birds were housed had 24 mg Cu kg^{-1}.

RES results are in accordance to those found by Pesti & Bakalli (1996), who observed that the use of cupric citrate in broiler chicken diet, for a 42-day period, produces lower Cu residues in the litter than when cupric sulfate is used, because of the lower levels of cupric citrate added to the feed. The use of cupric citrate as a replacement for cupric sulfate in diets results in the production of litter with a lower damage potential to the environment or to be used directly to feed ruminants (NRC, 1980).

Table 5 - Effect of treatments on litter Cu residues after rearing for 42 days.

Treatments	Cu Residue in litter
	mg kg^{-1}
Control	43.2 c
Citrate 1-21	54.7 c
Citrate 22-42	97.7 b
Citrate 1-42	115.2 b
Sulfate	197.2 a
CV	21.0

[a, b, c]Values followed by different letters differ by Tukey test ($P < 0.01$).

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