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Evaluation of Collagen Supplements to Reduce the Occurrence of Orthopedic Injuries in Trained Foals

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Abstract

Hydrolyzed collagen is a popular supplement for equine joint health due to its potential role in cartilage metabolism and tissue repair. However, scientific validation of its efficacy remains limited. This study aimed to evaluate the effect of hydrolyzed collagen supplementation in the diet of trained foals on the occurrence of orthopedic injuries and joint changes. Twenty Mangalarga Marchador foals were used and randomly allocated to either a collagen supplementation group (50 g/day for 180 days) or a control group. During this period, the foals were exercised five consecutive days per week. Every 36 days, the hock region was evaluated by radiography and ultrasonography. The concentrations of prostaglandin E2 (PGE2) and glycosaminoglycans (GAGs) in the articular synovial fluid were also measured. For imaging parameters, the data were analyzed using non-parametric Kruskal–Wallis tests. For synovial fluid parameters, the data were subjected to analysis of variance, and the means were compared using Tukey's test, with a significance level set to 0.05. Significant differences ($P < 0.05$) were observed in radiographic, ultrasonographic, and synovial fluid parameters, indicating reduced inflammation and joint degeneration in the supplemented group. It is concluded that hydrolyzed collagen supplementation reduced inflammation and joint degeneration in trained foals without altering joint homeostasis. The significance of this finding is substantial, as it proposes a nutritional strategy to prevent joint disorders while reducing reliance on invasive interventions. However, further research involving extended follow-up and varied supplementation protocols is critical to confirm the sustainability of these outcomes and validate the long-term efficacy of this approach.

Keywords

Foal breeding; glycosaminoglycan; joint; osteoarthritis; prostaglandin E2; training program

1. Introduction

With a sustained demand for high-performance horses, clinical problems in the musculoskeletal system may increase, as sports and training programs influence the prevalence and predisposition to orthopedic injuries and lameness **[1,2]**. Depending on the workload imposed on specific anatomical structures—which varies according to the type of sport and the level of training—injuries to supporting structures can impair performance. This impact may affect both the foal's development and its future athletic career **[2,3]**.

In this context, it was observed that physical training protocols promote adaptations in the bone and cartilage structure of joints, which can be monitored through physical examination and ultrasonography [4,5]. However, the importance of balancing the benefits of these adaptations with the imminent risk of injuries was emphasized. Although bones and cartilage adjust to training-induced stress, this process can temporarily weaken the joints, increasing the foals' vulnerability to overload and repetitive mechanical stress. Furthermore, there are periods when susceptibility to inflammation and orthopedic injuries is heightened, underscoring the need for preventive measures to reduce their occurrence.

Accordingly, in addition to early detection to minimize new cases, the equine supplements industry has gained prominence by providing dietary supplements and nutritional management techniques to support horses' health [6,7]. Thus, specific ingredients for this purpose, such as collagen, are widely used, although there is a lack of scientific evidence for their efficacy in horses.

Much is known about the naturally occurring physiological functions of collagen in horses, including its role in bone remodeling [8], identification and degradation of collagen in the joint capsule [9], organization of the collagen network in the joint cartilage of young foals [10], histological changes in the equine flexor tendon [11], and its relationship to bone biomarkers [12].

Regarding the use of exogenous collagen in horses, reports suggest that it may be a safe alternative to help protect the non-glandular stomach, preventing gastric mucosal inflammation [13]. In the orthopedic field, horses diagnosed with osteoarthritis may exhibit reduced lameness grades [14]. Additionally, collagen supplementation may serve as an important ally in the treatment of synovitis [15], contribute to collagen synthesis (types I and II), and act as an inhibitor of pro-inflammatory cytokines in vitro [16].

However, the limited research conducted so far presents several constraints, including the short duration of supplementation, variability in collagen sources, small sample sizes, a predominance of in vitro studies, and the lack of complementary assessments such as radiography and ultrasonography—particularly in studies focused on the locomotor system.

Thus, the present study hypothesized that the inclusion of collagen in the diet of foals in training reduces the occurrence of orthopedic injuries and promotes joint homeostasis. Accordingly, the aim was to evaluate the effect of hydrolyzed collagen supplementation in the diet of trained foals on the occurrence of orthopedic injuries and joint changes.

2. Materials and Methods

2.1. Animals, Diets, Treatments, Supplementation, and Experimental Design

All experimental procedures were carried out in accordance with the guidelines established by the Ethics Committee on the Use of Animals – School of Veterinary Medicine and Animal Sciences, University of Sao Paulo (protocol #5595210323). The experiment was conducted at Haras Morada Nova, located in the municipality of Inhaúma, Minas Gerais State, Brazil. Environmental conditions included a temperature range of 19 °C to 27 °C and an average relative humidity of 63%.

Twenty foals of the Mangalarga Marchador breed, born at the experimental site and with a mean body weight of 206 ± 18 kg, were included in the study. There were ten male and ten female foals, all aged seven months. The horses were managed identically from birth in pastures of approximately 3 hectares and weaned at 6 months of age.

Before starting the experiment, all the foals underwent a clinical evaluation to ensure they were healthy (no changes in behavior or manifestation of any clinical signs), and blood samples were collected for a leukogram and an erythrogram to obtain complete blood cell counts.

The foals were kept in individual 16 m² stalls with wood shavings as bedding from weaning until the end of the experimental period. Diets were based on grass hay (*Cynodon* spp. Tifton 85) and concentrate formulated for each animal category. The equivalent of 2.5% of body weight in dry matter was offered, following NRC recommendations [17], with a forage-to-concentrate ratio of 50:50.

To ensure the nutritional quality of the feed (roughage and concentrate), a bromatological analysis was conducted at the beginning of the experimental period. The chemical composition of the feeds is shown in **Table 1**. Water and mineral salt were provided *ad libitum*. The concentrate was provided in individual feeders twice a day, always at the same times (8:00 AM and 4:00 PM), while the hay was placed in a specific manger.

Foals were randomly allocated to one of two groups: a control group (not supplemented with hydrolyzed collagen) and a treatment group (supplemented with hydrolyzed collagen), with ten foals in each group. The experimental period lasted six months. Supplementation occurred over 180 days, during which 50 g of hydrolyzed collagen (Gelco International® – Gelatin and Collagen Excellence) was added daily as a topdress over the concentrate, divided equally between the two feedings.

Table 1: Chemical composition of the concentrate and roughage used for foals (grass hay *Cynodon* spp. Cv. Tifton 85).

Composition	Concentrate (%)	Roughage (%)
Dry matter	87.0	89.3
Crude protein	18.0	9.20
Crude fiber	10.0	38.1
Acid detergent fiber	9.0	42.40
Neutral detergent fiber	14.0	69.34
Ether extract	3.5	1.01
Ash	13.1	6.45
Calcium	1.5	0.19
Phosphorus	0.6	0.19
Starch	20.0	2.80

2.2. Training

All foals underwent weekly training in both the control and the supplemented groups. Training occurred over five consecutive days (Mondays to Fridays) of exercise, followed by two consecutive rest days (Saturdays and Sundays). The training protocol involved a gait exercise combined alternately with aquatic exercises and galloping on an inclined surface.

In the gait exercise, the foals were led with a halter by a handler along a straight path for 50 m, repeated five times, totaling 250 m of walking, on a concrete floor covered by a thick layer of sand. With an average speed of 6 m/second, this exercise was performed daily.

For aquatic training, the animals were placed in a 20-meter-long straight-line pool with a water depth of approximately 1 m and a rubber floor. The foals were individually led with a halter by the handler, walking for 20 minutes. With an average speed of 4.5 m/second, this exercise was performed on Mondays, Wednesdays, and Fridays.

In the galloping exercise on an inclined surface, the animals were led as a group on a flat surface with an approximate incline of 40°, covering 100 m uphill and 100 m downhill. With an average speed of 12 m/second, the foals practiced this activity on Tuesdays and Thursdays.

2.3. Evaluation of Joint Structure by Ultrasonographic Examination

The zootechnical region evaluated was the hock of the left hind limb. The examinations were performed using an ultrasound system (an Esaote® MyLab 70 model) and a 10 MHz linear transducer. The images were obtained in both transverse and longitudinal sections. All exams were performed with the animal in a standing position and without the need for sedation.

Subsequently, the images were sent for further evaluation to three veterinarians specialized in orthopedic diagnostic imaging. The parameters assessed **[5]** were: synovial fluid appearance (SFA), synovial fluid quantity (SFQ), joint capsule thickness (JCT), thickness and appearance of articular cartilage (TAAC), subchondral surface appearance (SSA), and presence of subchondral osteophytes (PSO) **(Table 2)**.

The image acquisition for all periods was performed every 36 days. On the day of imaging collection, no foals were exercised.

2.4. Evaluation of Bone Structure by Radiographic Examination

For the evaluation of bone structure, the zootechnical region we evaluated was also the hock of the left hind limb, corresponding to the tarsal bones. The radiographs were performed using a portable X-ray machine (Eco Ray® model Orange 1060HF) with a power of 100 Kvp and a detector of 37 × 28.5 cm. All examinations were conducted with the animal in a standing position and without the need for sedation. The images were obtained and sent to three veterinary doctors specializing in orthopedic diagnostic imaging for subsequent evaluation. According to the methodology of Machado *et al.* **[18]**, the radiographic projections performed were dorsoplantar and mediolateral. The parameters assessed **[19]** were: increase in soft tissue volume (ISTV), presence of soft tissue mineralization (PSTM), presence of osteophytes and bone proliferations (POBP), enthesophytes (PE), subchondral sclerosis (PSS), subchondral osteolysis (PO), and osteochondral fragments (OF) **(Table 3)**.

The image acquisition for all periods was performed every 36 days. On the day of imaging collection, no foals were exercised.

Table 2: Classification and score of each parameter evaluated in the ultrasound examination.

Variables	Categories	Score
Synovial fluid appearance	Normal	0
	Anechoic	1
	Predominantly anechoic	2
	Predominantly heterogeneous	3
Synovial fluid quantity	Normal	0
	Increased +	1
	Increased ++	2
	Increased +++	3
	Increased ++++	4
Joint capsule thickness	No increase	0
	Mild	1
	Moderate	2
	Severe	3
Thickness and appearance of articular cartilage	Well-defined, continuous, smooth, and easily identifiable chondral line	0
	Chondral line difficult to identify with 50% of the surface preserved	1
	Chondral line difficult to identify, discontinuous, and rough	2
	No identification of the line with the presence of fragments	3
Subchondral surface appearance	Smooth	0
	Regular	1
	Areas with depression	2
Presence of subchondral osteophytes	None	0
	Present	1

Adapted from **[5]**.

Table 3: Classification and score of each parameter evaluated in the radiographic examination.

Variables	Categories	Score
Increase in soft tissue volume	None	0
	Mild	1
	Moderate	2
	Severe	3
Presence of soft tissue mineralization	None	0
	Mild	1
	Evident	2
	Severe	3
Presence of osteophytes and bone proliferations	None	0
	Mild	1
	Moderate	2
	Severe	3
Presence of enthesophytes	None	0
	Mild	1
	Moderate	2
	Severe	3
Presence of subchondral sclerosis	None	0
	Mild	1
	Evident	2
	Severe	3
Presence of subchondral osteolysis	None	0
	Mild	1
	Evident	2
	Severe	3
Osteochondral fragments	None	0
	One	1
	Two	2
	Multiple	3

Adapted from **[19]**.

2.5. Evaluation of Articular Synovial Fluid

The evaluated zootechnical region was the hock of the left hind limb, corresponding to the tibiotarsal joint. All collections were performed with the animal in a standing position and without the need for sedation.

The asepsis of the region consisted of an initial wash with running water and neutral soap, followed by two 5-minute scrubs in a circular motion using 3"×3" gauze sponges soaked in povidone-iodine solution. Excess povidone-iodine was removed with a sterile wipe moistened with 70% isopropyl alcohol. The site was dried with a sterile gauze sponge before sampling **[20]**. The sample was collected via arthrocentesis, where the synovial fluid was aspirated aseptically using a 0.8 × 30 mm needle and syringe, resulting in 4 ml of joint fluid **(Figure 1)**. The samples were immediately transferred to a plain tube (without EDTA).

The tubes were centrifuged at 3000 rpm for 20 minutes at 4 °C, and the supernatant was divided into aliquots, which were immediately stored in a -80 °C freezer within two hours after sample collection for subsequent analysis of PGE2 and GAG **[21]**.

PGE2 was determined using the Prostaglandin E2 ELISA Monoclonal kit (Cayman Chemical, Michigan, USA) **[19]**.

To determine GAG (hyaluronic acid and chondroitin sulfate) concentrations, synovial fluid (SF) samples (50 µL) were subjected to proteolysis using a Maxatase solution (4 mg/mL in 0.05 mol/L Tris-HCl, pH 8.0, 100 µL). After 18 hours of incubation at 50 °C, Maxatase was thermally inactivated (15 minutes at 100 °C), and debris was removed by centrifugation (3000 × g, 15 minutes, 24 °C). The supernatant was lyophilized and resuspended in distilled water (25 µL).

Aliquots (5 µL) were subjected to agarose gel electrophoresis in PDA buffer and stained with 0.1% toluidine blue in 50% ethanol:1% acetic acid (for sulfated glycosaminoglycans), and subsequently in 0.05 M sodium acetate buffer, pH 5 (for HA). Compounds were quantified by densitometry of the agarose gel slides **[22,23]**.

The collection of synovial fluid for all analyses was performed every 36 days. On the day of collection, no foals were exercised.

Figure 1: Arthrocentesis performed on the tibiotarsal joint of a young foal.

2.6. Statistical Analysis

For the imaging parameters, the data were analyzed using non-parametric Kruskal–Wallis tests, considering repeated measures over time, with the significance level set to 0.05, using the PROC NPAR1WAY procedure of SAS 9.0 (SAS Institute, Cary, NC).

For the synovial fluid parameters, the data were initially subjected to the Shapiro–Wilk normality test, and all parameters exhibited a normal distribution. Then, the data were subjected to analysis of variance, and the means were compared using Tukey's test, considering repeated measures over time, with the significance level set to 0.05, using the PROC MIXED procedure of SAS 9.0 (SAS Institute, Cary, NC).

3. Results

It was possible to visualize and record radiographic and ultrasonographic images of all foals without any complications or unexpected findings. In the ultrasound assessment of joint structure, a difference ($P < 0.05$) was observed between groups for several parameters, including SFQ, JCT, SSA, and PSO **(Table 4)**, where collagen supplementation suggests a possible effect on increasing synovial fluid production, associated with a decrease in joint capsule thickness, subchondral surface appearance, and subchondral osteophytes. However, no changes were observed in the appearance of synovial fluid, articular cartilage, or the appearance of periarticular ligaments. Additionally, a difference ($P < 0.05$) was observed between the time points for the variables of SFA, SFQ, JCT, TAAC, and PSO, indicating ultrasonographic changes across the experimental period **(Table 4)**.

Regarding the assessment of bone structure through radiographs, there was a difference ($P < 0.05$) between the treatment and control groups in POBP, PSS, and PSO, but no changes were observed in soft tissues **(Table 5)**. Thus, supplemented foals showed indications of an effect in reducing/preventing osteophytes and bone proliferations, as well as in subchondral sclerosis and osteolysis. Evaluation of the different time periods and variables revealed a significant difference ($P < 0.05$) only for POBP, with the other variables showing no radiographic changes over time **(Table 5)**.

For the assessments of synovial joint fluid, the samples were successfully collected with no unexpected findings. There was a difference ($P < 0.05$) between treatments for PGE2 **(Figure 2)**, with a reduction in the inflammatory grades of supplemented foals. This finding suggests that therapeutic interventions may modulate joint inflammatory responses in young horses.

For GAG analyses, no difference ($P > 0.05$) was observed for HA **(Figure 3)** and CS **(Figure 4)**. This indicates that supplementation did not significantly affect the synthesis or degradation of HA and CS in the extracellular matrix.

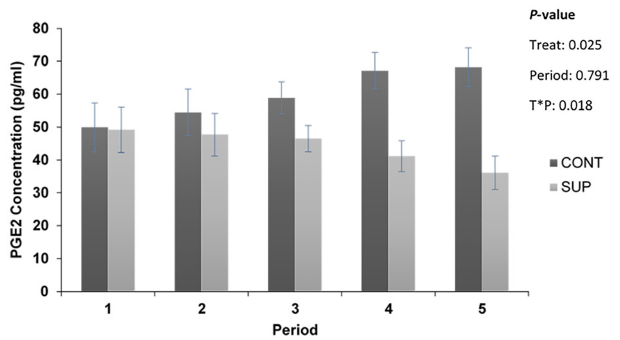

Figure 2: Means and standard error (SE) of PGE2 concentrations (pg/ml) in foals under different treatments and periods.

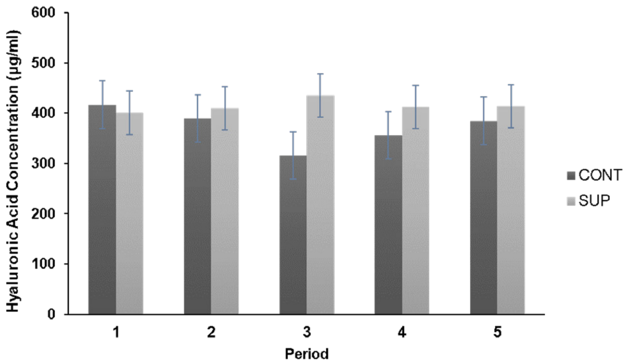

Figure 3: Means and standard error (SE) of HA concentrations (µg/ml) in foals under different treatments and periods.

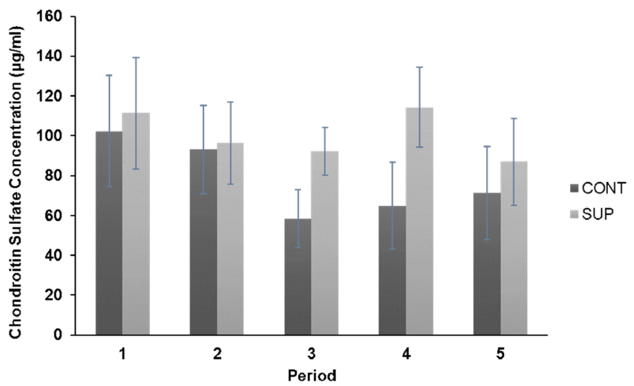

Figure 4: Means and standard error (SE) of CS concentrations (µg/ml) in foals under different treatments and periods.

4. Discussion

Regarding the results for the quantity of synovial fluid, the additional nutritional intake, exclusively derived from peptides present in the supplement, potentially increased the volume of joint fluids. In this context, Shaw *et al.* **[24]** observed that individuals supplemented with gelatin-derived hydrolyzed collagen one hour before engaging in intense exercise showed an increase in circulating levels of glycine, proline, and hydroxyproline—amino acids that constitute the structure of hydrolyzed collagen **[25]**. The authors concluded that adding gelatin to an intermittent exercise program improves circulating and intra-articular collagen synthesis, potentially resulting in increased fluid quantity and playing a beneficial role in injury prevention and tissue repair.

Table 4: Means and standard error of the mean (SEM) corresponding to the ultrasonographic assessments across different treatments, periods, and variables.

Variables	Periods										SEM	P value		
	1		2		3		4		5					
	Cont[1]	Supple[2]	Cont	Supple	Cont	Supple	Cont	Supple	Cont	Supple		Treat[3]	Period	T × P
SFA *(0–3)*	0.90^{B}	0.76^{B}	1.03^{AB}	1.06^{AB}	1.26^{A}	1.06^{A}	1.23^{A}	1.16^{A}	1.00^{AB}	1.03^{AB}	0.2482	0.921	0.026	0.316
SFQ *(0–4)*	0.53^{aB}	0.56^{bB}	0.63^{aA}	1.30^{bA}	0.63^{aA}	1.40^{bA}	0.53^{aA}	1.41^{bA}	0.60^{aA}	1.41^{bA}	0.1374	<.0001	<.0001	<.0001
JCT *(0–3)*	0.16^{aC}	0.33^{bC}	0.26^{aBC}	0.33^{bBC}	0.53^{aAB}	0.36^{bAB}	0.73^{aA}	0.46^{bA}	0.63^{aAB}	0.26^{bAB}	0.1330	0.028	0.002	<.0001
TAAC *(0–3)*	0.60^{A}	0.36^{A}	0.30^{AB}	0.36^{AB}	0.30^{B}	0.16^{B}	0.20^{B}	0.15^{B}	0.20^{B}	0.15^{B}	0.1375	0.261	0.061	0.139
SSA *(0–2)*	0.83^{a}	0.73^{b}	0.86^{a}	0.83^{b}	0.96^{a}	0.53^{b}	0.96^{a}	0.53^{b}	0.96^{a}	0.33^{b}	0.2281	0.012	0.569	0.002
PSO *(0–1)*	0.20^{aB}	0.00^{bB}	0.10^{aB}	0.10^{bB}	0.40^{aA}	0.10^{bA}	0.40^{aA}	0.10^{bA}	0.40^{aA}	0.10^{bA}	0.1061	<.0001	0.032	0.000

Synovial fluid appearance (SFA); synovial fluid quantity (SFQ); joint capsule thickness (JCT); thickness and appearance of articular cartilage (TAAC); subchondral surface appearance (SSA); presence of subchondral osteophytes (PSO). Different uppercase letters indicate significant differences over time, and different lowercase letters indicate significant differences among treatments, according to the Kruskal–Wallis test ($P < 0.05$). [1]Control group; [2]Supplement group; [3]Effect of the treatment.

Table 5: Means and standard error of the mean (SEM) corresponding to the radiographic assessments across different treatments, periods, and variables.

Variables	Periods										SEM	P value		
	1		2		3		4		5					
	Cont[1]	Supple[2]	Cont	Supple	Cont	Supple	Cont	Supple	Cont	Supple		Treat[3]	Period	T × P
ISTV (0–3)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.0105	0.317	0.406	0.408
PSTM (0–3)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0970	1.000	1.000	1.000
POBP (0–3)	0.30^{aA}	0.00^{bA}	0.43^{aAB}	0.00^{bAB}	0.55^{aAB}	0.03^{bAB}	0.60^{aAB}	0.03^{bAB}	0.73^{aB}	0.00^{bB}	0.1414	<.0001	0.034	0.460
PE (0–3)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0970	1.000	1.000	1.000
PSS (0–3)	0.06^{a}	0.00^{b}	0.06^{a}	0.00^{b}	0.16^{a}	0.00^{b}	0.06^{a}	0.00^{b}	0.04^{a}	0.00^{b}	0.0409	0.007	0.686	0.435
PO (0–3)	0.10^{a}	0.06^{b}	0.06^{a}	0.00^{b}	0.13^{a}	0.03^{b}	0.10^{a}	0.03^{b}	0.04^{a}	0.00^{b}	0.0467	0.038	0.478	0.933
OF (0–3)	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.03	0.03	0.0191	0.562	0.251	0.844

Increase in soft tissue volume (ISTV); presence of soft tissue mineralization (PSTM); presence of osteophytes and bone proliferations (POBP); presence of enthesophytes (PE); presence of subchondral sclerosis (PSS); presence of subchondral osteolysis (PSO); osteochondral fragments (OF). Different uppercase letters indicate significant differences over time, and different lowercase letters indicate significant differences among treatments, according to the Kruskal–Wallis test ($P < 0.05$). [1]Control group; [2]Supplement group; [3]Effect of the treatment.

Beyond the impact on synovial fluid volume, the thickness of the joint capsule decreased in supplemented foals. This inverse relationship between capsular thickness and inflammation was previously established by Farfaras *et al.* **[26]**, who associated thicker capsules with higher inflammatory levels. Supporting this idea, Van de Water *et al.* **[15]** reported that racehorses supplemented with hydrolyzed collagen for 60 days exhibited lower degrees of synovitis, as indicated by inflammatory markers in synovial fluid (PGE2 and interleukins), and, consequently, reduced joint capsule thickening. In the present study, the decrease in prostaglandin E2 (PGE2) concentrations in the supplemented group reinforces the hypothesis of a direct anti-inflammatory effect of the supplement in this case.

This anti-inflammatory effect, in turn, appears to be linked to the bioavailability of collagen peptides. As highlighted by **[27]**, these peptides can be absorbed intact, reach systemic circulation, accumulate in cartilage, and stimulate chondrocytes to synthesize extracellular matrix (ECM), promoting joint homeostasis. This mechanism may explain, for instance, the findings of Atayde *et al*. **[28]**, who observed a reduction in inflammatory cell infiltration and pro-inflammatory cytokine production (such as IL-1β and TNF-α) in mice following oral collagen supplementation.

However, beyond inflammation, structural changes such as osteophytes and bone proliferations (POBP), subchondral osteophytes (PSO), subchondral sclerosis (PSS), and osteolysis (PO) were also analyzed. In the present study, these osteoarthritis (OA) markers—particularly POBP—were more prevalent in the control group, where the imposed exercise load likely contributed to increased scores **[29]**. This finding contrasts with Di Filippo *et al*. **[30]**, who, when evaluating the influence of exercise, age, body weight, and growth on the development of tarsal osteoarthritis in gaited foals, observed that only 10% of the control group presented radiographic bone alterations indicative of OA. In contrast, in the present study, 40% of the foals, regardless of treatment, were affected by the presence of POBP. Some of them had already exhibited these radiographic changes even before starting training, suggesting a strong genetic association with the incidence of injuries.

However, in the study by Di Filippo *et al*. **[30]**, the foals followed a training program in which they exercised only three days per week, performing gallop exercises on a straight surface with a gradual increase in speed, with each session lasting 15 minutes. Additionally, as a methodological difference, radiographs were taken only at two-time points during the experimental period—at 18 and 36 months of age.

Still regarding the findings of POBP, Souza **[29]** observed that OA can also affect subchondral bone. The mechanical stimulation of the tarsal bones, caused by repetitive and intense exercises, often leads to microdamage that can eventually result in either normal or excessive bone remodeling, leading to sclerosis and subchondral osteolysis. These findings are consistent with those of our study, where approximately 30% of foals from both treatments exhibited such characteristics. Thus, an increase in subchondral bone sclerosis is related to higher degrees of generalized OA in the joints, justifying the occurrence of these three types of alterations (POBP, PSS, PO).

However, beyond physical load, other factors can influence the development of osteoarthritis (OA) in young horses. While early exercise is often associated with this pathology **[31]**, variables such as genetic predisposition, body weight, foal growth potential (early or late), and nutritional imbalances also play significant roles **[32]**. According to **[31]**, these factors do not all need to be present to contribute to the clinical manifestation of OA, but their interaction can modulate the severity of the condition.

Given this complexity, studies such as **[14]** aim to evaluate interventions that mitigate joint risks. In their research, hydrolyzed collagen was administered to assess its clinical efficacy in horses diagnosed with OA. The collagen was administered orally as a dietary supplement to 38 privately owned horses of various breeds. One group (G25; aged 6 ± 3 years and with a mean body weight of 519 ± 100 kg) received 25 g/day of hydrolyzed collagen, and another group (G50) received 50 g/day for 12 weeks. In a second center, another group of horses (18 ± 4 years; 413 ± 94 kg) did not receive the supplement; they were designated the control group. The evaluation methodology included orthopedic examination (e.g., flexion tests) and lameness assessment without imaging or complementary examinations. In the G25 group, a moderate effect was observed in reducing lameness grades and the incidence of local pain. In the G50 group, a strong effect was observed in reducing lameness grades and the incidence of local pain after six weeks of supplementation. Both groups showed a strong effect on improved mobility and willingness to run compared to the control group.

Corroborating this perspective—but in the context of induced orthopedic conditions—Van de Water *et al*. **[15]** quantified the preventive effects of a dietary supplement on experimentally induced synovitis in horses. Twenty-four Standardbred horses, divided into groups, received 90 g of hydrolyzed collagen for 60 days before undergoing a joint challenge. Synovitis was induced by intra-articular injection of 0.5 ng of lipopolysaccharide into the intercarpal joint. Subsequently, blood and synovial fluid samples were analyzed. The study found that collagen-supplemented animals exhibited significantly lower total protein levels in synovial fluid, total nucleated cell count, and prostaglandin E2 compared to the placebo group (without collagen supplementation). However, no statistically significant differences were observed between treatments regarding interleukins and glycosaminoglycans. The study concluded that hydrolyzed collagen supplementation may reduce inflammation in an experimental model of joint synovitis.

Additionally, investigations such as **[33]** expand the understanding of the effects of combined and comparative supplementation (collagen, glucosamine, chondroitin sulfate, hyaluronic acid, and turmeric) on joint biomechanics. It was found that the supplemented group exhibited a greater range of motion in the hock during gait compared to the control group. This suggested that the hock joint was responsive to biomechanical changes induced by supplementation. However, no changes were observed between treatments regarding serum and plasma biomarkers, including the absence of alterations in collagen metabolite concentrations and no changes in systemic inflammatory markers over the study period, which lasted only 28 days.

Complementing these findings, Bourdon *et al*. **[16]** observed that hydrolyzed collagen downregulates the synthesis of pro-catabolic and pro-inflammatory markers of OA and may ultimately promote collagen production and metabolic activity in equine articular chondrocyte organoids. This mechanism possibly contributes to joint homeostasis, even under intensive load conditions.

However, the response of synovial fluid biomarkers to exercise intensity and duration, based on findings in the literature, is not always clear. Study results vary and often contradict each other, influenced by factors such as exercise type, training period, synovial fluid collection methods, timing between exercise and sampling, joint evaluated, invasive procedures (arthrocentesis and arthroscopy), pathological conditions, and individual biological factors, including breed, age, sex, and temperament **[34–39]**.

Therefore, for the glycosaminoglycan (GAG) findings, exercise did not promote ECM degradation. Over the past decades, several studies on young horses (from neonates to 18 months old) have provided important insights into the role of exercise in articular cartilage maturation **[31,40–42]**. These authors observed that the absence of physical exercise during the first months of life results in a lack of heterogeneity formation in the joint, eventually leading to negative consequences for the tissue's future resistance to injuries **[43]**.

Moreover, findings by **[36]** demonstrated that horses subjected to a training regimen with gradually increasing intensity (five days of progressive intensity), alternating between walk, trot, and gallop on an automated treadmill, did not exhibit changes in GAG concentrations in the synovial fluid.

Contrary to the results of the present study, Brown *et al.* **[37]** reported that exercise influences the composition of synovial fluid, particularly GAGs such as CS and HA. Their study found that training resulted in a modest increase in CS chain length, with the exercised group showing a peak chain length of 15.6 kDa compared to 11.6 kDa in the control group. However, the collections were performed immediately after exercise. In the present study, the foals were not trained on the day of sample collection.

In a temporal evaluation of inflammatory and oxidative markers following high-intensity exercise, MacNicol *et al.* **[44]** measured GAG concentrations in synovial fluid at distinct time points (0.5, 1, 2, 4, 8, and 24 hours) and observed that within less than 24 hours, the joint environment returned to homeostasis following an 8-hour post-exercise peak in GAG concentrations.

Thus, the results of the present study revealed strong evidence of the beneficial effects of collagen peptides for horses when administered orally as a dietary supplement.

5. Conclusions

We conclude that supplementation with hydrolyzed collagen in trained foals reduces the occurrence of orthopedic injuries and the inflammatory level of synovial fluid without adverse effects on glycosaminoglycan levels. These findings suggest that, from a nutritional perspective, collagen may play a protective role in maintaining joint integrity in horses undergoing training, potentially contributing to enhanced athletic longevity and animal welfare. The clinical relevance of this discovery is significant, as it highlights a viable nutritional strategy for preventing articular orthopedic disorders, thereby reducing reliance on more invasive medical interventions. However, despite the observed benefits, gaps remain in the understanding of collagen's mechanisms of action and its long-term effects. Further studies with extended follow-up periods and varied supplementation protocols are needed to confirm the durability of these effects.

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Authors' Contributions

All authors contributed substantially to the conception, development, writing, and critical revision of the manuscript. Each author affirms their role in ensuring the academic integrity and quality of the work.

Data Availability

All data supporting the findings of this study are included within the article.

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Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical Approval

All ethical considerations related to the use of animals were carefully addressed. The study protocol was approved by the Ethics Committee on the Use of Animals – School of Veterinary Medicine and Animal Sciences, University of São Paulo (Protocol #5595210323), São Paulo, Brazil. All applicable international, national, and institutional guidelines for the care and use of animals were followed.

References

[1] Murray RC, Dyson SJ, Tranquille C, Adams V. Association of type of sport and performance level with anatomical site of orthopaedic injury diagnosis. Equine Veterinary Journal 2006;38:411–6. https://doi.org/10.1111/j.2042-3306.2006.tb05578.x.

[2] Menarim BC, Vasconcelos Machado VM, Cisneros Alvarez LE, Carneiro R, Busch L, Vulcano LC. Radiographic abnormalities in barrel racing horses with lameness referable to the metacarpophalangeal joint. Journal of Equine Veterinary Science 2012;32:216–21. https://doi.org/10.1016/j.jevs.2011.09.064.

[3] Couroucé-Malblanc A, Leleu C, Bouchilloux M, Geffroy O. Abnormal radiographic findings in 865 French Standardbred trotters and their relationship to racing performance. Equine Veterinary Journal 2006;38:417–22. https://doi.org/10.1111/j.2042-3306.2006.tb05579.x.

[4] Moshtagh PR, Korthagen NM, Plomp SG, Pouran B, Castelein RM, Zadpoor AA, *et al.* Early signs of bone and cartilage changes induced by treadmill exercise in rats. JBMR Plus 2018;2:134–42. https://doi.org/10.1002/jbm4.10029.

[5] Yamada ALM, Pinheiro M, Marsiglia MF, Hagen SCF, Baccarin RYA, da Silva LCLC. Ultrasound and clinical findings in the metacarpophalangeal joint assessment of show jumping horses in training. Journal of Veterinary Science 2020;21:e21–e21. https://doi.org/10.4142/jvs.2020.21.e21.

[6] Frank N, Andrews FM, Elliott SB, Lew J. Effects of dietary oils on the development of gastric ulcers in mares. American Journal of Veterinary Research 2005;66:2006–11. https://doi.org/10.2460/ajvr.2005.66.2006.

[7] Reese RE, Andrews FM. Nutrition and dietary management of equine gastric ulcer syndrome. Veterinary Clinics of North America: Equine Practice 2009;25:79–92. https://doi.org/10.1016/j.cveq.2008.11.004.

[8] Martin RB, Lau ST, Mathews PV, Gibson VA, Stover SM. Collagen fiber organization is related to mechanical properties and remodeling in equine bone. A comparsion of two methods. Journal of Biomechanics 1996;29:1515–21. https://doi.org/10.1016/s0021-9290(96)80002-9.

[9] Billinghurst RC, Buxton EM, Edwards MG, McGraw MS, McIlwraith CW. Use of an antineoepitope antibody for identification of type-II collagen degradation in equine articular cartilage. American Journal of Veterinary Research 2001;62:1031–9. https://doi.org/10.2460/ajvr.2001.62.1031.

[10] Brama PAJ, Holopainen J, van Weeren PR, Firth EC, Helminen HJ, Hyttinen MM. Effect of loading on the organization of the collagen fibril network in juvenile equine articular cartilage. Journal of Orthopaedic Research 2009;27:1226–34. https://doi.org/10.1002/jor.20866.

[11] Jacobson E, Dart AJ, Mondori T, Horadogoda N, Jeffcott LB, Little CB, *et al*. Focal experimental injury leads to widespread gene expression and histologic changes in equine flexor tendons. PLoS One 2015;10:e0122220–e0122220. https://doi.org/10.1371/journal.pone.0122220.

[12] Coppelman EB, David FH, Tóth F, Ernst NS, Trumble TN. The association between collagen and bone biomarkers and radiographic osteoarthritis in the distal tarsal joints of horses. Equine Veterinary Journal 2020;52:391–8. https://doi.org/10.1111/evj.13187.

[13] Camacho-Luna P, Andrews FM, Keowen ML, Garza F, Liu C-C., Lamp B, *et al*. The effect of porcine hydrolysed collagen on gastric ulcer scores, gastric juice pH, gastrin and amino acid concentrations in horses. Equine Veterinary Education 2020;34:248–57. https://doi.org/10.1111/eve.13409.

[14] Dobenecker B, Reese S, Jahn W, Schunck M, Hugenberg J, Louton H, *et al*. Specific bioactive collagen peptides (PETAGILE®) as supplement for horses with osteoarthritis: A two-centred study. Journal of Animal Physiology and Animal Nutrition 2018;102:16–23. https://doi.org/10.1111/jpn.12863.

[15] van de Water E, Oosterlinck M, Dumoulin M, Korthagen NM, van Weeren PR, van den Broek J, *et al*. The preventive effects of two nutraceuticals on experimentally induced acute synovitis. Equine Veterinary Journal 2017;49:532–8. https://doi.org/10.1111/evj.12629.

[16] Bourdon B, Contentin R, Cassé F, Maspimby C, Oddoux S, Noël A, *et al*. Marine collagen hydrolysates downregulate the synthesis of pro-catabolic and pro-inflammatory markers of osteoarthritis and favor collagen production and metabolic activity in equine articular chondrocyte organoids. International Journal of Molecular Sciences 2021;22:580. https://doi.org/10.3390/ijms22020580.

[17] National Research Council (NRC). Nutrient requirements of horses. Sixth Revised Edition. Washington, D.C.: National Academies Press; 2007.

[18] Machado VMV, Aguiar ACS, Viana GF, Crosignani NO, Puoli Filho JNP. Diagnostic value of computed tomography, radiography and ultrasonography in metacarpophalangeal joint disorders in horses. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 2016;68:66–72. https://doi.org/10.1590/1678-4162-7899.

[19] Yamada ALM, do Prado Vendruscolo C, Marsiglia MF, Sotelo EDP, Agreste FR, Seidel SRT, *et al*. Effects of oral treatment with chondroitin sulfate and glucosamine in an experimental model of metacarpophalangeal osteoarthritis in horses. BMC Veterinary Research 2022;18:215–215. https://doi.org/10.1186/s12917-022-03323-3.

[20] Zubrod CJ, Farnsworth KD, Oaks JL. Evaluation of arthrocentesis site bacterial flora before and after 4 Methods of preparation in horses with and without evidence of skin contamination. Veterinary Surgery 2004;33:525–30. https://doi.org/10.1111/j.1532-950x.2004.04074.x.

[21] Van de Water E, Oosterlinck M, Korthagen NM, Duchateau L, Dumoulin M, van Weeren PR, *et al*. The lipopolysaccharide model for the experimental induction of transient lameness and synovitis in Standardbred horses. The Veterinary Journal 2021;270:105626. https://doi.org/10.1016/j.tvjl.2021.105626.

[22] Machado TSL, Correia da Silva LCL, Baccarin RYA, Michelacci YM. Synovial fluid chondroitin sulphate indicates abnormal joint metabolism in asymptomatic osteochondritic horses. Equine Veterinary Journal 2012;44:404–11. https://doi.org/10.1111/j.2042-3306.2011.00539.x.

[23] Moreira JJ, Coelho JM, Machado TSL, Morais APL, Michelacci YM, Baccarin RYA. Oral glucosamine and chondroitin sulfate on synovial fluid biomarkers from osteoarthritic equine joints. Ciência Rural 2019;49. https://doi.org/10.1590/0103-8478cr20180247.

[24] Shaw G, Lee-Barthel A, Ross ML, Wang B, Baar K. Vitamin C-enriched gelatin supplementation before intermittent activity augments collagen synthesis. The American Journal of Clinical Nutrition 2017;105:136–43. https://doi.org/10.3945/ajcn.116.138594.

[25] Song H, Li B. Beneficial effects of collagen hydrolysate: a review on recent developments. Biomedical Journal of Scientific & Technical Research 2017;1:1–4. https://doi.org/10.26717/bjstr.2017.01.000217.

[26] Farfaras S, Roshani L, Mulder J, Mitsios N, Hallström EK, Kartus J-T. Increased levels of inflammatory markers in the subscapularis tendon and joint capsule in patients with subacromial impingement. Knee Surgery, Sports Traumatology, Arthroscopy 2021;29:2228–36. https://doi.org/10.1007/s00167-020-05992-9.

[27] Dobenecker B, Böswald LF, Reese S, Steigmeier-Raith S, Trillig L, Oesser S, *et al*. The oral intake of specific Bioactive Collagen Peptides (BCP) improves gait and quality of life in canine osteoarthritis patients-A translational large animal model for a nutritional therapy option. PLoS One 2024;19:e0308378–e0308378. https://doi.org/10.1371/journal.pone.0308378.

[28] Atayde SR, Velosa APP, Catanozi S, Del Bianco V, Andrade PC, Rodrigues JE de CM, *et al*. Collagen V oral administration decreases inflammation and remodeling of synovial membrane in experimental arthritis. PLOS ONE 2018;13:e0201106. https://doi.org/10.1371/journal.pone.0201106.

[29] Souza MV de. Osteoarthritis in horses - Part 1: relationship between clinical and radiographic examination for the diagnosis. Brazilian Archives of Biology and Technology 2016;59. https://doi.org/10.1590/1678-4324-2016150024.

[30] Di Filippo PA, Dias Meireles MA, Ribeiro LMF, de Lannes ST, Meireles NFT, Viana IS, *et al.* Influence of exercise, age, body weight, and growth on the development of tarsal osteoarthritis in young Mangalarga Marchador horses. Journal of Equine Veterinary Science 2019;80:36–9. https://doi.org/10.1016/j.jevs.2019.06.015.

[31] Van Weeren PR, Firth EC, Brommer H, Hyttinen MM, Helminen HJ, Rogers CW, *et al.* Early exercise advances the maturation of glycosaminoglycans and collagen in the extracellular matrix of articular cartilage in the horse. Equine Veterinary Journal 2008;40:128–35. https://doi.org/10.2746/042516408x253091.

[32] Donabédian M, Fleurance G, Perona G, Robert C, Lepage O, Trillaud-Geyl C, *et al.* Effect of fast vs. moderate growth rate related to nutrient intake on developmental orthopaedic disease in the horse. Animal Research 2006;55:471–86. https://doi.org/10.1051/animres:2006026.

[33] Much ML, Leatherwood JL, Martinez RE, Silvers BL, Basta CF, Gray LF, *et al.* Evaluation of an oral joint supplement on gait kinematics and biomarkers of cartilage metabolism and inflammation in mature riding horses. Translational Animal Science 2020;4:txaa150–txaa150. https://doi.org/10.1093/tas/txaa150.

[34] Van den Hoogen BM, van de Lest CH, van Weeren PR, Lafeber FP, Lopes-Cardozo M, van Golde LM, *et al.* Loading-induced changes in synovial fluid affect cartilage metabolism. Rheumatology 1998;37:671–6. https://doi.org/10.1093/rheumatology/37.6.671.

[35] Van Den Boom R, Brama PAJ, Kiers GH, Degroot J, Barneveld A, Van Weeren PR. The influence of repeated arthrocentesis and exercise on matrix metalloproteinase and tumour necrosis factor a activities in normal equine joints. Equine Veterinary Journal 2004;36:155–9. https://doi.org/10.2746/0425164044868602.

[36] van den Boom R, van de Lest CHA, Bull S, Brama R a. J, van Weeren PR, Barneveld A. Influence of repeated arthrocentesis and exercise on synovial fluid concentrations of nitric oxide, prostaglandin E2 and glycosaminoglycans in healthy equine joints. Equine Veterinary Journal 2005;37:250–6. https://doi.org/10.2746/0425164054530740.

[37] Brown MP, Trumble TN, Plaas AHK, Sandy JD, Romano M, Hernandez J, *et al.* Exercise and injury increase chondroitin sulfate chain length and decrease hyaluronan chain length in synovial fluid. Osteoarthritis and Cartilage 2007;15:1318–25. https://doi.org/10.1016/j.joca.2007.04.005.

[38] Lamprecht ED, Williams CA. Biomarkers of antioxidant status, inflammation, and cartilage metabolism are affected by acute intense exercise but not superoxide dismutase supplementation in horses. Oxidative Medicine and Cellular Longevity 2012;2012:920932–920932. https://doi.org/10.1155/2012/920932.

[39] Baccarin RYA, Rasera L, Machado TSL, Michelacci YM. Relevance of synovial fluid chondroitin sulphate as a biomarker to monitor polo pony joints. Canadian Journal of Veterinary Research 2014;78:50–60.

[40] Barneveld A, Van Weeren PR. Conclusions regarding the influence of exercise on the development of the equine musculoskeletal system with special reference to osteochondrosis. Equine Veterinary Journal 1999;31:112–9. https://doi.org/10.1111/j.2042-3306.1999.tb05323.x.

[41] Brama PAJ, TeKoppele JM, Bank RA, Barneveld A, Van Weeren PR. Development of biochemical heterogeneity of articular cartilage: influences of age and exercise. Equine Veterinary Journal 2002;34:265–9. https://doi.org/10.2746/042516402776186146.

[42] Rogers CW, Firth EC, McIlwraith CW, Barneveld A, Goodship AE, Kawcak CE, *et al.* Evaluation of a new strategy to modulate skeletal development in Thoroughbred performance horses by imposing track-based exercise during growth. Equine Veterinary Journal 2008;40:111–8. https://doi.org/10.2746/042516408x268923.

[43] te Moller NCR, van Weeren PR. How exercise influences equine joint homeostasis. The Veterinary Journal 2017;222:60–7. https://doi.org/10.1016/j.tvjl.2017.03.004.

[44] MacNicol JL, Lindinger MI, Pearson W. A time-course evaluation of inflammatory and oxidative markers following high-intensity exercise in horses: a pilot study. Journal of Applied Physiology 2018;124:860–5. https://doi.org/10.1152/japplphysiol.00461.2017.

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