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Performance, nutrient digestibility, and intestinal histomorphometry of broilers fed diet supplemented with chondroitin and glucosamine sulfates

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ABSTRACT - We aimed to evaluate the performance, nutrient digestibility, and intestinal histomorphometry of broilers fed diet supplemented with chondroitin sulfate and glucosamine sulfate. The experiment was carried out with 320 male broiler chicks distributed in a completely randomized design in a 2×2 factorial scheme (0 and 0.1% chondroitin sulfate and 0 and 0.3% glucosamine sulfate), with eight replications of 10 birds. Performance was evaluated at 7 and 21 days of age, nutrient digestibility of the diet was performed from 18 to 21 days of age, and small intestine histomorphometry was evaluated at 21 days of age. Broilers fed diet supplemented with 0.3% glucosamine sulfate showed high final weight and weight gain. A significant interaction was observed between sulfates for digestibility coefficients of nitrogen, mineral matter, and calcium. The use of 0.1% chondroitin sulfate without glucosamine sulfate resulted in a reduced digestibility of nitrogen but increased digestibility of total minerals and calcium. Diets without chondroitin sulfate with 0.3% glucosamine sulfate increased the digestibility coefficients of mineral matter and calcium. A significant interaction was found for jejunum villus height, which was higher in broilers fed diet supplemented with 0.3% glucosamine sulfate, regardless of the inclusion of chondroitin sulfate. Thus, supplementation with glucosamine sulfate in broiler diets contributes to high weight gain and villus height. Sulfates used in isolation promote high digestibility of minerals.

Keywords: intestinal health, metabolism, metabolizability, polysulfated glycosaminoglycans

Introduction

Poultry performance and digestibility coefficient depend on the maintenance of gastrointestinal tract structures, which includes structural and functional integrity. Growth of small intestine structures of broilers, such as villus height, crypt depth, and the villus to crypt, ratio have been investigated due to their relationship with digestion and absorption processes (Boleli and Morita, 2017).

Polysulfated glycosaminoglycans (GAG), chondroitin, and glucosamine have been studied as nutraceuticals in the prevention and/or treatment of pathologies of bone and joint structures in broilers (Sgavioli et al., 2017; Santos et al., 2018; Santos et al., 2019). Chondroitin sulfate is composed of hexosamine D-galactosamine and D-glucuronic acid. It is the most common natural GAG in aggrecan

molecule in cartilage. Due to sulfation, it has a high density of negative charges, which is responsible for water retention in cartilage and high osmotic pressure (Jerosch, 2011).

Glucosamine is a hexosamine that can be administered in the form of glucosamine hydrochloride, glucosamine sulfate, or N-acetyl-D-glucosamine. It is an important precursor to glycoproteins and GAG, such as hyaluronic acid, heparan sulfate, and keratan sulfate (Jerosch, 2011). Besides their chondroprotective and chondrostimulating role, GAG may increase epithelial defenses, contributing to intestinal integrity (Salvatore et al., 2000; Hori et al., 2001; Segarra et al., 2016) and mucin biosynthesis (Salvatore et al., 2000) in dogs, humans, and mice.

Glucosamine and chondroitin have anti-inflammatory action and are pharmacologically classified as slow-acting symptomatic (Brandt et al., 2002). These components can act as bioactive compounds and modulate intestinal microbiota, reducing the incidence of pathogenic microorganisms (Garrido et al., 2012; Coulson et al., 2013; Shang et al., 2016; Liu et al., 2017). When the gastrointestinal tract is colonized by enteric pathogens, intestinal lining damage impairs mucosal function, nutrient use, and cell turnover, which in turn has a direct influence on intestinal histophometry (Boleli and Thimotheo, 2017).

Besides, there is evidence that chondroitin and glucosamine can act as bioactive compounds and modulate intestinal microbiota proliferation (Garrido et al., 2012; Coulson et al., 2013; Shang et al., 2016; Liu et al., 2017), which could contribute to intestinal tropism and, consequently, higher nutrient digestibility.

However, no research has been published on the use of GAG in terms of nutrient digestibility and intestinal histomorphometry of broilers; therefore, we aimed to study the use of GAG in broiler diets to evaluate if they might contribute to broiler performance, nutrient digestibility, and intestinal histomorphometry.

Material and Methods

All procedures in this study were performed according to the Protocol CEUA 051/16, approved by the local Animal Use Ethics Committee in Goiânia, Goiás, Brazil (16°35'33" S and 49°16'51" W, with an altitude of 710 m).

A total of 320-day-old male Cobb 500® broiler chicks, with mean initial weight of 39±0.2 g from a commercial hatchery (São Salvador Alimentos S/A, Itaberaí, Goiás, Brazil), were used. The animals were housed in metal batteries containing galvanized wire cages, with dimensions of 0.25 m height × 0.75 m width × 0.80 m length, screen floor, excreta collection trays, and equipped with drinking and feeding troughs. These batteries were located in a masonry shed with clay tile roof, concrete floor, and sides with a short wall, screen, and curtains.

Chicks were vaccinated in the hatchery against Marek's disease and avian bronchitis and Gumboro disease via drinking water at 14 days of age. Animals received ration and water ad libitum throughout the experimental period, being raised following the lighting, temperature, humidity, and management recommendations by Cobb-Vantress Management Guide (Cobb-Vantress, 2008).

The experimental design was completely randomized in a 2×2 factorial scheme (two chondroitin sulfate inclusion levels – 0 and 0.1%, and two glucosamine sulfate inclusion levels – 0 and 0.3%), with eight replications of 10 animals in each treatment, totaling 32 experimental plots. Chondroitin sulfate [$(C_{14}H_{21}NO_{14}S)n$, Biofac A/S, Englandsvej, Kastrup, Denmark] was 91.27% pure, while potassium glucosamine sulfate [$(C_6H_{14}NO_5)$ $2SO_4$ × 2KCl, Zhejiang Golden-Shell Pharmaceutical Co. Ltd., Yuhuan, Zhejiang, China] had 16% sulfate.

Diets were formulated based on corn and soybean meal, following the recommendations of Rostagno et al. (2011) for the pre-starter (1 to 7 days of age) and starter (8 to 21 days of age) rearing phases, with a variable portion of 0.4% for chondroitin sulfate and/or glucosamine sulfate and/or inert inclusions according to the treatments (Table 1).

The nutritional composition of experimental diets was analyzed for dry matter, crude energy, crude protein, calcium, and phosphorus contents. Dry matter was determined by the gravimetric method, using heat, 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 by the calorimetric pump (model 6100, Parr® Instrument Company, Moline, Illinois, United States). 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). A factor of 6.25 was used to convert the nitrogen value into crude protein due to the widespread use of this value by nutrition laboratories. Ash content was determined by calcination of samples in a muffle (model F-3, Fornitec®, São Paulo, São Paulo, Brazil) at 600 °C. Calcium and phosphorus contents were analyzed using an atomic absorption spectrophotometer (model AA-7000, Shimadzu®, Barueri, São Paulo, Brazil) and a UV/VIS spectrophotometer (model UV-5100, Tecnal®, Piracicaba, São Paulo, Brazil), respectively, as proposed by Silva and Queiroz (2002).

Table 1 - Ingredients and calculated nutritional composition of diets for broilers in pre-starter (1 to 7 days old) and starter (8 to 21 days old) phases

Item	Pre-starter	Starter
Ingredient (%)		
Corn	54.46	59.80
Soybean meal (45.5%)	35.16	30.78
Poultry fat	1.13	1.40
Meat and bone meal (47%)	3.67	4.40
Offal meal (62.5%)	3.00	1.13
Limestone	0.53	0.53
Salt	0.39	0.35
Sodium bicarbonate	0.08	0.05
Choline chloride (75%)	0.05	0.08
DL-Methionine (99%)	0.41	0.35
L-Lysine HCL (64%)	0.33	0.34
L-Threonine (98%)	0.07	0.08
L-Valine (96.5%)	0.02	0.01
Vitamin supplement[1]	0.05	0.05
Mineral supplement[2]	0.05	0.05
Additives[3]	0.20	0.20
Variable portion[4]	0.40	0.40
Total	100.00	100.00
Calculated nutritional composition		
Metabolizable energy (kcal/kg)	3000	3050
Crude protein (%)	25.00	22.50
Calcium (%)	0.98	0.98
Available phosphorus (%)	0.49	0.48
Sodium (%)	0.22	0.21
Chlorine (%)	0.30	0.27
Potassium (%)	0.90	0.82
Digestible methionine + cystine (%)	1.03	0.92
Digestible methionine (%)	0.73	0.64
Digestible lysine (%)	1.36	1.21
Digestible threonine (%)	0.87	0.79

[1] Vitamin supplement (composition per kg product): vitamin A, 20,000,000 IU; vitamin D3, 5,000,000 IU; vitamin E, 50,000 IU; vitamin K3, 4000 mg; vitamin B1, 5000 mg; vitamin B2, 13,000 mg; vitamin B6, 7000 mg; vitamin B12 36 mg; niacina, 84,000 mg; pantothenate, 30,000 mg; folic acid, 2400 mg; biotin, 160 mg; selenium, 600 mg.
[2] Mineral supplement (composition per kg product): copper, 16.25 g; iron, 100 g; iodine, 2000 g; manganese, 150 g; zinc, 125 g.
[3] Additives: 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.
[4] Variable portion: chondroitin sulfate [$(C_{14}H_{21}NO_{14}S)n$, Biofac A/S, Englandsvej, Kastrup, Denmark] and/or potassium glucosamine sulfate [$(C_6H_{14}NO_5)$ $2SO_4$ × 2KCl, Zhejiang Golden-Shell Pharmaceutical Co. Ltd., Yuhuan, Zhejiang, China] and/or inert (kaulin) according to treatments.

The analyzed values of crude energy, crude protein, calcium, and total phosphorus were 3,800 and 3,400 kcal/kg, 24 and 22%, 0.96 and 0.95%, and 0.75 and 0.73% for the pre-starter and starter phases, respectively.

Performance variables (mean final weight, mean weight gain, mean feed intake, feed conversion, and productive viability) were evaluated at 7 and 21 days of age. For this, animals, offered ration, and leftovers were weighed weekly, and the number and weight of dead animals were recorded daily.

The mean final weight (FW) was obtained by dividing the total weight (TW) of animals in the cage by the number (N) of animals at the end of the experimental period (FW = TW/N). Weight gain (WG) was calculated by the difference between the final weight and the initial weight (IW) of animals (WG = FW − IW). The mean feed intake (FI) was obtained by the difference between the weight of the offered ration (OR) and the resulting leftover (LR) at each phase divided by the number of animals [FI = (OR − LR)/N]. Feed conversion (FC) was obtained by the ratio between the mean feed intake and the mean weight gain of animals {FC = FI/[WM + TW − (IW*N)]}, corrected by weight mortality (WM) according to Sakomura and Rostagno (2016). Productive viability (V) was calculated as a percentage of surviving animals in relation to the initial number of housed birds (V = 100 − mortality*100/N initial).

The digestibility coefficients of nutrients and energy of diets were performed by the total excreta collection method, from 18 to 21 days of age, corresponding to four days of collection, as described by Sibbald and Slinger (1963) and adapted by Sakomura and Rostagno (2016). Feed intake, total excreta, and weight gain were measured during the experimental period. Two excreta collections were performed daily (8:00 and 16:00 h) to avoid fermentation.

Excreta were stored in identified plastic bags and stored in a freezer until the end of the collection period. These samples were then thawed, homogenized, and aliquoted. Subsequently, they were pre-dried in an air-circulation oven (model MA 035/5, Marconi Equipamentos para Laboratório Ltda, Piracicaba, São Paulo, Brazil) at 55 °C for 72 h. The following analyses were then carried out: dry matter content in a drying oven (model 315/3, Fanen®, Guarulhos, São Paulo, Brazil) at 105 °C; nitrogen content 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); crude energy, by using a calorimetric pump (model 6100, Parr® Instrument Company, Moline, Illinois, United States); ash content by calcination of samples in a furnace at 600 °C (model F-3, Fornitec®, São Paulo, São Paulo, Brazil); and calcium content, by using an atomic absorption spectrophotometer (model AA-7000, Shimadzu®, Barueri, São Paulo, Brazil), as proposed by Silva and Queiroz (2002).

The values of the digestibility coefficient of nutrients and energy were calculated using the equations proposed by Sakomura and Rostagno (2016) and Matterson et al. (1965), respectively.

One animal per replication was euthanized by cervical dislocation after 8 h of fasting at 21 days of age, totaling eight animals per treatment, which represents the mean weight of the plot (±5%).

Histological slides were made using 2.0-cm segments from the duodenum (distal portion of the duodenal loop), jejunum (2.0 cm before the ileal diverticulum), and ileum (2.0 cm after the ileal diverticulum), which were fixed in 10% buffered formaldehyde solution for 24 h. They were then stored in 70% alcohol, processed according to the methodology of Luna (1968), and stained by the Hematoxylin-Eosin method. Subsequently, 5-µm thick semi-serial sections were made with an electronic rotary microtome (model RM2255, Leica Biosystems, Buffalo Grove, Illinois, United States).

Images were obtained in fivefold magnification using an optical microscope (model DM4000B, Leica Microsystems, Wetzlar, Hessen, Germany) coupled to a microcomputer. The images were analyzed using the software ImageJ (National Institutes of Health, Bethesda, Maryland, United States; freeware, https://imagej.net/Welcome), in which 20 measurements of the villus height and crypt depth were taken from each segment per replication, totaling 160 measurements for each segment in the treatment and 640 in total for each measure and segment. Villus height was obtained from the basal villus region to its apex, while crypt depth was measured from its base to the villus/crypt transition region (Fukayama et al., 2005). The villus height to crypt depth ratio was calculated by dividing villus height by crypt depth.

The effects of the inclusion of chondroitin sulfate (CO) (0 and 0.1%) and glucosamine (GLU) (0 and 0.3%) sulfate, as well as their interaction (CO × GLU), were analyzed according to the experimental model:

$$Yijk = \mu + (CO)i + (GLU)j + (CO \times GLU)ij + eijk,$$

in which 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 between chondroitin sulfate and glucosamine sulfate, and eijk is the residual error.

The data were verified for the presence of outliers (box-and-whisker plot), homogeneity of variances (Bartlett's test), and normality of residuals (Cramér-von Mises). Subsequently, they were subjected to analysis of variance, and the means were compared by the F test at 5% probability using the general linear model (GLM) procedure of the software SAS® (Statistical Analysis System, version 9.2).

Results

No effect of treatments was observed on the parameters evaluated for performance from 1 to 7 days of age (P>0.05). However, broilers fed diet supplemented with 0.3% glucosamine sulfate showed higher final weight (P = 0.0221) and weight gain (P = 0.0229) than broilers from 1 to 21 days of age fed diet with no supplementation (Table 2).

An interaction was observed between chondroitin sulfate and glucosamine sulfate for digestibility coefficients of nitrogen (P = 0.0173), mineral matter (P = 0.0089), and calcium (P = 0.0028) from 18 to 21 days of age. The use of 0.1% chondroitin sulfate without glucosamine sulfate resulted in a reduced digestibility of nitrogen (P = 0.0020) but increased the digestibility coefficients of mineral matter and

Table 2 - Final weight (FW), weight gain (WG), feed intake (FI), feed conversion (FC), and viability (V) of broilers at 21 days of age fed diets supplemented with chondroitin sulfate and glucosamine sulfate

		Chondroitin[1] (CO, %)	Glucosamine[2] (GLU, %)		P	Mean	SEM	CV	Probability		
			0	0.3					CO	GLU	CO × GLU
1 to 21 days of age	FW (g)	0	903.00	977.28	0.2982	940.14	16.55	6.29	0.2233	0.0221	0.0651
		0.1	950.14	986.86	0.1700	968.50					
		P	0.1546	0.7680							
		Mean	926.57B	982.07A							
	WG (g)	0	863.14	937.28	0.0295	900.21	16.54	6.55	0.2161	0.0229	0.0652
		0.1	911.00	947.00	0.2723	929.00					
		P	0.1483	0.7644							
		Mean	887.07B	942.14A							
	FI (g)	0	1166.85	1228.00	0.2085	1197.43	22.80	7.38	0.8891	0.1643	0.5362
		0.1	1184.71	1219.57	0.4684	1202.14					
		P	0.7092	0.8601							
		Mean	1175.79	1223.79							
	FC (g/g)	0	1.325	1.307	0.6031	1.329	0.02	4.96	0.1711	0.4378	0.4694
		0.1	1.292	1.271	0.5613	1.301					
		P	0.3434	0.3138							
		Mean	1.321	1.313							
	V (%)	0	98.41	98.41	1.0000	98.41	1.09	4.18	1.0000	0.3269	0.5938
		0.1	100.00	96.82	0.1781	98.29					
		P	0.4942	0.4770							
		Mean	99.14	97.62							

P - probability; SEM - standard error of the mean; CV - coefficient of variation.
[1] [$(C_{14}H_{21}NO_{14}S)n$, Biofac A/S] purity of 91.27%.
[2] [$(C_6H_{14}NO_5)2SO_4 \times 2KCl$, Zhejiang Golden-Shell Pharmaceutical Co. Ltd.] sulfate content 16%.
A,B: means followed by distinct letters in the same line, differ significantly by the F-test 5% probability.

calcium (P = 0.0375 and P = 0.0478, respectively). The diets without chondroitin sulfate with 0.3% glucosamine sulfate increased the digestibility of mineral matter and calcium (P = 0.0009 and P = 0.0002, respectively) (Table 3).

An interaction (P = 0.0118) of sulfates was observed for jejunum villus height, which was higher in broilers fed diet supplemented with 0.3% glucosamine sulfate, regardless of the chondroitin sulfate inclusion (P = 0.0131 and P = 0.0209, respectively) (Table 4). No effect of treatments was observed on the evaluated parameters for duodenum and ileum (P>0.05).

Discussion

Supplementation with chondroitin sulfate and glucosamine sulfate in the diet of broilers was evaluated for the ability to promote an improvement in the intestinal histomorphometry, nutrient digestibility, and performance.

A longer villi in the jejunum of broilers fed diet supplemented with glucosamine sulfate may be related to the ability of this nutraceutical to increase epithelial defenses, which contributes to intestinal integrity and histomorphometry, as observed in humans (Salvatore et al., 2000). According to Liu et al. (2012), glucosamine may promote stabilization of intestinal mucosa barrier, which reduces penetration of intestinal endotoxins, food metabolites, and bacteria. These factors interfere with mucosal cell metabolism and intestinal histomorphometry.

Besides, glucosamine acts to prevent tissue damage by protecting the digestive mucosa (Liu et al., 2012; Ryczko et al., 2016), due to its anti-inflammatory effects (Yomogida et al., 2008; Bak et al., 2014),

Table 3 - Digestibility coefficients of dry matter (DCDM), nitrogen (DCN), mineral matter (DCMM), calcium (DCCa), and apparent metabolizable energy corrected by nitrogen balance (AMEn) of broilers from 18 to 21 days of age fed diets supplemented with chondroitin sulfate and glucosamine sulfate

	Chondroitin[1] (CO, %)	Glucosamine[2] (GLU, %)		P	Mean	SEM	CV	Probability		
		0	0.3					CO	GLU	CO × GLU
DCDM (%)	0	79.29	79.14	0.8140	79.22	0.29	1.35	0.1894	0.2465	0.2764
	0.1	79.07	78.22	0.1491	78.65					
	P	0.7201	0.1348							
	Mean	79.17	78.64							
DCN (%)	0	75.68a	73.70	0.1454	74.69	0.72	3.35	0.0086	0.9378	0.0173
	0.1	71.13b	72.94	0.1817	72.04					
	P	0.0020	0.5659							
	Mean	73.40	73.32							
DCMM (%)	0	56.70bB	68.72a	0.0009	62.71	1.81	9.39	0.7591	0.0169	0.0089
	0.1	63.67a	63.14	0.8684	63.40					
	P	0.0375	0.0905							
	Mean	60.18	65.93							
DCCa (%)	0	60.61bB	79.41A	0.0002	70.01	2.57	11.61	0.9261	0.0034	0.0028
	0.1	69.69a	70.90	0.7829	70.30					
	P	0.0478	0.0625							
	Mean	65.15	75.16							
AMEn (kcal/kg DM)	0	3246.85	3274.50	0.4388	3260.68	17.41	2.03	0.1467	0.8904	0.3040
	0.1	3240.71	3206.15	0.3349	3223.43					
	P	0.8626	0.0634							
	Mean	3243.78	3240.33							

P - probability; SEM - standard error of the mean; CV - coefficient of variation.
[1] [$(C_{14}H_{21}NO_{14}S)n$, Biofac A/S] purity of 91.27%.
[2] [$(C_6H_{14}NO_5)2SO_4 \times 2KCl$, Zhejiang Golden-Shell Pharmaceutical Co. Ltd.] sulfate content 16%.
A,B and a,b - means followed by distinct letters in the same line and column, respectively, differ significantly by the F-test 5% probability.

because it suppresses the activation of the nuclear factor Kappa B (NF-κB), an inflation marker, blocking related inflammatory responses (Bak et al., 2014).

Bak et al. (2014) demonstrated that glucosamine supplementation reduces inflammatory responses (IL-8, IL-1β, and TNF-α) mediated by NF-κB. This anti-inflammatory activity is believed to derive from interaction with NF-κB protein structure due to negatively charged groups, such as sulfate and carboxyl

Table 4 - Villus height, crypt depth, and villus height to crypt depth ratio (villus:crypt) of duodenum, jejunum, and ileum of broilers at 21 days of age fed diet supplemented with chondroitin sulfate and glucosamine sulfate

		Chondroitin[1] (CO, %)	Glucosamine[2] (GLU, %)		P	Mean	SEM	CV	Probability		
			0	0.3					CO	GLU	CO × GLU
	Villus (µm)	0	1535.72	1537.30	0.9835	1537.57	36.80	9.02	0.3098	0.5679	0.6173
		0.1	1450.66	1511.12	0.4328	1478.56					
		P	0.2730	0.7326							
		Mean	1489.92	1525.22							
Duodenum	Crypt (µm)	0	182.53	178.11	0.7804	180.15	7.58	15.98	0.4971	0.7206	0.7474
		0.1	166.46	178.90	0.4353	172.20					
		P	0.3158	0.9604							
		Mean	173.88	178.48							
	Villus:crypt	0	8.58	8.87	0.7362	8.74	0.40	17.29	0.9682	0.9714	0.9866
		0.1	8.86	8.55	0.7184	8.72					
		P	0.7463	0.7084							
		Mean	8.73	8.72							
	Villus (µm)	0	968.41B	1094.90A	0.0209	1031.68	28.95	9.32	0.5397	0.0013	0.0118
		0.1	934.25B	1082.50A	0.0131	1008.38					
		P	0.5251	0.8162							
		Mean	951.33	1088.72							
Jejunum	Crypt (µm)	0	150.58	173.14	0.2481	161.86	9.46	12.25	0.7590	0.1825	0.5604
		0.1	149.53	165.50	0.4451	157.52					
		P	0.9581	0.7030							
		Mean	150.10	169.62							
	Villus:crypt	0	6.81	6.51	0.6405	6.66	0.31	17.89	0.7574	0.9630	0.9259
		0.1	6.39	6.64	0.7116	6.51					
		P	0.5269	0.8428							
		Mean	6.61	6.57							
	Villus (µm)	0	657.48	714.53	0.2720	686.01	25.52	13.57	0.5220	0.1068	0.3642
		0.1	676.92	742.66	0.2252	712.32					
		P	0.7160	0.5842							
		Mean	666.45	728.59							
Ileum	Crypt (µm)	0	126.11	142.68	0.2000	134.40	6.25	17.57	0.8310	0.1146	0.4439
		0.1	125.90	139.06	0.3247	132.98					
		P	0.9871	0.7752							
		Mean	126.02	140.87							
	Villus:crypt	0	5.37	5.06	0.4535	5.21	0.20	14.40	0.5258	0.5667	0.8018
		0.10	5.42	5.39	0.9421	5.40					
		P	0.9078	0.4274							
		Mean	5.39	5.22							

P - probability; SEM - standard error of the mean; CV - coefficient of variation.
[1] [$(C_{14}H_{21}NO_{14}S)n$, Biofac A/S] purity of 91.27%.
[2] [$(C_6H_{14}NO_5)2SO_4 \times 2KCl$, Zhejiang Golden-Shell Pharmaceutical Co. Ltd.] sulfate content 16%.
A,B: means followed by distinct letters in the same line, differ significantly by the F-test 5% probability.

groups (Campo et al., 2009). The blocking of inflammatory responses in intestinal mucosa reduces possible tissue damage, besides contributing to mitosis in the crypt region and intestinal epithelium villi.

The area of nutrient digestion and absorption is directly related to the size and quantity of intestinal villi (Boleli and Thimotheo, 2017). Mori et al. (2016) found a positive correlation between the nutrient digestibility and intestinal histomorphometry of broiler jejunum. The jejunum and upper ileum are the main sites of digestion and absorption of minerals in broilers (Mutucumarana et al., 2014). Therefore, a high villus height may have contributed positively to the digestibility of mineral matter and calcium.

In addition, sulfate and carboxylic groupings of glycosaminoglycans have high negative charge density, which allows them to bind to various substances, including cations such as calcium and sodium, which are osmotically active (Embery et al., 1998; Ruiz Hernandez et al., 2015; Kim et al., 2017).

According to Ruiz Hernandez et al. (2015), charged ions interact with D-galactosamine, a hexosamine of chondroitin sulfate, mainly through acetyl amine sulfate and carbonyl groups. Glucosamine sulfate, otherwise, interacts through sulphate groups so that intensity depends on orientation to the interaction surface and saccharide concentration. Thus, the inclusion of chondroitin sulfate and glucosamine sulfate in the diet may have improved the digestibility of minerals and calcium due to their ability to complex with ions.

Due to the diverse and important biological functions that minerals exert in the organism of animals (Silva and Pascoal, 2014), the improvement in the digestibility of mineral matter and calcium with the inclusion of glucosamine sulfate may have contributed to the mean weight and weight gain of broilers from 1 to 21 days of age. As some studies have shown, an increased digestibility of minerals contributes to improved broiler performance (Islam et al., 2012; Abdulla et al., 2016; Bradbury et al., 2016; Gautier et al., 2018).

However, the lack of information on the action mode of glycosaminoglycans in vivo in the digestibility of diet nutrients reinforces the need for studies that elucidate the physiological ways of using these compounds.

In addition, glucosamine may have determined an increase in weight gain, as it can increase nutrient transport and regulate intermediate metabolism, increasing hepatic levels of uridine diphosphate-N-acetylglucosamine (UDP-GlcNAc), a substrate required in various protein glycosylation pathways. Once absorbed by the cell, glucosamine is phosphorylated by hexokinases into glucosamine-6-phosphate, which then enters a hexosamine biosynthetic pathway to generate UDP-GlcNAc. This is a substrate required for several protein glycosylation pathways and widely affect proteome (Ryczko et al., 2016). Ryczko et al. (2016) observed that oral administration of glucosamine increased body weight without affecting feed intake, physical activity, and energy expenditure in mice.

Conclusions

Supplementation with glucosamine sulfate in broiler diets contributes to high weight gain and villus height. Sulfates used in isolation promote a high digestibility of minerals. However, an additive with glycosaminoglycans for broilers has not yet been developed.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Data curation: L.D. Santos Neto and L.P.S. Gomides. Formal analysis: E.S. Fernandes and S. Sgavioli. Funding acquisition: M.B. Café. Investigation: J.M.S. Martins and E.S. Fernandes. Methodology: J.M.S. Martins, L.D. Santos Neto and L.P.S. Gomides. Project administration: M.B. Café. Supervision: S. Sgavioli

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References

Abdulla, N. R.; Loh, T. C.; Akit, H.; Sazili, A. Q. and Foo, H. L. 2016. Effects of dietary oil sources and calcium: phosphorus levels on growth performance, gut morphology and apparent digestibility of broiler chickens. South African Journal of Animal Science 46:42-53. https://doi.org/10.4314/sajas.v46i1.6

Bak, Y. K.; Lampe, J. W. and Sung, M. K. 2014. Effects of dietary supplementation of glucosamine sulfate on intestinal inflammation in a mouse model of experimental colitis. Journal of Gastroenterology and Hepatology 29:957-963. https://doi.org/10.1111/jgh.12485

Boleli, I. C. and Morita, V. S. 2017. Mucosa gastrintestinal. In: Fisiologia das aves comerciais. Macari, M. and Maiorka, A., eds. Funep, Jaboticabal.

Boleli, I. C. and Thimotheo, M. 2017. Estrutura funcional do trato gastrintestinal: da percepção à absorção. In: Fisiologia das aves comerciais. Macari, M. and Maiorka, A., eds. Funep, Jaboticabal.

Bradbury, E. J.; Wikinson, S. J.; Cronin, G. M.; Walk, C. L. and Cowieson, A. J. 2016. Effects of phytase, calcium source, calcium concentration and particle size on broiler performance, nutrient digestibility and skeletal integrity. Animal Production Science 58:271-283. https://doi.org/10.1071/AN16175

Brandt, K. D.; Mazzuca, S. A.; Conrozier, T.; Dacre, J. E.; Peterfy, C. G.; Provvedini, D.; Ravaud, P.; Taccoen, A. and Vignon, E. 2002. Which is the best radiographic protocol for a clinical trial of a structure modifying drug in patients with knee osteoarthritis? Journal of Rheumatology 29:1308-1320.

Campo, G. M.; Avenoso, A.; Campo, S.; D'Ascola, A.; Traina, P.; Samà, D. and Calatroni, A. 2009. Glycosaminoglycans modulate inflammation and apoptosis in LPS-treated chondrocytes. Journal of Cellular Biochemistry 106:83-92. https://doi.org/10.1002/jcb.21981

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

Coulson, S.; Butt, H.; Vecchio, P.; Gramotnev, H. and Vitetta, L. 2013. Green-lipped mussel extract (*Perna canaliculus*) and glucosamine sulphate in patients with knee osteoarthritis: therapeutic efficacy and effects on gastrointestinal microbiota profiles. Inflammopharmacology 21:79-90. https://doi.org/10.1007/s10787-012-0146-4

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

Embery, G.; Rees, S.; Hall, R.; Rose, K.; Waddington, R. and Shellis, P. 1998. Calcium- and hydroxyapatite-binding properties of glucuronic acid-rich and iduronic acid-rich glycosaminoglycans and proteoglycans. European Journal of Oral Sciences 106:267-273. https://doi.org/10.1111/j.1600-0722.1998.tb02186.x

Fukayama, E. H.; Bertechini, A. G.; Geraldo, A.; Kato, R. K. and Murgas, L. D. S. 2005. Extrato de orégano como aditivo em rações para frangos de corte. Revista Brasileira de Zootecnia 34:2316-2326. https://doi.org/10.1590/S1516-35982005000700018

Garrido, D.; Ruiz-Moyano, S. and Mills, D. A. 2012. Release and utilization of N-acetyl-D-glucosamine from human milk oligosaccharides by *Bifidobacterium longum* subsp. *infantis*. Anaerobe 18:430-435. https://doi.org/10.1016/j.anaerobe.2012.04.012

Gautier, A. E.; Walk, C. L. and Dilger, R. N. 2018. Effects of a high level of phytase on broiler performance, bone ash, phosphorus utilization, and phytate dephosphorylation to inositol. Poultry Science 97:211-218. https://doi.org/10.3382/ps/pex291

Hori, Y.; Hoshino, J.; Yamazaki, C.; Sekiguchi, T.; Miyauchi, S. and Horie, K. 2001. Effects of chondroitin sulfate on colitis induced by dextran sulfate sodium in rats. The Japanese Journal of Pharmacology 85:155-160. https://doi.org/10.1254/jjp.85.155

Islam, K. M. S.; Schaeublin, H.; Wenk, C.; Wanner, M. and Liesegang, A. 2012. Effect of dietary citric acid on the performance and mineral metabolism of broiler. Journal of Animal Physiology and Animal Nutrition 96:808-817. https://doi.org/10.1111/j.1439-0396.2011.01225.x

Jerosch, J. 2011. Effects of glucosamine and chondroitin sulfate on cartilage metabolism in AO: outlook on other nutrient partners especially omega-3 fatty acids. International Journal of Rheumatology 2011:969012. https://doi.org/10.1155/2011/969012

Kim, H. D.; Lee, E. A.; An, Y. H.; Kim, S. L.; Lee, S. S.; Yu, S. J.; Jang, H. L.; Nam, K. T.; Im, S. G. and Hwang, N. S. 2017. Chondroitin sulfate-based biomineralizing surface hydrogels for bone tissue engineering. ACS Applied Materials & Interfaces 9:21639-21650. https://doi.org/10.1021/acsami.7b04114

Liu, F.; Zhang, N.; Li, Z.; Wang, X.; Shi, H.; Xue, C.; Li, R. W. and Tang, Q. 2017. Chondroitin sulfate disaccharides modified the structure and function of the murine gut microbiome under healthy and stressed conditions. Scientific Reports 7:6783. https://doi.org/10.1038/s41598-017-05860-6

Liu, Y.; Xu, W.; Liu, L.; Guo, L.; Deng, Y. and Liu, J. 2012. N-acetyl glucosamine improves intestinal mucosal barrier function in rat. Bangladesh Journal of Pharmacology 7:281-284. https://doi.org/10.3329/bjp.v7i4.12806

Luna, L. G. 1968. Manual of histologic. Staining Methods of the Armed Forces. Institute of Pathology. 3rd ed. Mc Graw-Hill, New York, US.

Matterson, L. D.; Potter, L. M.; Stutz, M. W. and Singsen, E. P. 1965. The metabolizable energy of feeds ingredients for chickens. Research Report, Storrs, Connecticut, The University of Connecticut, Agricultural Experiment Station.

Mori, A.; Martins, J. M. S.; Carvalho, G. B.; Carvalho, F. B.; Stringhini, J. H.; Leandro, N. S. M.; Andrade, M. A. and Café, M. B. 2016. Nutrient digestibility parameters as a tool for analysis of the intestinal health of broiler chickens. Semina: Ciências Agrárias 37:2499-2512. https://doi.org/10.5433/1679-0359.2016v37n4Supl1p2499

Mutucumarana, R. K.; Ravindran, V.; Ravindran, G. and Cowieson, A. J. 2014. Influence of dietary calcium concentration on the digestion of nutrients along the intestinal tract of broiler chickens. The Journal of Poultry Science 51:392-401. https://doi.org/10.2141/jpsa.0140022

Rostagno, H. S.; Albino, L. F. T.; Donzele, J. L.; Gomes, P. C.; Oliveira, R. F.; Lopes, D. C.; Ferreira, A. S.; Barreto, S. L. T. and Euclides, R. F. 2011. Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais. 3.ed. Universidade Federal de Viçosa, Viçosa, MG.

Ruiz Hernandez, S. E.; Streeter, I. and Leeuw, N. H. 2015. The effect of water on the binding of glycosaminoglycan saccharides to hydroxyapatite surfaces: a molecular dynamics study. Physical Chemistry Chemical Physics 17:22377-22388. https://doi.org/10.1039/c5cp02630j

Ryczko, M. C.; Pawling, J.; Chen, R.; Abdel Rahman, A. M.; Yau, K.; Copeland, J. K.; Zhang, C.; Surendra, A.; Guttman, D. S.; Figeys, D. and Dennis, J. W. 2016. Metabolic reprogramming by hexosamine biosynthetic and Golgi N-glycan branching pathways. Scientific Reports 6:23043. https://doi.org/10.1038/srep23043

Sakomura, N. K. and Rostagno, H. S. 2016. Métodos de pesquisa em nutrição de monogástricos. 2.ed. Funep, Jaboticabal.

Salvatore, S.; Heuschkel, R.; Tomlin, S.; Davies, S. E.; Edwards, S.; Walker-Smith, J. A.; French, I. and Murch, S. H. 2000. A pilot study of N-acetyl glucosamine, a nutritional substrate for glycosaminoglycan synthesis, in paediatric chronic inflammatory bowel disease. Alimentary Pharmacology and Therapeutics 14:1567-1579. https://doi.org/10.1046/j.1365-2036.2000.00883.x

Santos, E. T.; Sgavioli, S.; Castiblanco, D. M. C.; Domingues, C. H. F.; Quadros, T. C. O.; Borges, L. L.; Petrolli, T. G. and Baraldi-Artoni, S. M. 2018. Glycosaminoglycans and vitamin C *in ovo* feeding affects bone characteristics of chicks. Revista Brasileira de Zootecnia 47:e20170304. https://doi.org/10.1590/rbz4720170304

Santos, E. T.; Sgavioli, S.; Castiblanco, D. M. C.; Borges, L. L.; Quadros, T. C. O.; Laurentiz, A. C.; Shimano, A. C. and Baraldi-Artoni, S. M. 2019 Glycosaminoglycans and vitamin C affect broiler bone parameters. Poultry Science 98:4694-4704. https://doi.org/10.3382/ps/pez177

Segarra, S.; Martínez-Subiela, S.; Cerdà-Cuéllar, M.; Martínez-Puig, D.; Muñoz-Prieto, A.; Rodríguez-Franco, F.; Rodríguez-Bertos, A.; Allenspach, K.; Velasco, A. and Cerón, J. 2016. Oral chondroitin sulfate and prebiotics for the treatment of canine Inflammatory Bowel Disease: a randomized, controlled clinical trial. BMC Veterinary Research 12:49. https://doi.org/10.1186/s12917-016-0676-x

Sgavioli, S.; Santos, E. T.; Borges, L. L.; Andrade-Garcia, G. M.; Castiblanco, D. M. C.; Almeida, V. R.; Garcia, R. G.; Shimano, A. C.; Nääs, I. A. and Baraldi-Artoni, S. M. 2017. Effect of the addition of glycosaminoglycans on bone and cartilaginous development of broiler chickens. Poultry Science 96:4017-4025. https://doi.org/10.3382/ps/pex228

Shang, Q.; Shi, J.; Song, G.; Zhang, M.; Cai, C.; Hao, J.; Li, G. and Yu, G. 2016. Structural modulation of gut microbiota by chondroitin sulfate and its oligosaccharide. International Journal of Biological Macromolecules 89:489-498. https://doi.org/10.1016/j.ijbiomac.2016.04.091

Sibbald, I. R. and Slinger, S. J. 1963. A biological assay for metabolizable energy in poultry feed ingredients together with findings which demonstrate some of the problems associated with the evaluation of fats. Poultry Science 42:313-325. https://doi.org/10.3382/ps.0420313

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

Silva, J. H. V. and Pascoal, L. A. F. 2014. Função e disponibilidade dos minerais. p.127-142. In: Nutrição de não ruminantes. Sakomura, N. K.; Silva, J. H. V.; Costa, F. G. P.; Fernandes, J. B. K. and Hauschild, L., eds. Funep, Jaboticabal.

Yomogida, S.; Kojima, Y.; Tsutsumi-Ishii, Y.; Hua, J.; Sakamoto, K. and Nagaoka, I. 2008. Glucosamine, a naturally occurring amino monosaccharide, suppresses dextran sulfate sodium-induced colitis in rats. International Journal of Molecular Medicine 22:317-323. https://doi.org/10.3892/ijmm_00000025