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ANIMAL HEALTH AND WELL BEING

Responses to an intra-articular lipopolysaccharide challenge following dietary supplementation of *Saccharomyces cerevisiae* fermentation product in young horses

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Abstract

Dietary intervention may be a valuable strategy to optimize the intra-articular environment in young horses to prolong their performance career. To test the hypothesis that dietary supplementation of a *Saccharomyces cerevisiae* fermentation product would reduce markers of joint inflammation and increase markers of cartilage metabolism following a single inflammatory insult, Quarter Horse yearlings (mean ± SD; 9 ± 1.0 mo) were balanced by age, sex, body weight (**BW**), and farm of origin and randomly assigned to the following treatment groups: 1.25% BW/d (dry matter basis) custom-formulated concentrate only (**CON**; $n = 9$) or concentrate top-dressed with 21 g/d *S. cerevisiae* fermentation product (**SCFP**; $n = 10$) for 98 d. Horses had ad libitum access to Coastal bermudagrass hay. On day 84, one randomly selected radial carpal joint from each horse was injected with 0.5 ng lipopolysaccharide (LPS) solution. The remaining carpal joint was injected with sterile lactated Ringer's solution as a contralateral control. Synovial fluid obtained before supplementation (day 0) and on day 84 at preinjection hour 0 and 6, 12, 24, 168, and 336 h postinjection was analyzed for prostaglandin E_2 (**PGE_2**), carboxypropeptide of type II collagen (**CPII**), and collagenase cleavage neopeptide (**C2C**) by commercial assays. Rectal temperature, heart rate, respiration rate, carpal surface temperature, and carpal circumference were recorded prior to each sample collection and for 24 h postinjection. Data were analyzed using linear models with repeated measures. From day 0 to 84, synovial C2C declined ($P \le 0.01$) and the CPII:C2C ratio increased ($P \le 0.01$) in all horses with no effect of diet. In response to intra-articular LPS, synovial PGE_2 increased by hour 6 ($P \le 0.01$) and returned to baseline by hour 336; CPII increased by hour 12, remained elevated through hour 168 ($P \le 0.01$), and returned to baseline by hour 336; and C2C increased by hour 6 ($P \le 0.01$) but did not return to baseline through hour 336 ($P \le 0.01$). Post-intra-articular injection, PGE_2 levels were lower in SCFP than CON horses ($P = 0.01$) regardless of injection type. Synovial CPII and the CPII:C2C ratio demonstrated stability during the LPS challenge in SCFP compared with CON horses ($P \le 0.01$). Clinical parameters were not influenced by diet but increased in response to repeated arthrocentesis ($P \le 0.01$). Dietary SCFP may favorably modulate intra-articular inflammation following an acute stressor and influence cartilage turnover in young horses.

Key words: cartilage, equine, lipopolysaccharide, *Saccharomyces cerevisiae*, synovial

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Abbreviations

BCS	body condition score
BW	body weight
C2C	collagenase cleavage neopeptide
CC	carpal circumference
CPII	carboxypropeptide of type II collagen
CT	carpal surface temperature
DM	dry matter
ELISA	enzyme-linked immunosorbent assay
HR	heart rate
LPS	lipopolysaccharide
LRS	lactated Ringer's solution
OA	osteoarthritis
PGE_2	prostaglandin E_2
PIH	preinjection hour
RR	respiratory rate
RT	rectal temperature

Introduction

Inflammatory joint disorders are a common cause of lameness and a leading cause for early retirement and loss of athletic performance in young horses (Brama et al., 2000b). Once initial injury or degradation of cartilage occurs, inflammation results as an attempt to stop the damage. In the short term, this process is designed to limit the use of the joint and promote healing. However, with continuous exercise and damage, excessive cytokines and eicosanoids may be released that could result in chronic inflammation and pain and elicit degradation of articular cartilage (Palmer and Bertone, 1994).

Inflammation of the synovial membrane, or synovitis, is a common consequence of repeated trauma and stress to the joint. Recently, interest has been placed on dietary mitigation of systemic inflammation through gut modulation (Hernández-Alonso et al., 2017). It is suggested that an alteration to the microbiome's microenvironment through dietary manipulation may improve the host's overall immunological response. Furthermore, the microbial composition of the gut has an intrinsic role in the development of the immune system, nutritional efficiency, storage of adipose tissue, and behavior (Sekirov et al., 2010; Cryan and O′Mahony, 2011). An imbalance in the microbial population, or dysbiosis, is now recognized to have an extensive influence on the development of several metabolic diseases and systemic inflammation (Hand et al., 2016), which may be associated with joint disease (Szychlinska et al., 2019). Therefore, a modification to the diet, and consequently, to the microbiome's microenvironment, has the potential to mitigate the immunological response of extended joint inflammation (Collins et al., 2015).

An example of such a dietary component is *Saccharomyces cerevisiae* fermentation product. Dietary supplementation of *S. cerevisiae* fermentation product decreased plasma haptoglobin concentrations, a biological marker that increases following an inflammatory response, in lactating Holstein dairy cows following a subacute ruminal acidosis challenge (Guo et al., 2017). Similarly, the paws of *S. cerevisiae* fermentation product-supplemented rats produced lesser prostaglandin E_2 (**PGE_2**), following an inflammatory insult compared with un-supplemented controls (Evans et al., 2012). Finally, adult horses supplemented with *S. cerevisiae* fermentation product had lower white blood cell counts compared with non-supplemented controls following transport (Faubladier et al., 2013). The reduction of biological markers related to inflammation demonstrates the potential for *S. cerevisiae* fermentation product supplementation to attenuate an intra-articular inflammatory response.

Measuring the effectiveness of dietary interventions related to joint health is challenging in young horses, as cartilage responds to both growth and exercise (Firth, 2006). Intra-articular lipopolysaccharide (**LPS**) injection is an established model for inducing acute localized inflammation in the young horse (de Grauw et al., 2009; Lucia et al., 2013). Following LPS injection, synovial concentrations of PGE_2, an eicosanoid indicative of naturally occurring arthritis (Bertone et al., 2001), increase in young and mature horses (Kahn et al., 2017). Similarly, biomarkers of cartilage metabolism, including anabolic carboxypropeptide of type II collagen (**CPII**) and catabolic collagenase cleavage neopeptide (**C2C**), also increase as a result of localized inflammation from LPS (de Grauw et al., 2006). The objective of the current study was to evaluate the effect of *S. cerevisiae* fermentation product supplementation on markers of cartilage metabolism and joint inflammation in response to an intra-articular LPS challenge in young horses. Authors hypothesized that horses receiving *S. cerevisiae* fermentation product would have reduced intra-articular inflammation and increased cartilage metabolism markers compared with non-supplemented horses following the intra-articular LPS challenge.

Materials and Methods

All care, handling, and sampling of horses were reviewed and approved by the Institutional Animal Care and Use Committee at Texas A&M University (2016-0294).

Horses and management

Nineteen Quarter Horses (11 fillies and 8 colts) entering their yearling year (mean ± SD; 9 ± 1 mo of age; initial body weight [**BW**] 280 ± 31 kg) were utilized in a randomized complete design for a 98-d study. Yearlings originated from two sources: Texas A&M University (College Station, TX; n = 8) and Birdsong Farms (Hearne, TX; n = 11). Horses arrived 30 d prior to the start of the study in order to centralize housing and standardize diet. Horses were randomly assigned to treatment groups that were balanced by age, sex, BW, and farm of origin. Treatments consisted of: control (**CON**; basal diet with no supplementation, n = 9) or *S. cerevisiae* fermentation product supplementation (**SCFP**; basal diet + 21 g/d Original XPC, Diamond V Mills, Inc.; Cedar Rapids, IA; n = 10); the SCFP was top-dressed onto the concentrate immediately prior to each feeding (10.5 g/feeding).

The basal diet was formulated to meet or exceed the requirements of growing horses (NRC, 2007). Horses were offered 1.25% BW/d on a dry matter (**DM**) basis of a custom-formulated pelleted concentrate that was free of *S. cerevisiae* fermentation product and was split into two equal meals per day. Horses were placed into individual stalls (3.2 × 3.2 m) and allowed 1 h to consume their respective dietary treatments at 12 h intervals. Horses were group-housed in dry lots (58.7 × 79.2 m) and had free-choice access to Coastal bermudagrass hay (*Cynodon dactylon*) in the form of round bales.

Every 14 d, BW was obtained utilizing a calibrated platform scale (Bastrop Scale Inc., Bastrop, TX), and concentrate intake was adjusted accordingly. Body condition scores (**BCSs**) were also obtained by three independent observers using the 1 to 9 scale described by Henneke et al. (1983). Composited hay and grain samples were analyzed by a commercial laboratory (Equi-Analytical Laboratories, Ithaca, NY) for nutrient composition (Table 1). On day 56, all horses performed a 2-h submaximal

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Table 1. Nutrient composition of custom-formulated concentrate and Coastal bermudagrass (*Cynodon dactylon*) hay offered to yearling horses

Nutrient[1]	Concentrate[2]	Coastal bermudagrass hay[3]
DE, Mcal/kg	0.61	0.39
CP, %	18.00	13.00
CF, %	8.80	1.70
NDF,%	30.40	71.60
ADF,%	15.20	40.30
Starch, %	18.00	1.00
Crude fat, %	8.40	1.70
Ca, %	1.40	0.44
P, %	1.06	0.22
K,%	1.40	0.88
Mg, %	0.57	0.17
Na, %	0.62	0.30
Cl, %	1.08	0.52
S, %	0.30	0.24
Co, ppm	2.00	0.50
Fe, ppm	813.00	184.00
Zn, ppm	217.00	34.00
Cu, ppm	56.00	7.00
Mn, ppm	189.00	241.00

[1]Values presented on a 100% dry matter (DM) basis. ADF, acid detergent fiber; CF, crude fiber; CP, crude protein; DE, digestible energy; NDF, neutral detergent fiber.
[2]Concentrate = basal grain diet fed to all horses at 1.25% body weight (DM basis) per day.
[3]Coastal bermudagrass (*Cynodon dactylon*) hay was offered ad libitum to all horses.

exercise test for a related study (Valigura et al., 2021) to evaluate the effects of SCFP supplementation on markers of exercise-induced stress and inflammation. This exercise test represented the only forced exercise that horses received for the duration of the study.

Intra-articular LPS challenge

On day 84, all horses underwent an intra-articular LPS challenge as previously described (Lucia et al., 2013). Briefly, carpal joints for each horse were randomly assigned one of two colors. A pharmacist at the Texas A&M Veterinary Hospital prepared sterile lactated Ringer's solution (**LRS**) and 0.5 ng LPS derived from *Escherichia coli* O55:B5 (Sigma-Aldrich, St. Louis, MO) diluted in LRS using aseptic techniques. The pharmacist assigned each solution a color, which corresponded to the color-coded carpal joints, allowing investigators to remain blinded to treatments until completion of data analysis. Horses were first sedated with xylazine HCL (Bimeda-MC Animal Health Inc., Cambridge, ON, Canada) that was administered intravenously at recommended doses (0.5 mg/kg BW) and then carpal arthrocentesis was hygienically performed by a veterinarian from the Texas A&M University Large Animal Clinic.

A volume of 0.8 mL of either LRS or LPS was injected into each carpal joint (following color coding). The LRS-injected joint served as a contralateral control. For sampling consistency, ease of collection, and to obtain the volume of fluid required, carpal joints were aseptically prepared and then aspirated utilizing a location medial to the extensor carpi radialis tendon in the palpable depression between the radial carpal bone and the third carpal bone, to a depth of approximately 12.7 mm to avoid unnecessary contact with articular cartilage (McIlwraith and Trotter, 1996). Throughout the first 24 h postinjection, all horses were closely monitored in individual 3.2 × 3.2 m stalls. During that time, rectal temperature (**RT**), heart rate (**HR**), and respiratory rate (**RR**) were recorded at preinjection hour (**PIH**) 0, and 6, 12, and 24 h postinjection. Carpal circumference (**CC**) was also determined (cm) at these time points at the level of the accessory carpal bone utilizing a soft tape measure, and surface temperature (**CT**) of the dorsal surface of each carpal joint was obtained at the same location from a distance of 1.2 m by the use of an infrared camera (FLIR E60 Series, Flir Systems Inc., Wilsonville, OR). Immediately prior to imaging, the dorsal surface of the carpus was cleaned using a dry brush to remove any particulate from the joint surface.

Images were analyzed in duplicate by a certified thermographer (Infrared Training Center, Nashua, NH; certificate no. 81467) using the FLIR Tools software (Flir Tools 2.0). For each image, emissivity was set to 0.95, and ambient temperature and humidity were entered. Additionally, reflected apparent temperature was accounted for on each image using a diffuse reflector (International Organization for Standardization ISO 18434-1:2006 and ASTM E1862-97(2002)e1, Standard Test Methods for Measuring and Compensating for Reflected Temperature Using Infrared Imaging Radiometers, ASTM International, West Conshohocken, PA, 1997). The ellipse measurement tool was then used to evaluate the average temperature of the entire carpal surface. Average ambient temperature and humidity were 22 °C and 70%, 27 °C and 64%, 29 °C and 53%, and 23 °C and 66% at PIH 0 and at 6, 12, and 24 h postinjection, respectively.

Synovial fluid (1 to 4 mL) was obtained at the beginning of the study (day 0) as well as surrounding the LPS challenge on day 84 at PIH 0 and 6, 12, 24, 168 (7 d), and 336 h (14 d) postinjection. Collected synovial fluid was immediately transferred into sterile non-additive tubes (serum blood collection tubes, BD Vacutainer, Kendall Co., Mansfield, MA) and then placed on ice for transfer back to the laboratory. Within 2 h of collection, samples were aliquoted and stored at −80 °C for later analysis of PGE_2, CPII, and C2C.

Sample analysis

Synovial fluid samples were analyzed in duplicate for concentrations of PGE_2 utilizing a commercially available enzyme-linked immunosorbent assay (**ELISA**; R&D Systems, Minneapolis, MN) previously utilized in horses (Bertone et al., 2001; de Grauw et al., 2006) using a plate reader (Synergy H1 Hybrid Multi-Mode Reader, BioTek Instruments, Inc. Winooski, VT). Synovial fluid samples were diluted with provided calibrator diluents 1:1 to 1:10 depending on time postinjection to remain within detectable limits of the assay. Intra-assay precision for PGE_2 ranged between 2.06% and 9.07%, and inter-assay precision ranged between 8.27% and 9.80%.

Concentrations of CPII and C2C were determined using commercially available ELISA kits (IBEX Pharmaceuticals Inc., Quebec, Montreal, Canada) previously reported for use in equine synovial fluid (Billinghurst et al., 2001; Frisbie et al., 2008). Synovial fluid samples were diluted 1:4 with assay buffer provided by the manufacturer. Samples were analyzed in duplicate with an intra-assay range coefficient of variation (**CV**) of 0.10% to 9.84% and an inter-assay CV of 1.38% to 7.96% for CPII and intra-assay range CV of 0.33% to 9.56% and an inter-assay CV of 2.28% to 6.76% for C2C. All markers were read (Synergy H1 Hybrid Multi-Mode Reader) at an optical density of 450 nm.

Statistical analysis

Data were analyzed using PROC MIXED in SAS v9.4 (SAS Inst., Inc., Cary, NC) with repeated measures (time). The responses to diet (day 0 to PIH 0 on day 84) were analyzed separately from

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Table 2. Mean synovial inflammatory and cartilage biomarkers of yearling horses before (day 0) and after 84 d of receiving either no supplementation (CON; $n = 9$) or 21 g *Saccharomyces cerevisiae* fermentation product (SCFP; $n = 10$) per day

					P-value		
Synovial biomarker[1]	Diet	Day 0	Day 84	SEM	Diet	Time	Diet × Time
PGE_2, pg/mL[2]	CON	454.07	460.59	49.94	0. 79	0.19	0.25
	SCFP	392.02	496.16				
CPII, ng/mL	CON	955.38	993.20	114.24	0.46	0.76	0.83
	SCFP	866.60	873.43				
C2C, ng/mL	CON	228.86	161.31*	9.96	0.41	<0.01	0.13
	SCFP	226.70	182.52*				
CPII:C2C, AU	CON	4.07	5.76*	0.51	0.37	<0.01	0.44
	SCFP	3.84	4.94*				

[1]C2C, collagenase cleavage neopeptide; CPII, carboxypropeptide of type II collagen; PGE_2, prostaglandin E_2.
[2]Synovial concentrations differed between CON and SCFP at day 0, so day 0 was included as a covariate in statistical analyses.
*Within row, day 0 differs from day 84 ($P < 0.05$).

responses to the intra-articular LPS challenge at day 84. Data were tested for normality, and outliers were identified using box plots of the residuals and removed if greater than two standard deviations from the mean.

For responses to the pre-LPS dietary adaptation period, the model contained fixed effects of diet, time, and the diet × time interaction, and a random effect of horse(diet). Where day 0 values differed by treatment (PGE_2), day 0 was included in the model as a covariate. Sex and knee were also included in the model as fixed effects but were removed when $P > 0.15$ to conserve degrees of freedom. For responses to the intra-articular LPS challenge at day 84, the model contained fixed effects of diet, injection type, time, and all interactions, and a random statement of horse(diet × injection type). All data are presented as least squares means ± SEM. Significance was declared at $P \leq 0.05$, and $P \leq 0.10$ was considered a trend toward significance.

Results

Responses to dietary treatment

There was no influence of dietary treatment on BW or BCS ($P \geq 0.9$); however, all horses, regardless of treatment, gained BW (280 ± 8 to 344 ± 8 kg) and increased BCS (5.3 ± 0.1 to 5.7 ± 0.1) over the 98-d trial ($P \leq 0.01$). During the pre-LPS period, synovial fluid C2C concentrations decreased ($P \leq 0.01$) and the ratio of CPII to C2C increased ($P \leq 0.01$) in all horses from day 0 to 84, but PGE_2 and CPII concentrations remained stable (Table 2). Synovial concentrations of PGE_2, CPII, C2C, and the ratio of CPII to C2C were unaffected by diet or the interaction of diet and time from day 0 to 84 pre-LPS challenge (Table 2).

Responses to the intra-articular LPS challenge

Clinical parameters

Throughout the intra-articular LPS challenge, dietary treatment did not influence HR, RR, or RT, and all values remained within normal physiological ranges over the 24-h period. Regardless of diet, HR and RR increased from 0 to 6 h postinjection ($P \leq 0.01$; Supplementary Figure S1A and B) and remained elevated at 12 h postinjection ($P \leq 0.01$). HR returned to preinjection values by 24 h postinjection (Supplementary Figure S1A), while RR decreased from 12 to 24 h postinjection ($P \leq 0.01$) but remained greater than preinjection values at 24 h postinjection ($P = 0.05$; Supplementary Figure S1B). Conversely, RT was greater at 12 and 24 h compared with 0 and 6 h postinjection ($P \leq 0.01$; Supplementary Figure S1C). Neither dietary treatment nor intra-articular injection of LPS affected CC or CT (Supplementary Figure S2). Following injection, CC increased at 6 h ($P \leq 0.01$) and remained above preinjection values through 336 h in response to repeated arthrocentesis ($P \leq 0.01$; Supplementary Figure S2A). At 6 h post LPS injection, CT increased in all horses and peaked at 12 h post-LPS injection ($P \leq 0.01$) but decreased to levels similar to preinjection temperatures by 24 h postinjection (Supplementary Figure S2B).

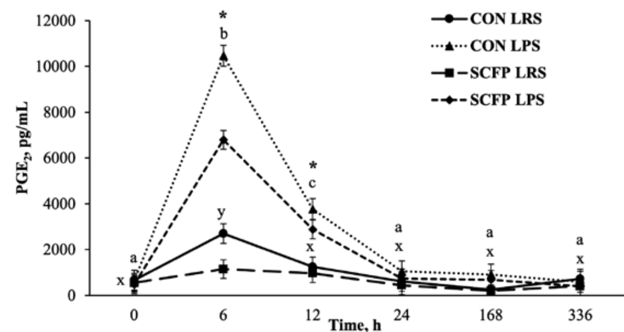

Figure 1. Synovial prostaglandin E_2 (PGE_2) concentrations of yearling horses in response to an intra-articular injection of either 0.5 ng lipopolysaccharide (LPS) derived from *Escherichia coli* O55:B5 or sterile lactated Ringer's solution (LRS) after receiving either no supplement (CON; $n = 9$) or 21 g/d of *Saccharomyces cerevisiae* fermentation product (SCFP; $n = 10$) for 84 d. Main effects included diet ($P \leq 0.01$), time ($P \leq 0.01$), injection type ($P \leq 0.01$), diet × time ($P \leq 0.01$), diet × injection type ($P = 0.17$), time × injection type ($P \leq 0.01$), and diet × time × injection type ($P = 0.4$). [a,b,c,x,y]Across dietary groups but within injection type, time points with different letters differ ($P < 0.05$). *Within time point, LPS differs from LRS ($P < 0.05$).

Acute synovial inflammation

Across dietary treatments, synovial PGE_2 concentrations increased at 6 h postinjection ($P \leq 0.01$; Figure 1). Synovial PGE_2 then returned to baseline by 12 h postinjection in LRS (contralateral control) knees and by 24 h postinjection in LPS knees. At 6 and 12 h after injection, intra-articular LPS resulted in greater synovial PGE_2 concentrations compared with the contralateral control (LRS) regardless of dietary treatment ($P \leq 0.01$; Figure 1). However, throughout the challenge, horses receiving SCFP had lesser synovial PGE_2 concentrations compared with CON horses ($P \leq 0.01$), which was particularly evident at 6 h postinjection ($P \leq 0.01$; Figure 1).

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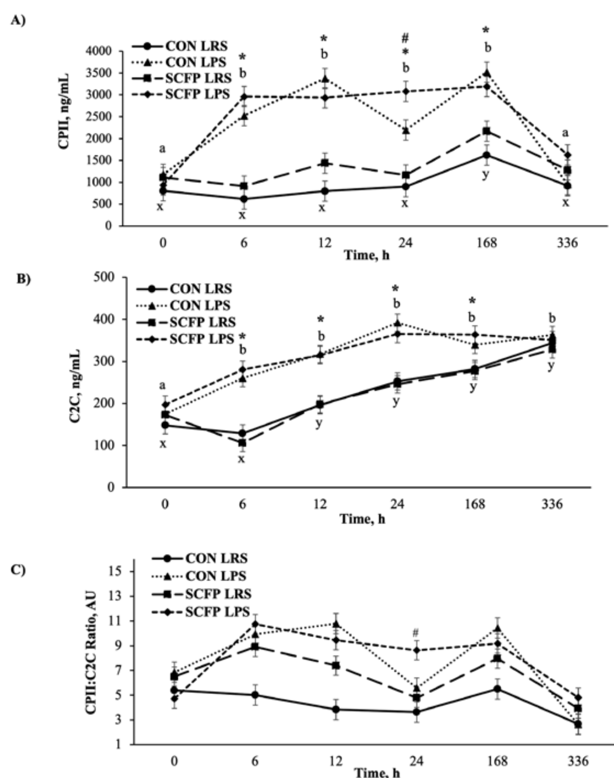

Figure 2. Synovial concentrations of (A) CPII, (B) C2C, and (C) the ratio of CPII:C2C of yearling horses in response to an intra-articular injection of either 0.5 ng lipopolysaccharide (LPS) derived from *Escherichia coli* O55:B5 or sterile lactated Ringer's solution (LRS) after receiving either no supplement (CON; $n = 9$) or 21 g/d of *Saccharomyces cerevisiae* fermentation product (SCFP; $n = 10$) for 84 d. Main effects included diet ($P = 0.02$, $P = 0.99$, $P \leq 0.01$), time ($P \leq 0.01$, $P \leq 0.01$, $P \leq 0.01$), injection type ($P \leq 0.01$, $P \leq 0.01$, $P \leq 0.01$), diet × time ($P = 0.3$, $P = 0.6$, $P = 0.09$), diet × injection type ($P = 0.3$, $P = 0.7$, $P = 0.01$), time × injection type ($P \leq 0.01$, $P \leq 0.01$, $P \leq 0.01$), and diet × time × injection type ($P = 0.04$, $P = 0.8$, $P \leq 0.01$) for panels A, B, and C, respectively. [a,b,x,y]Across dietary groups but within injection type, time points with different letters differ ($P < 0.05$). *Within time point, LPS differs from LRS ($P < 0.05$). #Within time point, CON LPS differs from SCFP LPS ($P < 0.05$).

Markers of cartilage metabolism

In response to the LPS injection, synovial concentrations of the cartilage synthesis marker, CPII, increased from 0 to 6 h ($P \leq 0.01$), remained elevated through 168 h postinjection ($P \leq 0.01$), and returned to baseline at 336 h (Figure 2A). Conversely, in knees that received the LRS injection (contralateral control), CPII concentrations remained unchanged through 24 h, increased at 168 h ($P \leq 0.01$), but returned to baseline by 336 h. At 6, 12, 24, and 168 h, LPS knees had greater CPII concentrations than LRS knees ($P \leq 0.01$). At 24 h postinjection, CPII concentrations in LPS knees of horses supplemented with SCFP were greater than LPS knees of CON horses ($P \leq 0.01$), as well as LRS-injected knees regardless of diet ($P \leq 0.01$; Figure 2A).

Across dietary treatments, the cartilage degradation marker, C2C, increased at 6 h in LPS knees ($P \leq 0.01$) and at 12 h in LRS knees ($P = 0.03$) and then remained elevated through 336 h postinjection in both knees of all horses ($P \leq 0.01$; Figure 2B). Similar to CPII, C2C concentrations were greater in LPS-injected knees compared with the contralateral control knees (LRS) at 6, 12, 24, and 168 h regardless of dietary treatment ($P \leq 0.01$; Figure 2B).

Throughout the challenge, there was a 3-way interaction of diet, time, and injection type for the ratio of CPII:C2C ($P \leq 0.01$; Figure 2C). There was no difference in the CPII:C2C ratio between groups at 0 h. In contralateral control knees of horses receiving the CON diet (CON-LRS), the CPII:C2C ratio did not change through 168 h; however, the ratio decreased from 168 to 336 h ($P = 0.01$) to be lower at 336 h than 0 ($P = 0.02$). In LPS knees of CON horses (CON-LPS) and the control knees of SCFP horses (SCFP-LRS), the CPII:C2C ratio increased from 0 to 6 h ($P \leq 0.02$), remained similar from 6 to 12 h, decreased from 12 to 24 h ($P \leq 0.01$), increased from 24 to 168 h ($P \leq 0. 01$), and then decreased from 168 to 336 h ($P \leq 0. 01$) to be lower at 336 than 0 h ($P \leq 0.02$). Finally, the CPII:C2C ratio in LPS knees of SCFP horses (SCFP-LPS) increased from 0 to 6 h ($P \leq 0. 01$), remained stable from 6 through 168 h, and then decreased from 168 to 336 h ($P \leq 0. 01$) to be the similar at 336 h compared with hour 0 (Figure 2C). These changes over time resulted in the following differences at each time point. At 6, 12, 24, and 168 h, CON-LRS had lesser CPII:C2C than all other groups ($P \leq 0.01$). At 12 and 168 h, CON-LPS had greater CPII:C2C than SCFP-LRS ($P \leq 0.03$), and at 24 h, SCFP-LPS had greater CPII:C2C than all other groups ($P \leq 0. 01$). At 336 h, the CPII:C2C ratio tended to be greater in LPS knees regardless of dietary treatment (CON-LPS and SCFP-LPS) than LRS knees of horses receiving the CON diet ($P = 0.06$; Figure 2C).

Discussion

The current study investigated the effects of dietary *S. cerevisiae* fermentation product supplementation on synovial biomarkers related to joint inflammation and cartilage metabolism in young horses challenged with an acute intra-articular inflammatory insult. Acute joint inflammation altered synovial PGE_2, CPII, C2C, as well as CPII:C2C, and SCFP supplementation impacted the degree of response. Specifically, SCFP horses responded more favorably to the acute inflammatory challenge evidenced by reduced PGE_2 concentrations in combination with sustained elevated CPII concentrations as well as the CPII:C2C ratio compared with non-supplemented horses. Therefore, these data provide foundational knowledge related to the ability of dietary provision of SCFP to result in stabilization of cartilage synthesis and mitigation of acute synovial inflammation in young growing horses.

During the 84 d pre-LPS supplementation period, horses readily consumed their concentrate with no significant feed refusals across treatment groups. Yearlings consumed energy and associated nutrients to meet or exceed recommended requirements (NRC, 2007) as BW and BCS increased in all horses as expected with growth but did not differ by dietary treatment.

Prior to the LPS challenge, C2C concentrations decreased and the ratio of type II collagen synthesis to degradation (CPII:C2C) increased over time. This was expected, as intra-articular collagen synthesis occurs with growth as horses adapt to increases in BW (Brama et al., 2000a). Concentrations of PGE_2 also increased numerically over the 84 d of supplementation, but this change was not statistically significant nor did it differ by dietary treatment. Therefore, this change may be related to growth of the yearlings. Similar to PGE_2, CPII, C2C, and the ratio of CPII to C2C were not affected by diet during the pre-LPS period. The horses in the current study received no forced exercise outside of the previously described submaximal exercise test, which occurred 4 wk prior to the LPS challenge (Valigura et al., 2021). As opposed to horses at rest, forced exercise in young horses is more commonly associated with alterations within the joint space due to repetitive hyperextensions and concussions that are generated during early training and exercise (Palmer and Bertone, 1994). Since horses were allowed turnout but were

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not enrolled in a regular exercise training program, a lack of dietary effect during the pre-LPS period was expected in these clinically healthy yearlings.

While diet did not affect cartilage inflammation or turnover while horses were at rest, the LPS challenge at day 84 allowed the determination of dietary impacts on synovial markers following an acute inflammatory insult. Clinical measures during the LPS challenge, including HR, RR, and RT, remained within physiologically normal ranges through 24 h postinjection, which confirmed a localized inflammatory response. CC was measured in an attempt to quantify the degree of joint distention, which might be expected to increase more in joints injected with LPS compared with LRS. However, both CC and CT increased over time without an effect of injection type. This indicates increased joint effusion from the initiation of an inflammatory response in both carpal joints due to repeated arthrocentesis (Bliss, 1998; van den Boom et al., 2005). These responses were expected and are similar to previous reports (Hawkins et al., 1993; de Grauw et al., 2009; Lucia et al., 2013). While the external physical characteristics that reflect joint distension/effusion were similar between injection types, cellular markers of inflammation and cartilage turnover within synovial fluid are likely more indicative of changes within the joint space. As further discussed below, inflammatory PGE_2 was greater in LPS compared with LRS joints, indicating a greater induction of inflammation in LPS over LRS knees even though this differential response was not captured by CC and CT measures.

The dietary supplementation of postbiotics such as *S. cerevisiae* fermentation product may elicit positive effects for treating acute stress-related gut dysmobility (West et al., 2016). Postbiotics are byproducts or metabolic waste released from a metabolic activity carried out by probiotics following the digestion of prebiotics in the gut (Żółkiewicz et al., 2020). Interestingly, postbiotics demonstrate beneficial health effects much like probiotics, strengthening the intestinal microbiome (Klemashevich et al., 2014). However, postbiotics are not made up of any live organisms like probiotics, decreasing risks associated with intake (Żółkiewicz et al., 2020). These organisms allow for the maintenance of tight junctions by increasing the production of mucin along the intestinal lining and improving the life of intestinal epithelial cells to increase the resistance of colonization from harmful bacteria (Oelschlaeger, 2010). Systemic and local inflammatory affects, such as intra-articular inflammation, are achieved by the modification of cytokine production by the intestinal epithelial cells and their effect on the innate immune system cells such as macrophages and dendritic cells (Watanabe et al., 2009).

In obese mice, supplementation of oligofructose allowed for the reestablishment of beneficial *Bifidobacteria* (Schott et al., 2018). *Bifidobacteria* are associated with supporting intestinal epithelial proliferation, supporting barrier function (Arboleya et al., 2016), and reducing the circulation of endotoxins and inflammatory cytokines, as well as protecting the infiltration of macrophages into the joint capsule (Schott et al., 2018). Therefore, synovial cells may be affected by a variety of mediators that directly impact cartilage structure and maintenance through the diffusion from systemic circulation into synovial fluid. However, there is limited information regarding the effects on the dietary inclusion of *S. cerevisiae* fermentation product on joint health in a young, growing equine model. This gap in knowledge allows for the potential that gut microbial manipulation from postbiotic supplementation could be utilized as a dietary intervention strategy to address systemic and intra-articular inflammation in young growing horses.

In the current study, intra-articular inflammation was assessed by quantification of synovial PGE_2 concentrations. Prostaglandin E_2 has both pro- and anti-inflammatory effects, but its pro-inflammatory effects cause the articular structural changes that characterize arthritic disease (Martel-Pelletier et al., 2003); therefore, PGE_2 is used as a marker of synovial inflammation (Sokolove and Lepus, 2013). In the present study, synovial PGE_2 peaked at 6 h and began to decline by 12 h postinjection regardless of injection type. However, knees that were challenged with LPS had greater concentrations of PGE_2 compared with contralateral control knees, indicating elevated inflammation in the presence of LPS. Furthermore, the PGE_2 peak at 6 h did not parallel the CT peak that occurred at 12 h but did mirror the increase in CC noted at 6 h postinjection of both LPS- and LRS-injected knees. The increased synthesis of PGE_2 likely enhanced carpal edema as well as the extravasion of plasma (Higgs et al., 1984) causing an increase in CC. Currently, the time frame of physiological occurrences that lead to increased swelling of the joint, including increases of synovial PGE_2 in growing horses, is minimally understood and warrants further investigation.

In previous work investigating the impacts of *S. cerevisiae* fermentation product supplementation on PGE_2, rats that had received 14 d of supplementation of 7 mg/kg BW *S. cerevisiae* fermentation product (EpiCor, Embria Health Sciences, Ankeny, IA) had decreased PGE_2 in their paw tissue following an inflammatory insult (Evans et al., 2012). In the current study, regardless of injection type, SCFP horses exhibited lower synovial PGE_2 than CON horses. In humans, PGE_2 has suppressive effects on collagen cleavage, allowing for the maintenance of a normal chondrocyte phenotype for appropriate collagen matrix maintenance of type II collagen (Tchetina et al., 2007). Although the present study did not evaluate the systemic immune response or associated changes in the gut microbiota, it can be hypothesized that the supplementation of SCFP modified the intestinal microbial population in a favorable manner, leading to a muted pro-inflammatory response following the acute LPS stressor. The mechanism of action whereby dietary SCFP impacted synovial PGE_2 warrants further investigation. While PGE_2 was the only biomarker evaluated to understand intra-articular inflammation in the current study, it would be of benefit to quantify and determine any dilutional effects of other synovial biomarkers related to inflammation, including cytokines, to obtain a more elaborate understanding of intra-articular inflammation. It was reported that horses supplemented with SCFP had lower serum concentrations of inflammatory cytokines, interleukin (IL)-1β, IL-6, and tumor necrosis factor-alpha, when compared with CON horses throughout the recovery period following the submaximal exercise test at day 56 (Valigura et al., 2021). However, possible cytokine levels in serum and their potential relationship with joint cytokines at the time of the LPS challenge were not evaluated, as serum cytokines were only analyzed surrounding the exercise test at day 56, and the evaluation of synovial cytokines was outside of the scope of the current study.

Any inflammatory insult to the joint, including LPS or an exercise-induced inflammatory event (Frisbie et al., 2008), may lead to degradation of the type II collagen fibril within articulating joints. Degradation is initiated by unwinding of the collagen, exposing the normally hidden epitope, catabolic C2C, and may be utilized as a marker of collagen degradation (Trumble et al., 2009). Regardless of dietary treatment, synovial C2C concentrations were greater in LPS joints compared with LRS joints. The increased inflammatory response to LPS

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compared with LRS joints, as evidenced by greater levels of PGE_2, likely leads to cartilage degradation, which would explain the elevation of catabolic C2C concentrations in LPS joints (Garvican et al., 2010). Interestingly, dietary SCFP did not impact synovial C2C concentrations in the current study.

During the regeneration process, CPII is released from the type II collagen precursor, procollagen (Nelson et al., 1998). In arthritic joints, the synovial concentration of CPII has been correlated to the direct upregulation of collagen production (Nelson et al., 1998; Sugiyama et al., 2003). Therefore, with a half-life of approximately 16 h in humans, CPII within synovial fluid can be utilized as an indicator of new collagen synthesis (Nelson et al., 1998). Although the half-life of CPII is unknown in the horse, type II collagen synthesis and degradation pathways appear to be homologous between mammalian species (Kadler, 1995), so it can be inferred that CPII serves as an appropriate indicator of new collagen synthesis in the horse. In the current study, synovial CPII concentrations increased 6 h postinjection, and remained elevated through 168 h postinjection, but returned to near baseline levels by 336 h.

The dietary treatment of SCFP appeared to modulate the CPII response to the LPS challenge. Previous investigations of *S. cerevisiae* fermentation product on joint health have shown improved lameness and arthritic scores in dogs and rats (Beynen and Legerstee, 2010; Evans et al., 2012). Although these studies demonstrated the potential for dietary supplementation of *S. cerevisiae* fermentation product to improve joint function, they did not evaluate synovial concentrations of cartilage metabolism. In the current study, supplementation of SCFP allowed for CPII concentrations to plateau through 168 h post-LPS injection, while LPS knees in CON horses exhibited a drop in concentrations from 12 to 24 h postinjection, only to increase once more at hour 168. Additionally, CPII concentrations remained greater in LRS knees of SCFP horses compared with LRS knees of CON horses throughout the postinjection period. The supplementation of SCFP demonstrated the ability to stabilize collagen synthesis throughout the 336 h following the intra-articular inclusion of LPS as well as favoring increases in synovial concentrations of CPII regardless of injection type. However, additional biological markers related to collagen metabolism would be necessary to definitively determine a potential improvement in cartilage repair.

In addition to elevated CPII concentrations, LPS also induced an increase in the ratio of CPII:C2C, and further, the CPII:C2C ratio remained stable in LPS knees of SCFP horses throughout the postinjection period before returning to preinjection levels at hour 336, similar to CPII concentrations. This is in contrast to control knees in SCFP horses and LPS knees in control horses, which both demonstrated fluctuations in CPII:C2C throughout the postinjection period. The CPII:C2C ratio is an important variable to consider to gain a more holistic understanding of cartilage health within the equine joint as it provides insight into net increases or decreases in cartilage synthesis and/or degradation. Given that SCFP horses maintained a sustained net positive cartilage synthesis:degradation ratio post-LPS injection, specifically at 24 h, it can be inferred that SCFP may help to positively regulate the cartilage metabolic response to an acute intra-articular inflammatory insult.

In conclusion, the dietary supplementation of SCFP to young horses for 84 d demonstrated potential to improve joint health following an acute stressor by reducing markers of intra-articular inflammation and improving markers of cartilage metabolism by stabilizing collagen formation. The dietary supplementation of SCFP may potentially reduce the susceptibility of intra-articular inflammation in growing horses. However, further studies are recommended to investigate the effects of SCFP supplementation on marker responses reflective of prolonged intra-articular inflammation and cartilage metabolism in horses.

Supplementary Data

Supplementary data are available at *Journal of Animal Science* online.

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Conflict of interest statement

The authors affirmatively acknowledge that they were free from influence by any funding sources or their employees that would result in any conflict of interest.

Literature Cited

Arboleya, S., C. Watkins, C. Stanton, and R. P. Ross. 2016. Gut *Bifidobacteria* populations in human health and aging. *Front. Microbiol.* **7**:1204. doi:10.3389/fmicb.2016.01204

Bertone, A. L., J. L. Palmer, and J. Jones. 2001. Synovial fluid cytokines and eicosanoids as markers of joint disease in horses. *Vet. Surg.* **30**:528–538. doi:10.1053/jvet.2001.28430

Beynen, A. C., and E. Legerstee. 2010. Influence of dietary beta-1,3/1,6-glucans on clinical signs of canine osteoarthritis in a double-blind, placebo-controlled trial. *Amer. J. Anim. Vet. Sci.* **5**(2):97–101. doi:10.3844/ajavsp.2010.97.101

Billinghurst, R. C., E. M. Buxton, M. G. Edwards, M. S. McGraw, and C. W. McIlwraith. 2001. Use of an anti-neoepitope antibody for identification of type-II collagen degradation in equine articular cartilage. *Amer. J. Vet. Res.* **62**:1031–1039. doi:10.2460/ajvr.2001.62.1031

Bliss, M. R. 1998. Hyperaemia. *J. Tissue Viability* **8**:4–13. doi:10.1016/s0965-206x(98)80028-4

van den Boom, R., C. H. van de Lest, S. Bull, R. A. Brama, P. R. van Weeren, and A. Barneveld. 2005. Influence of repeated arthrocentesis and exercise on synovial fluid concentrations of nitric oxide, prostaglandin E_2 and glycosaminoglycans in healthy equine joints. *Equine Vet. J.* **37**:250–256. doi:10.2746/0425164054530740

Brama, P. A., J. M. Tekoppele, R. A. Bank, A. Barneveld, and P. R. van Weeren. 2000a. Functional adaptation of equine articular cartilage: the formation of regional biochemical characteristics up to age one year. *Equine Vet. J.* **32**:217–221. doi:10.2746/042516400776563626

Brama, P. A. J., J. M. Tekeoppele, R. A. Bank, A. Barneveld, E. C. Firth, and P. R. van Weeren. 2000b. The influence of strenuous exercise on collagen characteristics of articular cartilage in Thoroughbreds age 2 years. *Equine Vet. J.* **32**:551–554. doi:10.2746/042516400777584613

Collins, K. H., H. A. Paul, R. A. Reimer, R. A. Seerattan, D. A. Hart, and W. Herzog. 2015. Relationship between inflammation, the gut microbiota, and metabolic osteoarthritis development: studies in a rat model. *Osteoarthritis Cartilage* **23**:1989–1998. doi:10.1016/j.joca.2015.03.014

Cryan, J. F., and S. M. O′Mahony. 2011. The microbiome-gut-brain axis: from bowel to behavior. *Neurogastroenterol. Motil.* **23**:187–192. doi:10.1111/j.1365-2982.2010.01664.x

Evans, M., S. Reeves, and L. E. Robinson. 2012. A dried yeast fermentate prevents and reduces inflammation in two separate

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experimental immune models. *Evid. Based Complement. Alternat. Med.* **2012**:973041. doi:10.1155/2012/973041

Faubladier, C., F. Chaucheyras-Durand, L. da Veiga, and V. Julliand. 2013. Effect of transportation on fecal bacterial communities and fermentative activities in horses: impact of *Saccharomyces cerevisiae* CNCM I-1077 supplementation. *J. Anim. Sci.* **91**:1736–1744. doi:10.2527/jas.2012-5720

Firth, E. C. 2006. The response of bone, articular cartilage and tendon to exercise in the horse. *J. Anat.* **208**:513–526. doi:10.1111/j.1469-7580.2006.00547.x

Frisbie, D. D., F. Al-Sobayil, R. C. Billinghurst, C. E. Kawcak, and C. W. McIlwraith. 2008. Changes in synovial fluid and serum biomarkers with exercise and early osteoarthritis in horses. *Osteoarthritis Cartilage* **16**:1196–1204. doi:10.1016/j.joca.2008.03.008

Garvican, E. R., A. Vaughan-Thomas, J. F. Innes, and P. D. Clegg. 2010. Biomarkers of cartilage turnover. Part 1: Markers of collagen degradation and synthesis. *Vet. J.* **185**:36–42. doi:10.1016/j.tvjl.2010.04.011

de Grauw, J. C., C. H. van de Lest, P. A. Brama, B. P. Rambags, and P. R. van Weeren. 2009. In vivo effects of meloxicam on inflammatory mediators, MMP activity and cartilage biomarkers in equine joints with acute synovitis. *Equine Vet. J.* **41**:693–699. doi:10.2746/042516409x436286

de Grauw, J. C., C. H. van de Lest, R. van Weeren, H. Brommer, and P. A. Brama. 2006. Arthrogenic lameness of the fetlock: synovial fluid markers of inflammation and cartilage turnover in relation to clinical joint pain. *Equine Vet. J.* **38**:305–311. doi:10.2746/042516406777749236

Guo, J., H. Khalouei, F. Kelsey, V. Senaratne, Z. Zhang, H. Derakhshani, M. Scott, G. Crow, I. Yoon, E. Khafipour, *et al.* 2017. Effects of *Saccharomyces cerevisiae* fermentation products on endotoxins and acute phase proteins in lactating dairy cows. In: Lucy, M. C., editor. Proceedings of the American Dairy Science Association Annual Meeting; June 25 to 28, 2017; Journal Of Dairy Science, Champaign, IL. Pittsburgh, PA; p. 161–162.

Hand, T. W., I. Vujkovic-Cvijin, V. K. Ridaura, and Y. Belkaid. 2016. Linking the microbiota, chronic disease, and the immune system. *Trends Endocrinol. Metab.* **27**:831–843. doi:10.1016/j.tem.2016.08.003

Hawkins, D. L., R. J. MacKay, G. G. Gum, P. T. Colahan, and J. C. Meyer. 1993. Effects of intra-articularly administered endotoxin on clinical signs of disease and synovial fluid tumor necrosis factor, interleukin 6 and prostaglandin E_2 values in horses. *Am. J. Vet. Res.* **54**:379–386.

Henneke, D. R., G. D. Potter, J. L. Kreider, and B. F. Yeates. 1983. Relationship between condition score, physical measurements and body fat percentage in mares. *Equine Vet. J.* **15**:371–372. doi:10.1111/j.2042-3306.1983.tb01826.x

Hernández-Alonso, P., D. Cañueto, S. Giardina, J. Salas-Salvadó, N. Cañellas, X. Correig, and M. Bulló. 2017. Effect of pistachio consumption on the modulation of urinary gut microbiota-related metabolites in prediabetic subjects. *J. Nutr. Biochem.* **45**:48–53. doi:10.1016/j.jnutbio.2017.04.002

Higgs, G. A., S. Moncada, and J. R. Vane. 1984. Eicosanoids in inflammation. *Ann. Clin. Res.* **16**:287–299.

Kadler, K. 1995. Extracellular matrix 1: fibril-forming collagens. *Protein Profile* **2**:491–619.

Kahn, M. K., J. A. Coverdale, J. L. Leatherwood, C. E. Arnold, R. A. Dabareiner, A. N. Bradbery, A. A. Millican, and T. H. Welsh. 2017. Age-related effects on markers of inflammation and cartilage metabolism in response to an intra-articular lipopolysaccharide challenge in horses. *J. Anim. Sci.* **95**:671–680. doi:10.2527/jas.2016.1078

Klemashevich, C., C. Wu, D. Howsmon, R. C. Alaniz, K. Lee, and A. Jayaraman. 2014. Rational identification of diet-derived postbiotics for improving intestinal microbiota function. *Curr. Opin. Biotechnol.* **26**:85–90. doi:10.1016/j.copbio.2013.10.006

Lucia, J. L., J. A. Coverdale, C. E. Arnold, and K. N. Winsco. 2013. Influence of an intra-articular lipopolysaccharide challenge on markers of inflammation and cartilage metabolism in young horses. *J. Anim. Sci.* **91**:2693–2699. doi:10.2527/jas.2012-5981

Martel-Pelletier, J., J. P. Pelletier, and H. Fahmi. 2003. Cyclooxygenase-2 and prostaglandins in articular tissues. *Semin. Arthritis Rheum.* **33**:155–167. doi:10.1016/s0049-0172(03)00134-3

McIlwraith, C. W., and G. W. Trotter. 1996. *Joint disease in the horse*. Philadelphia (PA): W.B. Saunders Co.

Nelson, F., L. Dahlberg, S. Laverty, A. Reiner, I. Pidoux, M. Ionescu, G. L. Fraser, E. Brooks, M. Tanzer, L. C. Rosenberg, *et al.* 1998. Evidence for altered synthesis of type II collagen in patients with osteoarthritis. *J. Clin. Invest.* **102**:2115–2125. doi:10.1172/JCI4853

NRC. 2007. *Nutrient requirements of horses*. 6th rev. ed. Washington (DC): National Academies Press.

Oelschlaeger, T. A. 2010. Mechanisms of probiotic actions—a review. *Intl. J. Medical Micro.* **300**(1):57–62. doi:10.1016/j.ijmm.2009.08.005

Palmer, J. L., and A. L. Bertone. 1994. Experimentally-induced synovitis as a model for acute synovitis in the horse. *Equine Vet. J.* **26**:492–495. doi:10.1111/j.2042-3306.1994.tb04056.x

Schott, E. M., C. W. Farnsworth, A. Grier, J. A. Lillis, S. Soniwala, G. H. Dadourian, R. D. Bell, M. L. Doolittle, D. A. Villani, H. Awad, *et al.* 2018. Targeting the gut microbiome to treat the osteoarthritis of obesity. *Amer. Soc. Clin. Invest.* **3**(8):e95997. doi:10.1172/jci.insight.95997

Sekirov, I., S. L. Russell, L. C. Antunes, and B. B. Finlay. 2010. Gut microbiota in health and disease. *Physiol. Rev.* **90**:859–904. doi:10.1152/physrev.00045.2009

Sokolove, J., and C. M. Lepus. 2013. Role of inflammation in the pathogenesis of osteoarthritis: latest findings and interpretations. *Ther. Adv. Musculoskelet. Dis.* **5**:77–94. doi:10.1177/1759720X12467868

Sugiyama, S., M. Itokazu, Y. Suzuki, and K. Shimizu. 2003. Procollagen II C propeptide level in the synovial fluid as a predictor of radiographic progression in early knee osteoarthritis. *Ann. Rheum. Dis.* **62**:27–32. doi:10.1136/ard.62.1.27

Szychlinska, M. A., M. Di Rosa, A. Castorina, A. Mobasheri, and G. Musumeci. 2019. A correlation between intestinal microbiota dysbiosis and osteoarthritis. *Heliyon* **5**:e01134. doi:10.1016/j.heliyon.2019.e01134

Tchetina, E. V., J. A. Di Battista, D. J. Zukor, J. Antoniou, and A. R. Poole. 2007. Prostaglandin PGE_2 at very low concentrations suppresses collagen cleavage in cultured human osteoarthritic articular cartilage: this involves a decrease in expression of proinflammatory genes, collagenases and *COL10A1*, a gene linked to chondrocyte hypertrophy. *Arthritis Res. Ther.* **9**:R75. doi:10.1186/ar2273

Trumble, T. N., A. B. Scarbrough, and M. P. Brown. 2009. Osteochondral injury increases type II collagen degradation products (C2C) in synovial fluid of Thoroughbred racehorses. *Osteoarthritis Cartilage* **17**:371–374. doi:10.1016/j.joca.2008.07.014

Valigura, H. C., J. L. Leatherwood, R. E. Martinez, S. Norton, and S. H. White-Springer. 2021. Dietary supplementation of a *Saccharomyces cerevisiae* fermentation product attenuates exercise-induced stress markers in young horses. *J. Anim. Sci.* **99**:1–11. doi:10.1093/jas/skab199

Watanabe, T., H. Nishio, T. Tanigawa, H. Yamagami, H. Okazaki, K. Watanabe, K. Tominaga, Y. Fujiwara, N. Oshitani, T. Asahara, *et al.* 2009. Probiotic *Lactobacillus casei* strain Shirota prevents indomethacin-induced small intestinal injury: involvement of lactic acid. *Am. J. Physiol. Gastrointest. Liver Physiol.* **297**:G506–G513. doi:10.1152/ajpgi.90553.2008

West, C., A. M. Stanisz, A. Wong, and W. A. Kunze. 2016. Effects of *Saccharomyces cerevisiae* or boulardii yeasts on acute stress induced intestinal dysmotility. *World J. Gastroenterol.* **22**:10532–10544. doi:10.3748/wjg.v22.i48.10532

Żółkiewicz, J., A. Marzec, M. Ruszczyński, and W. Feleszko. 2020. Postbiotics—a step beyond pre- and probiotics. *Nutrients* **12**(8):2189. doi:10.3390/nu12082189

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