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, Itaberá, 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_{6}H_{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 supplement¹         | 0.05        | 0.05    |
| Mineral supplement²         | 0.05        | 0.05    |
| Additives                   | 0.20        | 0.20    |
| Variable portion³           | 0.40        | 0.40    |
| Total                       | 100.00      | 100.00  |

Calculated nutritional composition

| Item                        | Pre-starter | Starter |
|-----------------------------|-------------|---------|
| 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; niacin, 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 mg; 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 hydroxyanisole), 0.004 g; copper sulfate, 0.03 g.

⁴ Variable portion: chondroitin sulfate [(C14H21NO14S)n, Biofac A/S, England;vej, Kastrup, Denmark] and/or potassium glucosamine sulfate [(C6H14NO5)2SO4 × 2KCl, Zhejiang Golden-Shell Pharmaceutical Co. Ltd., Yuhuan, Zhejiang, China] and/or inert (kaolin) 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 digestibility coefficients 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:

$$Y_{ijk} = \mu + (CO)_i + (GLU)_j + (CO \times GLU)_{ij} + e_{ijk},$$

in which $Y$ is the response variable, $\mu$ is the mean of the variable, CO is the chondroitin sulfate, GLU is the glucosamine sulfate, $CO \times GLU$ is the interaction between chondroitin sulfate and glucosamine sulfate, and $e_{ijk}$ 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 calcium.

**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

| Glucosamine (GLU, %) | 0 | 0.3 |
|----------------------|---|-----|
| **FW (g)** 0 | 903.00 | 977.28 |
| 1 | 950.14 | 986.86 |
| P | 0.1546 | 0.7680 |
| Mean | 926.57 | 982.07 |
| 1 to 21 days of age | 0 | 863.14 | 937.28 |
| 0.1 | 911.00 | 947.00 |
| P | 0.1483 | 0.7644 |
| Mean | 887.07 | 942.14 |
| **WG (g)** 0 | 863.14 | 937.28 |
| 1 | 911.00 | 947.00 |
| P | 0.1483 | 0.7644 |
| Mean | 887.07 | 942.14 |
| **FI (g)** 0 | 1166.85 | 1228.00 |
| 1 | 1184.71 | 1219.57 |
| P | 0.7092 | 0.8601 |
| Mean | 1175.79 | 1223.79 |
| **FC (g/g)** 0 | 1.325 | 1.307 |
| 1 | 1.292 | 1.271 |
| P | 0.3434 | 0.3138 |
| Mean | 1.321 | 1.313 |
| **V (%)** 0 | 98.41 | 98.41 |
| 1 | 100.00 | 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_{6}H_{14}NO_{5})2SO_{4}×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<sup>1</sup> (CO, %) | Glucosamine<sup>2</sup> (GLU, %) | P  | Mean | SEM | CV  | Probability |
|----------------|----------------------------------|----------------------------------|----|------|-----|-----|-------------|
|                | 0                                | 0.3                              |    |      |     |     |             |
| DCDM (%)       | 0.1                              | 79.29                            | 79.14 | 0.8140 | 79.22 | 0.29 | 1.35 | 0.1894 | 0.2465 | 0.2764 |
|                | P                                | 0.7201                           | 0.1348 | 0.79.17 | 78.64 |
| Mean           |                                  |                                  |      |       |     |     |             |
| DCN (%)        | 0.1                              | 75.68a                           | 73.70 | 0.1454 | 74.69 | 0.72 | 3.35 | 0.0086 | 0.9378 | 0.0173 |
|                | P                                | 0.0020                           | 0.5659 | 73.40 | 73.32 |
| Mean           |                                  |                                  |      |       |     |     |             |
| DCMM (%)       | 0.1                              | 63.67a                           | 63.14 | 0.8684 | 63.40 |
|                | P                                | 0.0375                           | 0.0905 | 60.18 | 65.93 |
| Mean           |                                  |                                  |      |       |     |     |             |
| DCCa (%)       | 0.1                              | 69.69a                           | 70.90 | 0.7829 | 70.30 |
|                | P                                | 0.0478                           | 0.0625 | 65.15 | 75.16 |
| Mean           |                                  |                                  |      |       |     |     |             |
| AMEn<sup>4</sup> (kcal/kg DM) | 0.1                              | 3240.71                          | 3206.15 | 0.3349 | 3223.43 |
|                | P                                | 0.8626                           | 0.0634 | 3243.78 | 3240.33 |

$P$ - probability; SEM - standard error of the mean; CV - coefficient of variation.

<sup>1</sup> [(C<sub>14</sub>H<sub>21</sub>NO<sub>14</sub>S)<sub>n</sub>, Biofac A/S] purity of 91.27%.

<sup>2</sup> [((C<sub>6</sub>H<sub>14</sub>NO<sub>5</sub>)<sub>2</sub>SO<sub>4</sub> × 2KCl, Zhejiang Golden-Shell Pharmaceutical Co. Ltd.] sulfate content 16%.

<sup>A</sup>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 inflammation 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

| 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 |

|                | Chondroitin¹ (CO, %) | Glucosamine² (GLU, %) | P | Mean | SEM | CV | Probability |
|----------------|----------------------|-----------------------|---|------|-----|----|-------------|
|                | 0                    | 0.3                   |   |      |     |    |             |
| 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) | 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                  | 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) | 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                  | 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) | 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                  | 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 [(C₁₄H₂₁NO₁₄S)n, Biofac A/S] purity of 91.27%.
² [(C₆H₁₄NO₅)₂SO₄ × 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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