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Research Paper

Effect of the addition of granulated calcium on growth and tibia characteristics in starter chickens challenged with a coccidia vaccine

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Abstract
The objective of this study was to evaluate the productive variables, the tibia measurements and dry matter and ash content in starter broilers fed increasing levels of granulated calcium (GraCa) and challenged with a coccidia vaccine containing different species of Eimeria oocysts. Ninety male Ross 308 chicks from 5 to 19 days of age were housed individually in battery cages and randomly assigned to six treatments given by the combination of 3 levels of GraCa (0, 50 and 100%) and 2 levels (0x and 10x) of a coccidia vaccine (Cova). The GraCa was added as a substitution for the finely ground Ca carbonate and had a particle size of 2-3 mm. The treatments were: 1) Standard diet with levels of Ca and AvP according to breed recommendations supplemented in the form of fine ground Ca carbonate; 2) the same diet with 50% GraCa in substitution of the fine ground Ca carbonate; and 3) the same diet with 100% GraCa. The 10x dosage of Cova/bird was given orally to half of broilers offered each diet at 12 days of age and the other half of broilers were gavaged with saline solution. The growth performance was registered from 0-19 days. The right tibia was used for dry matter and ash determinations, and the left tibia was used for measurements. In broilers with 0x Cova, the body weight at day 19 and the weight gain from 5-19 days were higher with the use of 0% GraCa compared to those receiving 50% and 100% GraCa, while in 10x Cova birds, an opposite response was observed ($P < 0.05$). In broilers with 0x Cova, the feed conversion ratio was lower with the use of 0% GraCa compared to those receiving 50% and 100% GraCa, while in 10x Cova birds, an opposite response was observed ($P < 0.05$). In broilers with 0x Cova, the tibia weight and diameter of the diaphysis were lower with the use of 0% GraCa compared to those receiving 50% and 100% GraCa, while in 10x Cova birds, an opposite response was observed ($P < 0.01$). In broilers with 0x Cova, the tibia length was lower with the use of 0% GraCa compared to those receiving 50% and 100% GraCa, while in 10x Cova birds, no differences were found with GraCa levels of 0%, 50% and 100% ($P < 0.05$). The dry matter had a linear increasing response as the dietary GraCa levels increased ($P < 0.05$) and the number of oocysts in the excreta were higher ($P < 0.05$) in broilers receiving a 10x Cova compared to those with 0x Cova. In conclusion, the increasing levels of GraCa impaired final weight, weight gain, and feed conversion but improved tibia weight and length and the diameter of the diaphysis in broilers that did not receive Cova, compared to broilers receiving a 10x dose of Cova. The results indicate that, although GraCa improves the growth of birds exposed to a coccidia challenge, it may exacerbate the incidence of locomotor problems.

Keywords: *Broiler chickens, Granulated calcium carbonate, Growth, Tibia characteristics*

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I. INTRODUCTION

Locomotor problems during the first three weeks of growth in broiler chickens are common and have been associated with slow bone mineralization compared to the rapid rate of muscle growth (Díaz-Alonso et al., 2019). There are several options to improve the bone mineralization such as the use of precise calcium (Ca) and available phosphorus (avP) dietary concentrations, adequate Ca:AvP ratios, proper vitamin D and metabolites supplementations, among other; however, there are many factors that affect whether broiler chickens can consume the necessary amounts of Ca and avP to meet the demands of these minerals for proper bone development, especially during the first three weeks of life (Powers and Angel, 2008). In laying hens, eggshell formation takes

place in the infundibulum for a period of 18 hours, starting in the late evening lasting until the morning of the following day. However, during this period the hens consume little or no feed, so the dietary Ca, which is normally supplemented by adding finely ground calcium carbonate to the feed, is quickly released in the intestine and absorbed. Therefore, during much of the shell formation process there is a Ca deficiency, and the hen draws on the Ca stored in her bones (Saki et al., 2019). This predisposes the hens to hypocalcemia over time, eventually leading to shell calcification problems and reduced productivity. To mitigate this issue, it is recommended to replace part of the fine ground calcium carbonate with granulated calcium carbonate in a proportion ranging from 50% at the start of egg production to 70% at the end of the laying cycle (Hy-Line W36, 2020). Granulated Ca is slowly released through a maceration process in the gizzard, and this ensures greater Ca availability for eggshell formation overnight. Because bone formation and mineralization occur continuously throughout the day in broiler chickens, the question arises as to whether the use of granulated calcium can improve the mineralization process and reduce locomotor problems. It has also been observed that the use of whole wheat grain in moderate amounts can improve the growth and the immune response in broilers (Gabriel et al., 2007). Therefore, another question is whether the use of granulated calcium can enhance the growth and reduce the severity of coccidiosis problems that commonly occur during the productive life of chickens. Therefore, the objective of this study was to evaluate the productive variables, the tibia measurements and dry matter and ash content in starter broilers fed increasing levels of granulated calcium and challenged with a coccidia vaccine containing different species of *Eimeria* oocysts.

II. MATERIALS AND METHODS

Birds, management and treatments

Ninety male Ross 308 chicks were used. At hatchery, an *in ovo* vaccine against Newcastle disease, Marek's disease, and Laringotracheitis was applied. The environment of the facilities was manually controlled, with canvas curtains and gas brooders. During rearing, the temperature was initially set at 32 °C and gradually decreased at a rate of 2 °C each week until reaching 26 °C after 21 days. The lighting program was 23 h of light for 1 h of darkness for the first seven days, and from day eight on, 20 hours of light for 4 hours of darkness were used. From 5 to 19 days of age, chicks were housed individually in battery cages and were randomly assigned to six treatments given by the combination of 3 levels of GraCa (0, 50 and 100%) and 2 levels (0x and 10x) of a coccidia vaccine (Cova). The GraCa was added as a substitution for the finely ground Ca carbonate commonly used in commercial diets and had a particle size of 2-3 mm. The description of the diets is as follows: 1) Standard diet with levels of Ca and AvP according to breed recommendations supplemented in the form of fine ground Ca carbonate; 2) the same diet with 50% GraCa in substitution of the fine ground Ca carbonate; and 3) the same diet with 100% GraCa. The 10x dosage of Cova/bird was given orally to half of broilers offered each diet at 12 days of age using 1 ml of saline solution containing 6000, 2000, 4000 and 2000 sporulated oocysts of *E. acervulina, E. maxima, E. mivati* and *E. tenella*, respectively. The other half of broilers were gavaged with 1 ml of saline solution without Cova. Water and feed were freely available during the study. The composition and nutrient content of the experimental diets are shown in Table 1.

Data collection, excreta and tibia samples and measurements

Individual broilers were weighed at day 5 and 19 of age. Weight gain (g/day) was calculated for the period from 5-19 days. During the same period, the feed intake (g/day) and the feed conversion ratio were calculated. The cages were equipped with underneath trays, in which a plastic bag was placed to collect excreta samples for 3 hours at day 19. All broilers were killed by cervical dislocation at the end of the study, and the right and left legs were removed. The tibias were drawn from the legs and subsequently cleared of soft tissues. The right tibias were weighed and dried at 105 °C to determine the dry matter content, then defatted in ethyl ether, and incinerated at 600 °C to determine the ash content. The left tibias were weighed, and the total length was measured; the diameter of the upper and lower epiphysis and diaphysis were measured with a caliper. The number of oocysts in excreta was assessed using the McMaster counting chamber technique.

The results were subjected to analysis of variance using the GLM procedures of SAS. A completely randomized design with 6 treatments in a factorial arrangement of 3 levels of GraCa and 2 levels of Cova. There were 30 replicates (chicks) for the main effect of GraCa, 45 replications for the main effect of Cova and 15 replications for each combination of the GraCa and Cova interaction. Before analysis, the dry matter and ash percentages values were transformed to arcsine. Linear regression analysis was also used to compare treatments with different GraCa levels.

Table 1. Feed ingredient composition and nutrient content of the experimental diets.

	Granulated calcium, %		
Item	0	50	100
Ground corn	53.13	53.13	53.13
Soybean meal	37.02	37.02	37.02
Vegetable oil	4.68	4.68	4.68
Calcium orthophosphate	1.75	1.75	1.75
Fine calcium carbonate	1.60	0.80	0.00
Granulated calcium carbonate	0.00	0.80	1.60
Sodium bicarbonate	0.36	0.36	0.36
DL-Methionine	0.23	0.23	0.23
L-Lysine·HCl	0.10	0.10	0.10
Salt	0.19	0.19	0.19
Choline chloride	0.10	0.10	0.10
Vitamins and minerals*	0.75	0.75	0.75
Antibiotic	0.05	0.05	0.05
Coccdiostat	0.05	0.05	0.05
Nutrient content			
ME, Kcal/kg	3050	3050	3050
Digestibe Lys, %	1.20	1.20	1.20
Digestible Met, %	0.55	0.55	0.55
Digestible Thr, %	0.77	0.77	0.77
Calcium, %	1.00	1.00	1.00
Available P, %	0.50	0.50	0.50

* Each kg provided: 6500 IU Vit A; 2000 IU Vit D3; 15 IU Vit E; 1.5 mg Vit K; 1.5 mg thiamine; 5 mg riboflavin; 35 mg niacin; 3.5 mg pyridoxine; 10 mg pantothenic acid; 1500 mg choline; 0.6 mg folic acid; 0.15 mg biotin; 0.15 mg Vit B12,100.0 mg Mn; 100 mg Zn; 50 mg Fe; 10 mg Cu; 1.0 mg I.

III. RESULTS AND DISCUSSION

The body weight on day 19, and the weight gain and feed conversion ratio from 5-19 days of age were affected by the GraCa levels and Cova interaction and are presented in Figure 1. In broilers with 0x Cova, the body weight at day 19 and the weight gain from 5-19 days were higher with the use of 0% GraCa compared to those receiving 50% and 100% GraCa, while in 10x Cova birds, an opposite response was observed since those offered 0% GraCa had lower body weight and weight gain compared to broilers fed 50% and 100% GraCa ($P < 0.05$). In broilers with 0x Cova, the feed conversion ratio was lower with the use of 0% GraCa compared to those receiving 50% and 100% GraCa, while in 10x Cova birds, an opposite response was observed because those offered 0% GraCa had higher feed conversion compared to broilers receiving 50% and 100% GraCa ($P < 0.05$). The regression analysis revealed quadratic responses in the final body weight, weight gain and feed conversion ratio in 0x and 10x broilers regarding the increasing levels of GraCa. The respective regression equations are shown in Table 2. The feed intake was similar among treatments. In broilers receiving 0x Cova, reduced body weight and weight gain and increased feed conversion ratio as the dietary GraCa level increased were unexpected. These detrimental effects appear to have been due to the slow release of Ca, since GraCa must undergo a maceration process in the gizzard before the Ca is released in the intestine and absorbed to become available for bone development. On the other hand, in birds receiving a 10x Cova and fed 0% GraCa, the reduced final body and weight gain and increased feed conversion agrees with other results in which broilers were challenged with coccidia oocysts (Alagbe et al., 2023; M´Sadeq, 2023). However, the addition of 50% and 100% GraCa improved the productive parameters, such that chickens in these treatments had a productivity that was statistically similar to that of the 0x Cova/0% GraCa chickens. In this case, the maceration process of GraCa in the gizzard likely destroyed some of the oocysts that the chickens received, which not only reduced the detrimental effects of the

challenge but also improved the production responses. In previous research in broilers, it has been shown that the dietary addition of whole grain can enhance the gizzard function and digestive tract activity in healthy conditions, but when the integrity of the gastrointestinal tract is compromised with a coccidia challenge, these adaptations may be counterproductive (Gabriel et al., 2007; Yegani and Korver, 2008). One side benefit of whole grain inclusion in broilers is the modulation of the immune responses since in broilers challenged with coccidia, increased serum IgG responses against some *Eimeria* species compared to pellet diets have been reported (Gabriel et al., 2007). In the present study, the exact mechanism of the improvements in growth responses due to GraCa inclusion is unknown, and this topic requires further investigation.

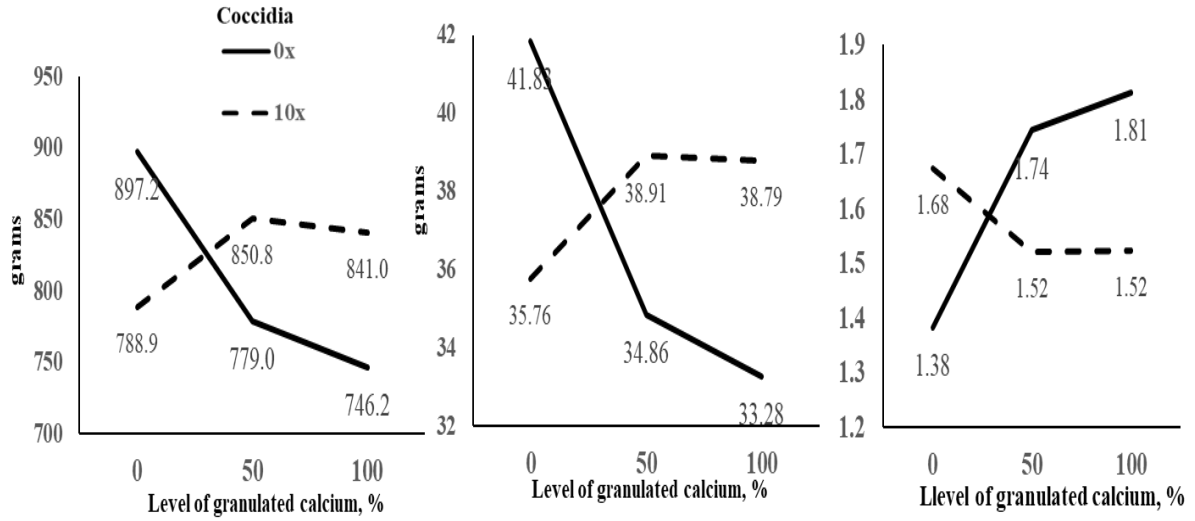

Figure 1. Left, body weight (g) at 19 days of age (GraCa level and coccidia level interaction, $P < 0.05$; SEM = 45.438); Middle, weight gain (g/day) from 5 to 19 days of age (GraCa level and coccidia level interaction, $P < 0.05$; SEM = 1.312); Right, feed conversión ratio(g/day) from 5 to 19 days of age (GraCa level and coccidia level interaction, $P < 0.05$; SEM = 0.124).

The tibia weight and length and diameter of the diaphysis were influenced by the GraCa levels and Cova interaction and are presented in Figure 2. In broilers with 0x Cova, the tibia weight and diameter of the diaphysis were lower with the use of 0% GraCa compared to those receiving 50% and 100% GraCa, while in 10x Cova birds, an opposite response was observed since broilers offered 0% GraCa had higher tibia weight and diameter of the diaphysis compared to those fed 50% and 100% GraCa ($P < 0.01$). In broilers with 0x Cova, the tibia length was lower with the use of 0% GraCa compared to those receiving 50% and 100% GraCa, while in 10x Cova birds, no differences were found with GraCa levels of 0%, 50% and 100% ($P < 0.05$). The regression analysis showed quadratic responses in the tibia weight and diameter of the diaphysis in 0x and 10x broilers regarding the increasing levels of GraCa. In 0x broilers, a flat line was observed in the tibia length regardless of the GraCa levels (linear response), while in 10x broilers, a quadratic response was seen about the increasing GraCa levels. The regression equations are presented in Table 2. The diameter of the upper and lower epiphysis was not statistically different among treatments. In a previous study from our laboratory, the tibia weight was similar between control and broilers receiving a 16x dosage of Cova (Cortes-Coronado et al., 2024), which differs from the present results in which the tibia weight was higher with the combination of 0%GraCa/10x Cova; however in the forementioned study, broilers were vaccinated al 21 days of age and the tibia measurements were carried out at 28 days of age.

The tibia length and diameter of the diaphysis were higher in broilers fed 0%GraCa/10x Cova compared to those fed 0%GraCa/0x Cova in the present study, while the opposite was found in our earlier research (Cortés-Coronado et al., 2024). Nonetheless, in other research in which broilers received a low dosage of coccidia oocysts, resembling the dosage used in the present trial, a lack of differences between the control and challenged broilers in the tibia length, width and mineral content were reported (Tompkins et al., 2022). It has been stated that the extent of the coccidial infection, using different species and the dosage of oocysts, affects tibia measurements to varying degrees with greater challenges resulting in more pronounced decreases in bone characteristics (Tompkins et al., 2022).

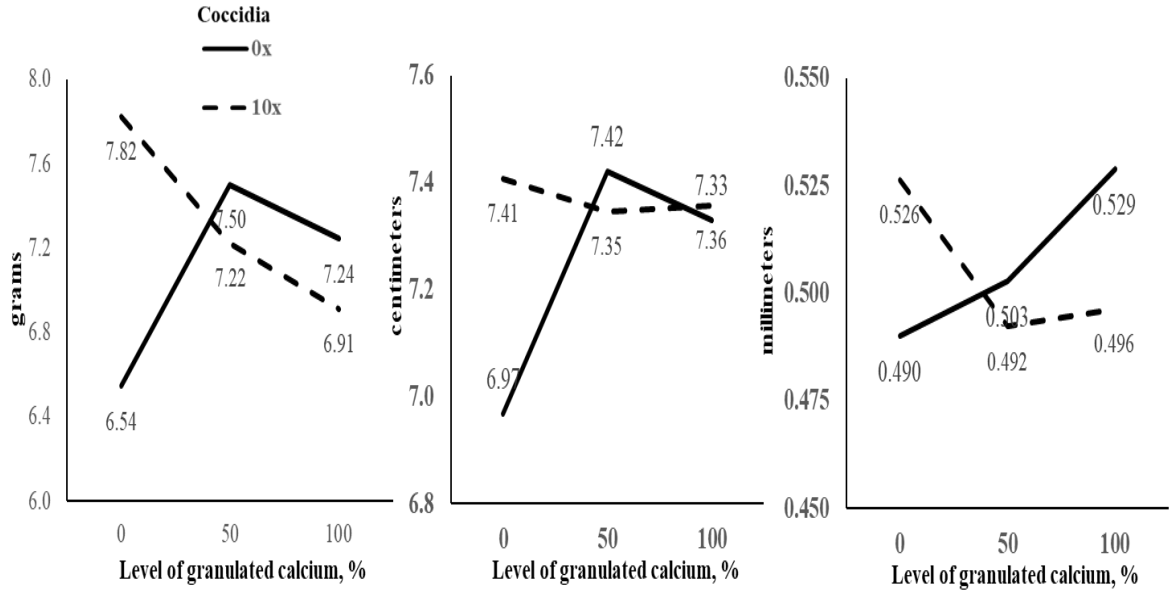

Figure 2. Left, tibia weight (GraCa level and coccidia level interaction, $P < 0.01$; SEM = 0.263); Middle, tibia length (GraCa level and coccidia level interaction, $P < 0.05$; SEM = 0.096); Right, diameter of the diaphysis of tibia (GraCa level and coccidia level interaction, $P < 0.01$; SEM = 0.0113).

Surprisingly, the tibia weight and the diameter of the diaphysis deteriorated in broilers fed 50% and 100% GraCa receiving 10x Cova, whilst the opposite response was seen in birds receiving 0x Cova. The diameter of the diaphysis in broilers receiving 100% GraCa/10x Cova was statistically different from those receiving 100%GraCa/0x Cova. In contrast, the diameter of the diaphysis showed no response to the increased dietary GraCa levels in broilers receiving 10x Cova. These findings suggest that adding GraCa to broilers exposed to a coccidiosis challenge is not recommended. In some ways these results agree with the suggestion that whole grain feeding in coccidia-challenged broilers is unlikely to improve tibia measurements and may exacerbate skeletal deficits, particularly under moderate to severe infection (Gabriel et al., 2007; Singh et al., 2015).

The dry matter percentage was different in respect to the levels of GraCa and is presented in Figure 3. The dry matter had a linear increasing response (Table 2) as the dietary GraCa levels increased ($P < 0.05$); however, no differences among treatments were detected on the weight of the dry matter and the weight and percentage of the ashes. Opposite to these results, reduced ash content was reported in our earlier study in broilers receiving a 16x dosage of Cova (Cortés-Coronado et al., 2024). Similarly, in studies involving *Eimeria* challenged broilers, decreased tibia relative weight of ash content and ash concentration at 14 d of age were reported (Akbari Moghaddam Kakhki et al., 2019). These discrepancies were probably due to the use of different oocyst dosages, the period of exposure to coccidia, the age of the chickens at the time of inoculation, and the timing of the tibia measurements. As expected, the number of oocysts in the excreta (Figure 3) were higher ($P < 0.05$) in broilers receiving a 10x dosage of Cova compared to those with 0x Cova. The low coccidia oocyst counts in the excreta of 0x Cova chickens can be explained by cross-contamination with those that received 10x Cova; however, environmental contamination of coccidia cannot be ruled out.

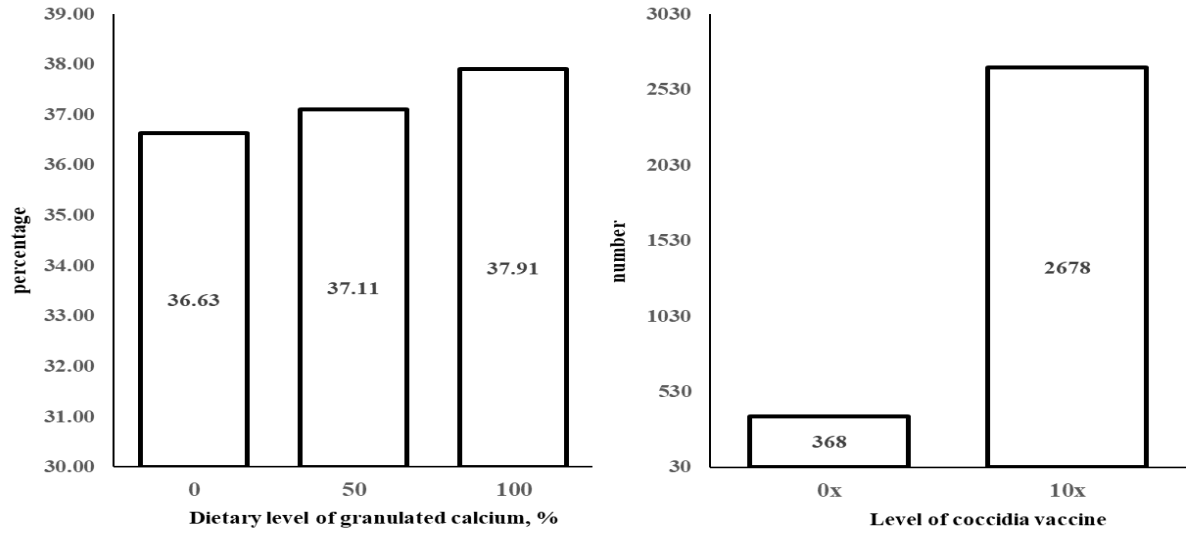

Figure 3. Left, tibia dry matter percentage (%) (Level of GraCa, P < 0.05; SEM = 0.342); Number of coccidia oocysts (Level of coccidia vaccine, P < 0.05; SEM = 818.55).

Table 2. Regression equations and determination coefficient of growth performance and tibia characteristic variable responses.

Variable response	Cova	Regression equations	R^2
Growth performance			
Body weight, 19 days of age, g	0x	y = -35.856x2 + 169.48x + 655.27	0.99
	10x	y = 42.694x2 - 246.27x + 1100.8	0.99
Weight gain, g/day	0x	y = 2.6958x2 - 15.06x + 54.197	0.99
	10x	y = -1.6316x2 + 8.0443x + 29.344	0.99
Feed conversion ratio	0x	y = -0.1455x2 + 0.7975x + 0.7307	0.99
	10x	y = 0.0772x2 - 0.3844x + 1.9826	0.99
Tibia characteristics			
Weight, g	0x	y = -0.6075x2 + 2.7793x + 4.3716	0.99
	10x	y = 0.1458x2 - 1.0383x + 8.7141	0.99
Length, cm	0x	y = -0.2728x2 + 1.2729x + 5.9677	0.99
	10x	y = -0.0245x + 7.419	0.57
Diameter of the diaphysis, mm	0x	y = 0.0067x2 - 0.0073x + 0.4906	0.99
	10x	y = 0.0191x2 - 0.0914x + 0.5986	0.99
Dry matter, %		y = 0.6412x + 35.932	0.98

IV. CONCLUSION AND RECOMMENDATION

In starter broilers, the increasing dietary levels of GraCa impaired final weight, weight gain, and feed conversion but improved tibia weight and length and the diameter of the diaphysis in broilers that did not receive Cova, compared to broilers receiving a 10x dose of Cova. The results indicate that, although GraCa improves the growth of birds exposed to a coccidia challenge, it may exacerbate the incidence of locomotor problems. The recommendation based on these results is that GraCa should not be used in healthy starter broilers, as it reduces productivity, nor in broilers exposed to coccidiosis challenges, as it impairs some tibial characteristics.

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