Dietary supplementation with glycosaminoglycans reduces locomotor problems in broiler chickens

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ABSTRACT This study aimed to assess the influence of glycosaminoglycan (chondroitin and glucosamine sulfates) supplementation in the diet on the performance and incidence of locomotor problems in broiler chickens. A completely randomized design was carried out in a 3 × 3 factorial scheme (3 levels of chondroitin sulfate −0, 0.05, and 0.10%; and 3 levels of glucosamine sulfate −0, 0.15, and 0.30%). Each treatment was composed of 6 replications of 30 broilers each. The performance of broilers (average weight, weight gain, feed intake, feed conversion, and productive viability) was assessed at 7, 21, 35, and 42 d of age, whereas the gait score, valgus and varus deviations, femoral degeneration, and tibial dyschondroplasia were assessed at 21 and 42 d of age. Increasing levels of glucosamine sulfate inclusion linearly increased the weight gain from 1 to 35 and from 1 to 42 d of age of broilers ($P = 0.047$ and $P = 0.039$, respectively), frequency of broilers with no femoral degeneration in the right and left femurs, and the proliferating cartilage area of proximal epiphysis at 42 d of age ($P = 0.014$, $P < 0.0001$, and $P = 0.028$, respectively). The increasing inclusion of chondroitin and glucosamine sulfates led to an increase in the frequency of broilers on the gait score scale 0 ($P = 0.007$ and $P = 0.0001$, respectively) and frequency of broilers with no valgus and varus deviations ($P = 0.014$ and $P = 0.0002$, respectively) also at 42 d of age. Thus, chondroitin and glucosamine sulfates can be used in the diet of broiler chickens to reduce their locomotor problems.

Key words: chondroitin, femoral degeneration, gait score, glucosamine, tibial dyschondroplasia

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INTRODUCTION

Technological advances in nutrition and genetic improvement that led to an increase of the body growth and meat deposition rate of broiler chickens originated metabolic disturbances that have caused damages in the production system (Julian, 2005; Angel, 2007). Ascites syndrome, sudden death syndrome, and locomotion disorders are among these disorders (Julian, 2005).

Locomotion disorders, commonly referred to as deformities or locomotion problems, may occur because of changes in bone and cartilaginous growth plate in cases of rickets, tibial dyschondroplasia, chondrodystrophy, and femoral degeneration; congenital changes in cases of spondylolisthesis and valgus and varus angular deformities; or as the result of infectious diseases such as osteomyelitis and arthritis (Angel, 2007). Together, femoral degeneration, tibial dyschondroplasia, and angulation deviations are the main diseases associated with lameness in broiler chickens, which may or may not occur in association (Fernandes et al., 2012; Colet et al., 2015).

The prevalence of locomotion disorders in broiler chickens has become a big concern, as it results in poor performance and increased carcass condemnation in slaughterhouses to the detriment of broiler welfare (Nääs et al., 2009; Shim et al., 2012). Broilers with mobility problems are totally or partially deprived of the freedoms described in the Farm Animal Welfare Council. Thus, locomotor diseases must be prevented because losses are inevitable once established.

Polysulfated glycosaminoglycans have prevented and/or reduced the progression of pathologic changes in bone and joint structures of animal models such as rats (Wen et al., 2010; Chiusaroli et al., 2011; Jawed et al., 2011; Muraleva et al., 2012; Arafa et al., 2013; Wolff, 2014), rabbits (Herrero-Beaumont et al., 2008;

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Largo et al., 2010; Kamarul et al., 2011), dogs (Vieira et al., 2010; Eleotéreo et al., 2012; Gupta et al., 2012; Maxwell et al., 2016; Wenz et al., 2017), broiler chickens (Sgavioli et al., 2017; Santos et al., 2018, 2019), humans (Kahan et al., 2009; Wildi et al., 2011; Kantor et al., 2014; Lubis et al., 2017), and in vitro (Tat et al., 2007; Varghese et al., 2007; Uitterlinden et al., 2008; Calamia et al., 2010, 2012, 2014; Chiusaroli et al., 2011; Imagawa et al., 2011; Pecchi et al., 2012; Cunha et al., 2017; Igarashi et al., 2017; Kim et al., 2017).

Among glycosaminoglycans, chondroitin and glucosamine sulfates stand out, which, in combination, stimulate anabolic cartilage processes such as proteoglycan and collagen synthesis (Kamarul et al., 2011), chondrocyte proliferation, bone matrix biosynthesis (Wolff, 2014), and reduce bone resorption (Maxwell et al., 2016). Moreover, they prevent cartilage degeneration through anti-inflammatory and inhibitory mechanisms (Calamia et al., 2010, 2014; Kantor et al., 2014). Therefore, the mechanisms of action of chondroitin and glucosamine sulfates may contribute to reducing the incidence of locomotor problems in broiler chickens.

Although locomotor problems deserve attention from countless researchers, there is little work in the literature to prevent these disorders with the use of glycosaminoglycans in broiler chickens. Sgavioli et al. (2017) and Santos et al. (2019) provided the important evidence that the addition of chondroitin and glucosamine sulfates in the diet can promote beneficial changes in bone and cartilaginous development of broiler chickens. However, there are few studies in the literature on the effect of chondroitin and glucosamine sulfates on their locomotor problems.

This study aimed to assess the influence of glycosaminoglycan supplementation in the diet of broiler chickens on the performance (average weight [**AW**], weight gain [**WG**], feed intake, feed conversion, and productive viability) and the incidence of locomotor problems (gait score, valgus and varus deviations, femoral degeneration, and tibial dyschondroplasia).

MATERIALS AND METHODS

All procedures in this study were approved by the Animal Use Ethics Committee, as per protocol 051/16.

Animals, Facilities, and Experimental Design

A total of 1,620 1-day-old, male, commercial Cobb 500 broiler chicks with a mean initial weight of 43 ± 0.2 g were reared until 42 d of age. They were housed in 54 pens, set up in the central area of a 1,500-m^2 commercial facility, acclimatized by negative pressure. Each pen consisted of 2.88 m^2, 10 nipple drinkers, and 1 tube feeder for chicks up to the seventh day, and 1 adult from 8 to 42 d of age.

Chicks were vaccinated in the hatchery against Marek's disease, infectious bursal disease, and avian pox. The following vaccination program was completed during the experimental period: Marek and avian pox in the hatchery and infectious bursal disease (mild strain) on the 14th day in the drinking water, using powdered milk as a carrier (2 g L^{-1}).

The experiment was conducted in a completely randomized manner in a 3 × 3 factorial arrangement (3 levels of chondroitin sulfate −0, 0.05, 0.10%, and 3 levels of glucosamine sulfate −0, 0.15, and 0.30%, both in the broiler chicken diet). Each treatment was composed of 6 replications of 30 birds, totaling 54 experimental pens. Chondroitin sulfate ($[C_{14}H_{21}NO_{14}S]$ n; Biofac A/S, Englandsvej, Kastrup, Denmark) was 91.27% pure and potassium glucosamine sulfate ($[C_6H_{14}NO_5]$ $2SO_4$ x 2KCl; Zhejiang Golden-Shell Pharmaceutical Co. Ltd., Yuhuan, Zhejiang, China) had 16% sulfate.

The broilers received food and water ad libitum throughout the experimental period and were reared following the management recommendations of the Cobb-Vantress Management Guide (2008). Diets were formulated based on corn and soybean meal and following the recommendations proposed by Rostagno et al. (2011) for the phases preinitial (1–7 d of age), initial (8–21 d of age), growth (22–35 d of age), and final (36–42 d of age), all of them with a variable portion of 0.4% of chondroitin sulfate and/or glucosamine sulfate and/or inert, as per treatments (Table 1).

The nutritional composition of the experimental diets was analyzed for DM, crude energy, CP, calcium, and phosphorus. DM was determined by the gravimetric method using heat and was based on the weight loss of the material subjected to heating at 105°C in a rectilinear oven (model 315/3; Fanen, Guarulhos, São Paulo, Brazil). Crude energy was determined using the calorimetric pump (model 6100; Parr Instrument Company, Moline, Illinois). The total nitrogen content was analyzed in a nitrogen distiller (model TE-036/1; Tecnal, Piracicaba, São Paulo, Brazil), using the Kjeldahl method (INCT-CA N-001/1), according to Detmann et al. (2012), and the factor of 6.25 was used in the conversion of nitrogen value into CP. Calcium and phosphorus were analyzed using an atomic absorption spectrophotometer (model AA-7000; Shimadzu, Barueri, São Paulo, Brazil) and UV/VIS spectrophotometer (model UV-5100; Tecnal, Piracicaba, São Paulo, Brazil), respectively, as proposed by Silva and Queiroz (2002).

The analyzed values for preinitial, initial, growth, and final phases were 3,755, 3,880, 4,010, and 4,100 kcal/kg for crude energy, respectively, 23.85, 22.60, 21.25, and 19.00% for CP, respectively, 0.99, 0.98, 0.90, and 0.83% for calcium, respectively, and 0.75, 0.73, 0.65, and 0.65% for total phosphorus, respectively.

Performance

Performance variables (AW, WG, mean feed intake, feed conversion, and productive viability) were assessed at 7, 21, 35, and 42 d of age by weighing broiler chickens, diets, and the number and weight of dead broilers.

Table 1. Ingredients and calculated nutritional composition of diets from preinitial (1–7 d of age), initial (88–21 d of age), growth (22–35 d of age), and final (36–42 d of age) phases.

Ingredients (%)	Pre-initial	Initial	Growth	Final
Corn	54.46	59.80	63.77	69.94
Soybean meal (45.5%)	35.16	30.78	23.82	15.18
Poultry fat	1.13	1.40	1.87	2.07
Meat and bone meal (47%)	3.67	4.40	3.13	6.87
Offal meal (62.5%)	3.00	1.13	3.53	1.80
Feather meal (84.81%)	-	-	1.53	2.00
Limestone	0.53	0.53	0.63	0.23
Salt	0.39	0.35	0.27	0.21
Sodium bicarbonate	0.08	0.05	0.10	0.15
Choline chloride (75%)	0.05	0.08	0.05	0.06
DL-Methionine (99%)	0.41	0.35	0.27	0.25
L-Lysine HCL (64%)	0.33	0.34	0.28	0.45
L-Threonine (98%)	0.07	0.08	0.05	0.09
L-Valine (96.5%)	0.02	0.01	-	-
[1]Vitamin supplement	0.05	0.05	0.05	0.05
[2]Mineral supplement	0.05	0.05	0.05	0.05
[3]Additives	0.20	0.20	0.20	0.20
[4]Variable portion	0.40	0.40	0.40	0.40
Total	100.00	100.00	100.00	100.00
Calculated nutritional composition				
ME (kcal/kg)	3,000	3,050	3,150	3,200
CP (%)	25.00	22.50	21.60	19.53
Calcium (%)	0.98	0.98	0.95	0.86
Available phosphorus (%)	0.49	0.48	0.46	0.44
Sodium (%)	0.22	0.21	0.20	0.19
Chlorine (%)	0.30	0.27	0.23	0.20
Potassium (%)	0.90	0.82	0.70	0.57
Digestible methionine + cystine (%)	1.03	0.92	0.85	0.75
Digestible methionine (%)	0.73	0.64	0.56	0.49
Digestible lysine (%)	1.36	1.21	1.10	1.00
Digestible threonine (%)	0.87	0.79	0.72	0.68

[1]Vitamin supplement (composition per kg product): preinitial and initial (vitamin A, 20000000 IU; vitamin D3, 5000000 IU; vitamin E, 50,000 IU; vitamin K3, 4,000 mg; vitamin B1, 5,000 mg; vitamin B2, 13,000 mg; vitamin B6, 7,000 mg; vitamin B12, 36 mg; niacin, 84,000 mg; pantothenate, 30,000 mg; folic acid, 2,400 mg; biotin, 160 mg; selenium 600 mg); growth and final (vitamin A, 16000000 IU; vitamin D3, 3800000 IU; vitamin E, 40,000 IU; vitamin K3, 3,600 mg; vitamin B1, 3,600 mg; vitamin B2, 11,000 mg; vitamin B6, 5,200 mg; vitamin B12, 30 mg; niacin, 70,000 mg; pantothenate, 26,000 mg; folic acid, 1,800 mg; biotin, 100 mg; selenium, 600 mg).

[2]Mineral supplement (composition per kg product): copper, 16.25 g; iron, 100 g; iodine, 2,000 g; manganese, 150 g; zinc 125 g.

[3]Additives: preinitial, initial, and growth (Maxiban [narasine + nicarbazine] 0.05 g; Enradin [enramycin] 0.01 g; Microtech [phytase] 0.01 g; Salmex (formaldehyde, propionic acid, terpenes and surfactants] 0.10 g; Endox [ethoxyquin and butylated hidroxyanisole] 0.004 g; copper sulfate 0.03 g); final (Maxiban [narasine + nicarbazine] 0.05 g; Enradin [enramycin] 0.006 g; Microtech [phytase] 0.01 g; Salmex [formaldehyde, propionic acid, terpenes, and surfactants] 0.10 g; Endox [ethoxyquin and butylated hidroxyanisole] 0.004 g; copper sulfate 0.03 g).

[4]Variable portion: chondroitin sulfate ($[C_{14}H_{21}NO_{14}S]n$, Biofac A/S, Englandsvej, Kastrup, Denmark) and/or potassium glucosamine sulfate ($[C_6H_{14}NO_5]$ $2SO_4$ x 2KCl, Zhejiang Golden-Shell Pharmaceutical Co. Ltd., Yuhuan, Zhejiang, China) and/or inert (kaulin) as per treatments.

The AW was obtained at the end of each experimental phase by dividing the total broiler weight of the pen by the number of animals, WG was calculated by the difference between the final and initial weight of animals, and feed intake by the difference between the weight of the offered diet and the leftover of each period, divided by the number of animals in the period. Feed conversion was obtained from the ratio between feed intake and broiler WG. Both feed intake and feed conversion were corrected for mortality, according to Sakomura and Rostagno (2016). Productive viability was calculated as a percentage of surviving animals in relation to the initial number of housed animals.

Gait Score

Gait score was assessed in all broilers at 21 and 42 d of age by 2 different evaluators previously trained on the method application, according to Kestin et al. (1992). The evaluators were trained by performing the methods over a period of 2 d, with the objective of standardizing the understanding of the methodology; in addition, during the analyses, the birds were evaluated by both, as a way to increase the speed, without compromising reliability. This method consisted of assessing the broiler displacement at a linear distance of 1 m, considering a scale ranging from 0 to 5 as follows: 0 – normal

movement; 1 – quick movement but with small deficiency; 2 – quick movement but with high deficiency, 3 –slow movement with difficulty; 4 – slow movement with high difficulty; and 5 – rare movement, crawling with wings.

Valgus and Varus

Valgus and varus deviations were assessed after assessing the gait score in all broilers at 21 and 42 d of age. The angulation of leg joints was assessed according to Almeida Paz et al. (2010) using a caliper and protractor, and the angle formed between the tibia and the finger 3 on the right and left legs. The varus deformity was characterized when the angulation was negative, with medial deviation, whereas the valgus deformity was characterized when the angulation was positive, with lateral deviation.

Femoral Degeneration and Tibial Dyschondroplasia

Twelve broilers/treatment/age with mean BW close to the mean BW of the experimental unit were selected on the 21st and 42nd day of growth and identified with numbered leg bands. The selected broilers were fasted for 8 h, slaughtered by cervical dislocation, and left and right tibias of each broiler were removed and marked for analyses of femoral degeneration and tibial dyschondroplasia.

A macroscopic assessment of femoral degeneration was performed in the proximal epiphysis region of the right and left femurs, according to Almeida Paz et al. (2009). The assigned injury scores ranged from 0 to 2, where 0 is equivalent to bone and cartilage with no injury, 1 is equivalent to partial integrity of articular cartilage, and 2 is equal to a bone with no cartilage and severe injury.

Longitudinal sections of the proximal right tibial epiphysis were used to make 108 histologic slides at each age (12 per treatment) to assess the incidence of tibial dyschondroplasia. The samples were fixed in 10% buffered formaldehyde solution for 24 h. After fixation, they were stored in 70% alcohol and, subsequently, decalcified and processed as per the methodology of Luna (1968). Semiserial 5-µm-thick sections were performed with an electronic rotary microtome (model RM2255; Leica Biosystems Inc., Buffalo Grove, Illinois).

The histologic slides were scanned using a Panoramic Desk (3D HISTECH Ltda., Budapest, Hungary) histologic slide scanner; Fujitsu Esprimo P9900 Intel Dual Core 2 GB RAM HD 500 GB computer; Eonis 22″ 2 MP clinical review display monitor, MDRC-2122 WP white; CMOS color camera model CIS 4MP; Zeiss Plan-Apochromat objective 40x NA 0.95; and 1.6x digital camera adapter and extended focus. The recorded images were analyzed using the software Pannoramic Viewer (3D HISTECH Ltda., Budapest, Hungary; freeware, https://www.3dhistech.com/pannoramic_viewer). Three areas were measured: proliferating cartilage area (**PCA**), hypertrophic cartilage area, and total proximal epiphysis area, according to Oviedo-Rondón et al. (2001). The calcified cartilage zone was considered the lower limit to determine the thickening of the hypertrophic zone in the lesion characterization, according to Thorp et al. (1993).

Statistical Analysis

Supplementation effects with chondroitin (0, 0.05, and 0.10%) and glucosamine sulfates (0, 0.15, and 0.30%) and their interaction (CO × GLU) were analyzed as per the experimental model: $Yijk = \mu + (CO)\ i + (GLU)\ j + (CO \times GLU)\ ij + eijk$, where Y is the response variable, µ is the mean of the variable, CO is the chondroitin sulfate, GLU is the glucosamine sulfate, CO × GLU is the interaction of chondroitin and glucosamine sulfates, and eijk is the residual error.

The data of performance and incidence of tibial dyschondroplasia were verified for the presence of outliers (box-and-whisker plot), the normality assumptions of error were studentized (Cramér-von Mises test) and homogeneity of variances (Bartlett's test). After corrections, the data were subjected to ANOVA using the GLM procedure of SAS (SAS Institute, 2002). An orthogonal analysis was performed when the means differed significantly by the F test at 5% probability to test linear and quadratic effects of the levels of chondroitin and glucosamine sulfates. Fisher's exact test at 5% significance was used to analyze the frequencies of the gait score, valgus and varus, and femoral degeneration.

The statistical analysis of performance was carried out considering each pen as an experimental unit (6 pens per treatment). Statistical analysis of tibial dyschondroplasia considered individual broilers as experimental units (N: 12 broilers per treatment and 2 broilers per pen). Frequency calculations for the analysis of gait score, valgus and varus, and femoral degeneration considered each broiler as an experimental unit (N: 180 broilers per treatment for gait score and valgus and varus and N: 12 broilers per treatment for femoral degeneration, 2 broilers per plot).

RESULTS

Performance

Periods from 1 to 35 and from 1 to 42 d of age showed increasing linear effects ($P = 0.047$ and $P = 0.039$, respectively) of glucosamine sulfate levels for WG ($WG_{1\ to\ 35\ d} = 88.133\ GLU + 2{,}606.5$, $R^2 = 0.94$; and $WG_{1\ to\ 42\ d} = 104.47\ GLU + 3{,}364.40$, $R^2 = 0.77$), with increased WG on supplementation with glucosamine sulfate. An increasing linear effect ($P = 0.040$) was observed from 1 to 42 d of age for AW ($AW_{1\ to\ 42\ d} = 107.4\ GLU + 3{,}407.2$, $R^2 = 0.78$). Similarly, higher levels of glucosamine sulfate resulted in an increased AW of broilers (Table 2).

Table 2. Average weight (AW), weight gain (WG), feed intake (FI), feed conversion (FC), and viability (V) of broilers from 1 to 42 d of age, supplemented with chondroitin and glucosamine sulfates of feed ration.

		Glucosamine[2] (GLU, %)						Probability		
Parameters	Chondroitin[1] (CO, %)	0	0.15	0.30	Regression	Mean	SEM	CO	GLU	CO x GLU
AW (g)	0	3,352.83	3,398.83	3,403.67	ns	3,385.11	14.09	ns	L(0.040)	ns
	0.05	3,426.83	3,467.50	3,446.50	ns	3,446.94				
	0.10	3,427.17	3,433.50	3,450.33	ns	3,437.00				
	Regression	ns	ns	ns						
	Mean	3,402.28	3,433.28	3,434.50						
WG (g)	0	3,309.83	3,355.50	3,361.17	ns	3,342.17	14.09	ns	L(0.039)	ns
	0.05	3,384.33	3,424.50	3,403.50	ns	3,404.11				
	0.10	3,383.83	3,390.67	3,407.33	ns	3,393.94				
	Regression	ns	ns	ns						
	Mean	3,359.33	3,390.22	3,390.67						
FI (g)	0	5,293.83	5,239.17	5,392.00	ns	5,308.33	29.91	ns	ns	ns
	0.05	5,362.33	5,269.60	5,318.67	ns	5,319.65				
	0.10	5,327.00	5,383.00	5,247.50	ns	5,319.17				
	Regression	ns	ns	ns						
	Mean	5,327.72	5,298.88	5,319.39						
FC	0	1.591	1.558	1.580	ns	1.576	0.01	ns	ns	ns
	0.05	1.573	1.536	1.549	ns	1.553				
	0.10	1.553	1.551	1.525	ns	1.543				
	Regression	ns	ns	ns						
	Mean	1.572	1.548	1.552						
V (%)	0	97.02	97.02	95.83	ns	96.63	0.96	ns	ns	ns
	0.05	96.43	94.64	93.45	ns	94.84				
	0.10	93.45	95.83	94.64	ns	94.64				
	Regression	ns	ns	ns						
	Mean	95.64	95.83	94.64						

Abbreviations: L, linear; ns, not significant.
[1]([$C_{14}H_{21}NO_{14}S$)n, Biofac A/S) purity of 91.27%.
[2]([$C_6H_{14}NO_5$)2SO_4 x 2KCl, Zhejiang Golden-Shell Pharmaceutical Co. Ltd.) sulfate content 16%.

No interaction ($P > 0.05$) and isolated effects of chondroitin and glucosamine sulfates were observed for AW, WG, feed intake, feed conversion, and productive viability of broilers from 1 to 7 and from 1 to 21 d of age. No effect of sulfates ($P > 0.05$) was observed for AW, feed intake, feed conversion, and productive viability from 1 to 35 and from 1 to 42 d of age (Table 2).

Gait Score

The gait score frequency showed an effect of chondroitin and glucosamine sulfates ($P = 0.007$ and $P = 0.0001$, respectively) at 42 d of age. The inclusion of 0.10 and 0.30% of conroutine sulfates and glucosamine increased the percentage of birds evaluated as normal by 7.69 and 12.59%, respectively, when compared with birds that did not receive sulfates. In addition, these inclusions reduced the percentage of birds that were classified on scale 1, 2 and 3 in 3.18 and 4.62%; 2.14 and 2.66%; 1.51 and 2.88%, respectively, for chondroitin and glucosamine sulfates, when compared with the birds of the treatments where sulfates were not included (Table 3). No effect of treatments ($P > 0.05$) was observed on the gait score frequency at 21 d of age.

Valgus and Varus

An effect of chondroitin and glucosamine sulfates was observed on valgus and varus deviations ($P = 0.014$ and $P = 0.0002$, respectively) at 42 d of age. The inclusion of glycosaminoglycans in the diet of broilers reduced the incidence of deviations and, consequently, increased the frequency of animals without deviations. The inclusion of 0.10 and 0.30% of conroutine sulfates and glucosamine increased the percentage of birds evaluated as without deviations (valgus or varus) in 8.74 and 12.83%, respectively, when compared with the birds of the treatments where sulfates were not included. In addition, these inclusions reduced the percentage of birds that were evaluated with valgus deviation, by 8.55 and 11.5%, for chondroitin and glucosamine sulfates, respectively, when compared with the birds of the treatments where sulfates were not included. In addition, it reduced the percentage of birds that were evaluated with varus deviation, by 0.19 and 1.33%, for

Table 3. Gait score frequency of broilers at 42 d of age, supplemented with chondroitin and glucosamine sulfates in the diet.

	Gait score (%)					
Treatments	0	1	2	3	4	5
Chondroitin[1] (CO, %)						
0	82.30	8.86	4.43	2.43	1.10	0.88
0.05	84.89	8.80	3.62	2.03	0.44	0.22
0.10	89.99	5.68	2.29	0.92	1.12	0.00
Glucosamine[2] (GLU, %)						
0	78.55	10.06	5.16	3.57	1.78	0.88
0.15	87.49	7.83	2.67	1.12	0.67	0.22
0.30	91.14	5.44	2.50	0.69	0.23	0.00
	Probability[3]					
CO	0.007					
GLU	0.0001					

[1]([$C_{14}H_{21}NO_{14}S$]n, Biofac A/S) purity of 91.27%.
[2]([$C_6H_{14}NO_5$]2SO_4 x 2KCl, Zhejiang Golden-Shell Pharmaceutical Co. Ltd.) sulfate content 16%.
[3]Significant at $P < 0.05$ (Fisher's exact test).

Table 4. Frequency of valgus and varus deviations of broiler chickens at 42 d of age, supplemented with chondroitin and glucosamine sulfates in the diet.

Treatments	Without deviation	Valgus	Varus
Chondroitin[1] (CO, %)			
0	74.71	23.07	2.22
0.05	77.15	21.28	1.57
0.10	83.45	14.52	2.03
Glucosamine[2] (GLU, %)			
0	71.53	26.02	2.45
0.15	79.41	18.35	2.24
0.30	84.36	14.52	1.12
	Probability[3]		
CO	0.014		
GLU	0.0002		

[1]([$C_{14}H_{21}NO_{14}S$]n, Biofac A/S) purity of 91.27%.
[2]([$C_6H_{14}NO_5$]2SO_4 x 2KCl, Zhejiang Golden-Shell Pharmaceutical Co. Ltd.) sulfate content 16%.
[3]Significant at $P < 0.05$ (Fisher's exact test).

chondroitin and glucosamine sulfates, respectively, when compared with the birds of the treatments where sulfates were not included (Table 4). No effect of treatments ($P > 0.05$) was observed on the frequency of valgus and varus deviations at 21 d of age.

Femoral Degeneration

An effect of glucosamine sulfate was observed on the femoral degeneration of the right and left femurs ($P = 0.014$ and $P < 0.0001$, respectively) at 42 d of age. The inclusion of 0.30% of glucosamine sulfate in the diet of broilers reduced the incidence of scores 1 and 2 in both femurs and hence increased the frequency of animals without femoral degeneration (Table 5). However, at 21 d of age, no effect of treatments ($P > 0.05$) was observed on the frequency of femoral degeneration.

Tibial Dyschondroplasia

An increasing linear effect ($P = 0.029$) of glucosamine sulfate was observed on the PCA ($PCA_{42\ d} = 3.6667$ GLU + 29.217; $R^2 = 0.92$) at 42 d of age, with increased PCA of the proximal tibial epiphysis of chicken broilers due to the inclusion of glucosamine sulfate (Table 6). No effect of treatments ($P > 0.05$) was observed for the hypertrophic cartilage area and the total area of proximal epiphysis tibial at 42 d of age. No effect of treatments ($P > 0.05$) was observed for tibial PCA, hypertrophic cartilage area, and total proximal epiphysis area at 21 d of age.

Table 5. Frequency of femoral degeneration (FD) of 42-day-old broiler chickens supplemented with chondroitin and glucosamine sulfates in the diet.

	FD right femur (%)			FD left femur (%)		
Treatments	0	1	2	0	1	2
Chondroitin[1] (CO, %)						
0	50.02	5.55	44.43	55.55	8.34	36.11
0.05	55.56	0.00	44.44	69.44	8.34	22.22
0.10	63.89	0.00	36.11	63.89	5.55	30.56
Glucosamine[2] (GLU, %)						
0	36.13	5.55	58.32	30.56	13.89	55.55
0.15	63.89	0.00	36.11	80.53	2.79	16.68
0.30	69.43	0.00	30.57	77.77	5.55	16.68
	Probability[3]					
CO	0.427			0.721		
GLU	0.014			<0.0001		

[1]([$C_{14}H_{21}NO_{14}S$]n, Biofac A/S) purity of 91.27%.
[2]([$C_6H_{14}NO_5$]2SO_4 x 2KCl, Zhejiang Golden-Shell Pharmaceutical Co. Ltd.) sulfate content 16%.
[3]Significant at $P < 0.05$ (Fisher's exact test).

DISCUSSION

The addition of chondroitin sulfate and glucosamine have been assessed for locomotor problems of broiler chickens, and the results showed evidence that these glycosaminoglycans could significantly reduce such problems.

Increasing levels of inclusion of chondroitin and glucosamine sulfates in broiler diets increased the frequency of birds without deviations (valgus and varus) and minimize the with gait score. Chondroitin and glucosamine sulfates are nutraceuticals that have the functions to prevent and treat injuries to the locomotor system (Kamarul et al., 2011; Kantor et al., 2014; Wolff, 2014; Lubis et al., 2017) and reduce pain caused by locomotor problems (Wolff et al., 2014). In addition, these glycosaminoglycans modulate the expression of osteoprotegerin and receptor activator of nuclear factor kappa B ligand, the 2 main factors involved in bone remodeling, with a protective effect on bone loss (Tat et al., 2007). These functions explain the positive results in reducing the frequency of valgus and varus deviations and the gait score.

The reduction in femoral degeneration of broilers by including increasing levels of glucosamine sulfate may have occurred because the glycosaminoglycan has anabolic (Varghese et al., 2007; Uitterlinden et al., 2008; Wen et al., 2010; Chiusaroli et al., 2011) and anticatabolic properties (Imagawa et al., 2011; Jawed et al., 2011; Taniguchi et al., 2012), which contribute to the protection and repair of cartilage. According to Varghese et al. (2007), glucosamine increases cartilage extracellular matrix components, such as aggrecan and type II collagen, which contribute to better cartilage structure and repair. Wen et al. (2010) and Chiusaroli et al. (2011) demonstrated that oral administration of glucosamine sulfate significantly decreased osteoarthritis in rats, increasing chondrocyte anabolism and reducing cartilage degradation.

In addition, Ryczko et al. (2016) found that glucosamine contributes to the synthesis of proteoglycans and glycoproteins, as it regulates intermediate metabolism, increasing hepatic levels of uridine diphosphate-N-acetylglucosamine, a necessary substrate in multiple protein glycosylation pathways. Uitterlinden et al. (2008) showed that glucosamine increases the production of hyaluronic acid in the synovium, which is important for joint lubrication. Moreover, it has inhibitory effects of the activation of metalloproteinase expression on the reported degenerative process and

Table 6. Proliferating cartilage area (PCA), hypertrophic cartilage área (HCA) and total area (TA) of tibia of broilers at 42 d of age, supplemented with chondroitin and glucosamine sulfates in the diet.

Parameters	Chondroitin[1] (CO, %)	Glucosamine[2] (GLU, %)						Probability		
		0	0.15	0.30	Regression	Mean	SEM	CO	GLU	CO x GLU
PCA (mm^2)	0	28.71	28.56	29.52	ns	29.94	0.45	ns	L (0.028)	ns
	0.05	29.17	30.55	30.13	ns	29.95				
	0.10	29.49	30.64	31.01	ns	30.38				
	Regression	ns	ns	ns						
	Mean	29.12	29.96	30.22						
HCA (mm^2)	0	45.62	44.32	44.32	ns	44.75	0.94	ns	ns	ns
	0.05	43.50	44.32	45.86	ns	44.56				
	0.10	44.52	45.43	43.20	ns	44.38				
	Regression	ns	ns	ns						
	Mean	44.55	44.69	44.46						
TA (mm^2)	0	131.24	130.61	132.24	ns	131.36	1.31	ns	ns	ns
	0.05	129.77	131.32	133.67	ns	131.59				
	0.10	133.30	132.82	132.12	ns	132.76				
	Regression	ns	ns	ns						
	Mean	131.43	131.58	132.70						

Abbreviations: L, linear; ns, not significant.
[1]([$C_{14}H_{21}NO_{14}S$]n, Biofac A/S) purity of 91.27%.
[2]([$C_6H_{14}NO_5$]2SO_4 x 2KCl, Zhejiang Golden-Shell Pharmaceutical Co. Ltd.) sulfate content 16%.

anti-inflammatory action (Imagawa et al., 2011; Jawed et al., 2011; Tanigushi et al., 2012).

Biomechanical tension exerted on the structurally immature epiphyseal cartilage of the leg bones causes microfracture and formation of cartilage fissures, which contribute to the formation of osteochondrosis lesions and hence a higher prevalence of lameness in broilers (Widerman et al., 2012). It is the first time that chondroitin and glucosamine sulfates have been assessed for locomotor problems of broiler chickens, and the results show evidence that glycosaminoglycans could significantly reduce such problems.

Areas of tibial growth plate zones have been used to study the incidence of tibial dyschondroplasia in broiler chickens (Oviedo-Rondón et al., 2001; Franco et al., 2004; Ospina-Rojas et al., 2018). Tian et al. (2013) have demonstrated that broilers with tibial dyschondroplasia had a reduction in the growth plate, with a low number of chondrocytes in the proliferating cartilage area. The inclusion of increasing levels of glucosamine sulfate improved the proliferating cartilage area of chondrocytes owing to its chondro-stimulating property (Wen et al., 2010; Chiusaroli et al., 2011; Imagawa et al., 2011).

Wolff (2014) reported that the association of chondroitin and glucosamine sulfates has increased the proliferation of the growth plate of the tibial epiphyseal disc and bone formation in ovariectomized rats, with organized cell arrangement, increased the number of resting and proliferative chondrocytes, proliferative zone thickness, trabecular bone area, and reduction in the resting and hypertrophic zone thickness.

A high WG and mean weight was observed with the inclusion of increasing levels of glucosamine sulfate, probably because of the positive results regarding locomotor problems of broiler chickens. Nääs et al. (2009) provided evidence that broilers with lameness spend less time on standing activities because of chronic pain, which compromises welfare and results in low feed and water intake (Weeks et al., 2000). Sgavioli et al. (2017) and Santos et al. (2019) confirmed this hypothesis and concluded that chondroitin and glucosamine sulfates were effective in improving feed conversion and breeding viability owing to the positive results regarding bone and cartilaginous development of broilers.

In addition, glucosamine sulfate favors nutrient transport and regulates intermediate metabolism by increasing hepatic levels of uridine diphosphate-N-acetylglucosamine, a necessary substrate for multiple protein glycosylation pathways (Ryczko et al., 2016).

Thus, chondroitin and glucosamine sulfates can be used in the diet of broiler chickens to reduce their locomotor problems. However, ideal inclusion levels could not be established because of the increasing linear effects, and therefore, further studies should be conducted.

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DISCLOSURES

The authors declare no conflicts of interest.

REFERENCES

Almeida Paz, I. C. L., R. G. Garcia, R. Bernardi, I. A. Nääs, F. R. Caldara, L. W. Freitas, L. O. Seno, V. M. O. S. Ferreira, D. F. Pereira, and F. Cavichiolo. 2010. Selecting appropriate bedding to reduce locomotion problems in broilers. Braz. J. Poult. Sci. 12:189–195.

Almeida Paz, I. C. L., A. A. Mendes, M. R. F. B. Martins, B. C. S. Fernandes, I. C. L. Almeida, E. L. Milbradt, A. Balog, and

C. M. Komiyama. 2009. Follow-up of the development of femoral degeneration lesions in broilers. Int. J. Morphol. 27:571–575.

Angel, R. 2007. Metabolic disorders: limitations to growth of and mineral deposition into the broiler skeleton after hatch and potential implications for leg problems. J. Appl. Poult. Res. 16:138–149.

Arafa, N. M. S., H. M. Hamuda, S. T. Melek, and S. K. Darwish. 2013. The effectiveness of Echinacea extract or composite glucosamine, chondroitin and methyl sulfonyl methane supplements on acute and chronic rheumatoid arthritis rat model. Toxicol. Ind. Health 29:187–201.

Calamia, V., L. Lourido, P. Fernández-Puente, J. Mateos, B. Rocha, E. Montell, J. Vergés, C. Ruiz-Romero, and F. J. Blanco. 2012. Secretome analysis of chondroitin sulfate-treated chondrocytes reveals anti-angiogenic, anti-inflammatory and anti-catabolic properties. Arthritis Res. Ther. 14:R202.

Calamia, V., J. Mateos, P. Fernández-Puente, L. Lourido, B. Rocha, C. Fernández-Costa, E. Montell, J. Vergés, C. Ruiz-Romero, and F. J. Blanco. 2014. A pharmacoproteomic study confirms the synergistic effect of chondroitin sulfate and glucosamine. Sci. Rep. 4:R5069.

Calamia, V., C. Ruiz-Romero, B. Rocha, P. Fernández-Puente, J. Mateos, E. Montell, J. Vergés, and F. J. Blanco. 2010. Pharmacoproteomic study of the effects of chondroitin and glucosamine sulfate on human articular chondrocytes. Arthritis Res. Ther. 12:R138.

Chiusaroli, R., T. Piepoli, T. Zanelli, P. Ballanti, M. Lanza, L. C. Rovati, and G. Caselli. 2011. Experimental pharmacology of glucosamine sulfate. Int. J. Rheumatol. 2011:939265.

Cobb-Vantress. 2008. Manual de manejo de frangos de corte. Cobb-Vantress Brasil, Guapiaçu, SP.

Colet, S., R. G. Garcia, I. C. L. Almeida Paz, F. R. Caldara, R. Borille, A. F. B. Royer, I. A. Nääs, and S. Sgavioli. 2015. Bone characteristics of broilers supplemented with vitamin D. Braz. J. Poult. Sci. 17:325–332.

Cunha, A. L., J. A. K. Aguiar, F. S. Correia da Silva, and Y. M. Michelacci. 2017. Do chondroitin sulfates with different structures have different activities on chondrocytes and macrophages? In. J. Biol. Macromol. 103:1019–1031.

Detmann, E., M. A. Souza, S. C. Valadares Filho, A. C. Queiroz, T. T. Berchielli, E. O. S. Saliba, L. S. Cabral, D. S. Pina, M. M. Ladeira, and J. A. G. Azevedo. 2012. Métodos para análise de alimentos: Instituto Nacional de Ciência e Tecnologia de Ciência Animal. Suprema, Visconde do Rio Branco, MG.

Eleotério, R. B., A. P. B. Borges, K. C. S. Pontes, N. A. Fernandes, P. F. Soares, M. B. Silva, N. J. S. Martins, and J. P. Machado. 2012. Glucosamine and chondroitin sulfate in the repair of osteochondral defects in dogs - clinical-radiographic analysis. Ver. Ceres 59:587–596.

Fernandes, B. C. S., M. R. F. B. Martins, A. A. Mendes, I. C. L. Almeida Paz, C. M. Komiyama, E. L. Milbradt, and B. B. Martins. 2012. Locomotion problems of broiler chickens and its relationship with the gait score. Braz. J. Sci. 41:1951–1955.

Franco, J. R. G., A. E. Murakami, M. I. Sakamoto, E. N. Martins, I. Moreira, and M. A. S. Pereira. 2004. Efeito dos ionóforos e do balanço eletrolítico da dieta sobre o desempenho e a incidência de discondroplasia tibial em frangos de corte na fase inicial. Braz. J. Poult. Sci. 33:135–145.

Gupta, R. C., T. D. Canerdy, J. Lindley, M. Konemann, J. Minniear, B. A. Carroll, C. Hendrick, J. T. Goad, K. Rohde, R. Doss, M. Bagchi, and D. Bagchi. 2012. Comparative therapeutic efficacy and safety of type-II collagen (UC-II), glucosamine and chondroitin in arthritic dogs: pain evaluation by ground force plate. J. Anim. Physiol. Anim. Nutr. (Berl) 96:770–777.

Herrero-Beaumont, G., M. E. Marcos, O. Sánchez-Pernaute, R. Granados, L. Ortega, E. Montell, J. Vergés, J. Egido, and R. Largo. 2008. Effect of chondroitin sulphate in a rabbit modelo of atherosclerosis aggravated by chronic arthritis. Br. J. Pharmacol. 154:843–851.

Igarashi, M., K. Sakamoto, and I. Nagaoka. 2017. Effect of glucosamine on expression of type II collagen, matrix metalloproteinase and sirtuin genes in a human chondrocyte cell line. J. Mol. Med. 39:472–478.

Imagawa, K., M. C. Andrés, K. Hashimoto, D. Pitt, E. Itoi, M. B. Goldring, H. I. Roach, and R. O. C. Oreffo. 2011. The epigenetic effect of glucosamine and a nuclear factor-kappa B(NF-kB) inhibitor on primary human chondrocytes – implications for osteoarthritis. Biochem. Biophys. Res. Commun. 405:362–367.

Jawed, H., S. Anjum, S. I. Awan, and S. U. Simjee. 2011. Anti-arthritic effect of GN1, a novel synthetic analog of glucosamine, in the collagen-induced arthritis model in rats. Inflamm. Res. 60:1113–1120.

Julian, R. J. 2005. Production and growth related disorders and other metabolic diseases of poultry - a review. Vet. J. 169:350–369.

Kahan, A., D. Uebelhart, F. De Vathaire, P. D. Delmas, and J. Y. Reginster. 2009. Long-term effects of chondroitins 4 and 6 sulfate on knee osteoarthritis: the study on osteoarthritis progression prevention, a two-year, randomized, double-blind, placebo-controlled trial. Arthritis Rheum. 60:524–533.

Kamarul, T., S. Ab-Rahim, M. Tumin, L. Selvaratnam, and T. S. Ahmad. 2011. A preliminary study of the effects of glucosamine sulphate and chondroitin sulphate on surgically treated and untreated focal cartilage damage. Eur. Cell. Mater. 21:259–271.

Kantor, E. D., J. W. Lampe, S. L. Navarro, X. Song, G. L. Milne, and E. White. 2014. Associations between glucosamine and chondroitin supplement use and biomarkers of systemic inflammation. J. Altern. Complement. Med. 20:479–485.

Kestin, S. C., T. G. Knowles, A. E. Tinch, and N. G. Gregory. 1992. Prevalence of leg weakness in broiler-chickens and its relationship with genotype. Vet. Rec. 131:190–194.

Kim, H. D., E. A. Lee, Y. H. An, S. L. Kim, S. S. Lee, S. J. Yu, H. L. Jang, K. T. Nam, S. G. Im, and N. S. Hwang. 2017. Chondroitin sulfate-based biomineralizing surface hydrogels for bone tissue engineering. ACS. Adv. Mater. Inter. 9:21639–21650.

Largo, R., J. A. Roman-Blas, J. Moreno-Rubio, O. Sánchez-Pernaute, M. J. Martínez-Calatrava, S. Castañeda, and G. Herrero-Beaumont. 2010. Chondroitin sulfate improves synovitis in rabbits with chronic antigen-induced arthritis. Osteoarthr. Cartil. 1:17–23.

Lubis, A. M. T., C. Siagin, E. Wonggokusuma, A. F. Marsetyo, and B. Setyohadi. 2017. Comparison of glucosamine-chondroitin sulfate with and without methylsulfonylmethane in grade I-II knee osteoarthritis: a double blind randomized controlled trial. Acta Med. Indones. 49:105–111.

Luna, L. G. 1968. Manual of Histologic. Staining Methods of the Armed Forces. 3th ed. Institute of Pathology, Mc Graw-Hill, New York, NY.

Maxwell, L. K., P. Regier, and S. Achanta. 2016. Comparison of glucosamine absorption after administration of oral liquid, chewable, and tablet formulations to dogs. J. Anim. Hosp. Assoc. 52:90–94.

Muraleva, N. A., E. N. Ofitserov, V. P. Tikhonov, and N. G. Kolosova. 2012. Efficacy of glucosamine alendronate alone and in combination with dihydroquercetin for treatment of osteoporosis in animal model. Indian J. Med. Res. 135:221–227.

Nääs, I. A., I. C. L. Almeida Paz, M. S. Baracho, A. G. Menezes, L. G. F. Bueno, I. C. L. Almeida, and J. D. Moura. 2009. Impact of lameness on broiler well-being. J. Appl. Poult. Res. 18:432–439.

Ospina-Rojas, I. C., A. E. Murakami, C. R. A. Duarte, M. I. Sakamoto, P. C. Aguihe, P. C. Pozza, and T. C. Santos. 2018. Tibiotarsus bone characteristics and tibial dyschondroplasia incidence of broilers fed diets supplemented with leucine and valine. J. Anim. Physiol. Anim. Nutr. (Berl). 102:768–776.

Oviedo-Rondón, E. O., A. E. Murakami, A. C. Furlan, I. Moreira, and M. Macari. 2001. Sodium and chloride requirements of young broiler chickens fed corn-soybean diets (one to twenty one days of age). Poult. Sci. 80:592–598.

Pecchi, E., S. Priam, Z. Mladenovic, M. Gosset, A. S. Saurel, L. Aguilar, F. Berenbaum, and C. Jacques. 2012. Potential role of chondroitin sulfate on bone in osteoarthritis: inhibition of prostaglandim E_2 and matrix metalloproteinases synthesis in interleukin-1β-stimulated osteoblasts. Osteoarthr. Cartil. 20:127–135.

Rostagno, H. S., L. F. T. Albino, J. L. Donzele, P. C. Gomes, R. F. M. Oliveira, D. C. Lopes, A. S. Ferreira, and S. L. T. Barreto. 2011. Brazilian Tables for Poultry and Swine-Composition of Feedstuffs and Nutritional Requirements. 3th ed. UFV, Viçosa, MG, Brazil.

Ryczko, M. C., J. Pawling, R. Chen, A. M. Abdel Rahman, K. Yau, J. K. Copeland, C. Zhang, A. Surendra, D. S. Guttman, D. Figeys, and J. W. Dennis. 2016. Metabolic reprogramming by hexosamine

biosynthetic and golgi N-glycan branching pathways. Sci. Rep. 6:23043.

Sakomura, N. K., and H. S. Rostagno. 2016. Planejamento dos experimentos com monogástricos. Pages 1–30 in Métodos de pesquisa em nutrição de monogástricos. 2th ed. Funep, Jaboticabal, SP, Brazil.

Santos, E. T., S. Sgavioli, D. M. C. Castiblanco, C. H. F. Domingues, T. C. O. Quadros, L. L. Borges, T. G. Petrolli, and S. M. Baraldi-Artoni. 2018. Glycosaminoglycans and vitamin C *in ovo* feeding affects bone characteristics of chicks. Braz. J. Sci. 47:e20170304.

Santos, E. T., S. Sgavioli, D. M. C. Castiblanco, L. L. Borges, T. C. O. Quadros, A. C. Laurentiz, A. C. Shimano, and S. M. Baraldi-Artoni. 2019. Glycosaminoglycans and vitamin C affect broiler bone paramenters. Poult. Sci. 98:4694–4704.

SAS Institute. 2002. SAS/STAT User's Guide: Statistics. Version 9.2. SAS Inst. Inc., Cary, NC.

Sgavioli, S., E. T. Santos, L. L. Borges, G. M. Andrade-Garcia, D. M. C. Castiblanco, V. R. Almeida, R. G. Garcia, A. C. Shimano, I. A. Nääs, and S. M. Baraldi-Artoni. 2017. Effect of the addition of glycosaminoglycans on bone and cartilaginous development of broiler chickens. Poult. Sci. 96:4017–4025.

Shim, M. Y., A. B. Karnuah, N. B. Anthony, G. M. Pesti, and S. E. Aggrey. 2012. The effects of broiler chicken growth rate on valgus, varus, and tibial dyschondroplasia. Poult. Sci. 91:62–65.

Silva, D. J., and A. C. Queiroz. 2002. Análises de alimentos: métodos químicos e biológicos. 3th ed. Imprensa Universitária da UFV, Viçosa, MG, Brazil.

Taniguchi, S., J. Ryu, M. Seki, T. Sumino, Y. Tokuhaschi, and M. Esumi. 2012. Long-tern oral administration of glucosamine or chondroitin sulfate reduces destruction of cartilage andu p-regulation of MMP-3 mRNA in a model of spontaneous osteorthritis in Hartley Guinea pigs. J. Orthop. Res. 30:673–678.

Tat, S. K., J. P. Pelletier, J. Vergés, D. Lajeunesse, E. Montell, H. Fahmi, M. Lavigne, and J. Martel-Pelletier. 2007. Chondroitin and glucosamine sulfate in combination decrease the pro-resorptive properties of human osteoarthritis subchondral bone osteoblasts: a basic science study. Arthritis Res. Ther. 9:R117.

Thorp, B. H., B. Ducro, and C. C. Whitehead. 1993. Avian tibial dyschondroplasia: the interaction of genetic selection and dietary 1,25-dihydroxycholecalciferol. Avian Pathol. 22:311–324.

Tian, W., J. Li, P. Qin, R. Wang, G. Ning, J. Qiao, H. Li, D. Bi, S. Pan, and D. Guo. 2013. Screening of differentially expressed genes in the growth plate of broiler chickens with tibial dyschondroplasia by microarray analysis. BMC Genom. 14:276.

Uitterlinden, E. J., J. L. M. Koevoet, C. F. Verkoelen, S. M. A. Bierma-Zeinstra, H. Jahr, H. Weinans, J. A. N. Verhaar, and G. J. V. M. Van Osch. 2008. Glucosamine increases hyaluronic acid production in human osteoarthritic synovium explants. BMC. Musculoskelet. Disord. 9:120.

Varghese, S., P. Theprungsirikul, S. Sahani, N. Hwang, K. J. Yarema, and J. H. Elisseeff. 2007. Glucosamine modulates chondrocyte proliferation, matrix synthesis, and gene expression. Osteoarthr. Cartil. 15:59–68.

Vieira, N. T., E. G. Melo, C. M. F. Rezende, M. G. Gomes, F. M. C. Caldeira, and M. C. Jesus. 2010. Efeitos dos glicosaminoglicanos e sulfato de condroitina A sobre a cartilagem articular normal e com doença articular degenerativa em cães. Arq. Bras. Med. Vet. Zootec. 62:1117–1127.

Weeks, C. A., T. D. Danbury, H. C. Davies, T. P. Hun, and S. C. Kestin. 2000. The behavior of broiler chickens and its modification by lamness. Appl. Anim. Behav. Sci. 67:111–125.

Wen, Z. N., C. C. Tang, Y. C. Chang, S. Y. Huang, S. P. Hsieh, C. H. Lee, G. S. Huang, H. F. Ng, C. A. Neoh, C. S. Hsieh, W. F. Chen, and Y. H. Jean. 2010. Glucosamine sulfate reduces experimental osteorthritis and nociception in rats: association with changes of mitogen-activated protein kinase in chondrocytes. Osteoarthr. Cartil. 18:1192–1202.

Wenz, W., C. Hornung, C. Cramer, M. Schroeder, and M. Hoffmann. 2017. Effect of glucosamine sulfate on osteoarthritis in the cruciate-deficient canine modelo of osteoarthritis. Cartil 8:173–179.

Wideman, R., K. Hamal, J. Stark, J. Blankenship, H. Lester, K. Mitchell, G. Lorenzoni, and I. Pevzner. 2012. A wire-flooring model for inducing lameness in broilers: evaluation of probiotics as a prophylactic treatment. Poult. Sci. 91:870–883.

Wildi, L. M., J. P. Raynauld, J. Martel-Pelletier, A. Beaulieu, L. Bessette, F. Morin, F. Abram, M. Dorais, and J. P. Pelletier. 2011. Chondroitin sulphate reduces both cartilage volume loss and bone marrow lesions in knee osteoarthritis patients starting as early as 6 months after initiation of therapy: a randomised, double-blind, placebo-controlled pilot study using MRI. Ann. Rheum. Dis. 70:982–989.

Wolff, R. B. 2014. Glucosamine and chondroitin sulfate association increases tibial epiphyseal growth plate proliferation and bone formation in ovariectomized rats. Clinics 69:847–853.