



## RESEARCH ■ REVIEW >

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## Purina® Equine Senior® Horse Feed with ActivAge® Supports Optimal Joint Function in Senior Horses

A SUMMARY OF RESEARCH CONDUCTED AT THE PURINA ANIMAL NUTRITION CENTER EVALUATING THE EFFECTS OF FEEDING PURINA® EQUINE SENIOR® HORSE FEED WITH ACTIVAGE® TO SENIOR HORSES UNDERGOING A JOINT INFLAMMATION CHALLENGE.<sup>1</sup>

### < BACKGROUND >

Equine joint health is of critical importance to horse owners and veterinarians. Thanks to improved nutrition and veterinary care, horses are living longer, and more productive lives than ever before, commonly exercising, competing, and performing into their senior years. As horses age, the maintenance of joint health is paramount. Nutritional support of joint health for horses is a common request by horse owners, however many nutritional products do not have proper research and efficacy studies to support their claimed benefits.

Research at Purina Animal Nutrition into nutritional support of joint health has been ongoing utilizing both in vitro methodologies as well as in vivo techniques. This study was part of a large-scale investigation into feeding Purina® Equine Senior® with ActivAge® to senior horses and the effects of this diet on markers of joint health. It was hypothesized that horses fed Purina® Equine Senior® with ActivAge® would respond more favorably to a joint inflammation challenge aimed at mimicking the effects of degenerative joint disease, or other non-acute joint discomfort in horses.

### < MATERIALS AND METHODS >

American Quarter Horse geldings (n=10; average age = 19 ± 0.6 yr; average BW = 587.5 ± 22 kg) were used in this trial in a randomized cross-over design. Horses in the TRT group received 0.36 kg/45.5 kg BW per day of Purina® Equine Senior® Horse Feed formulated without ActivAge® in addition to a treatment pellet fed at 0.45 kg/day containing ActivAge®. Horses in the CON group received 0.36 kg/45.5 kg BW per day of Purina® Equine Senior® formulated without ActivAge® in addition to a control pellet of wheat middlings fed at 0.45 kg/day. All horses were offered 1.25% of their BW daily as Timothy grass hay. Diets were split evenly into AM (0700) and PM (1500) feedings daily. Horses were housed in individual stalls with individual drylot turnouts and had free-choice access to white salt blocks. Horses were offered their respective diets for 54 days prior to the inflammation challenge and synovial fluid collection. Immediately following the final synovial fluid collection during period 1, horses were assigned to a washout period consisting only of 0.36 kg/45.5 kg BW per day of the base diet daily in addition to their hay for 28 days prior to transitioning to the opposite group.

<sup>1</sup>HR 303. The effects of ActivAge on the joint, metabolic, inflammatory, and overall health in senior horses. Jacobs, R.D. et al. 2020. Internal research, PANC.

On d 54 of dietary treatment, all horses underwent an inflammation challenge designed to mimic joint inflammation. The carpus of both front legs was prepared utilizing aseptic technique and the radiocarpal joint was selected for an intra-articular inflammation challenge. During period 1, for each horse, the left radiocarpal joint was selected as the challenged joint while the contra lateral joint was used as the control joint. During period 2, the joints were switched so that the right radiocarpal joint was designated as the challenge joint while the left was used as a control joint. At timepoint 0, immediately preceding the challenge, an 18 ga, 1-inch needle was inserted into the joint space, while the leg was being flexed and approximately 0.5 mL of synovial fluid was obtained from each joint. Immediately following this collection, 1 mL of a sterile phosphate-buffered saline (PBS) solution was injected into the joint space. For the joint designated as the treatment joint, the PBS contained 0.5 ng of lipopolysaccharide (LPS; from *E. Coli* 055:B5; Sigma-Aldrich) in order to produce an inflammatory response. Synovial fluid was collected from both radiocarpal joints at post-injection hour (PIH) 5, 24, and 48. All horses were monitored for adverse responses to the challenge during the entire collection period and for 48-hr following injection. All injections and synovial fluid collections were conducted under the supervision of a veterinarian.

Synovial fluid samples were collected into sterile 1.5 mL conicals and immediately frozen at -20°C. Samples were sent to the University of Missouri School of Medicine, Thompson Laboratory for Regenerative Medicine for analysis. The markers measured in the synovial fluid were as follows: matrix metalloproteinase (MMP) activity, prostaglandin E2 (PGE2), substance P, interleukin-1 beta (IL-1b), interleukin-6 (IL-6), interleukin-8 (IL-8), and tumor necrosis factor alpha (TNF- $\alpha$ ) utilizing commercially available assays previously validated in equine tissues. Data were analyzed via SAS 9.4 (Cary, NC) utilizing a MIXED procedure ANOVA.

## < RESULTS >

The LPS-challenge produced an appropriate level of inflammation in the injected joints, and all horses completely recovered from the challenge within the 48-hr period following the final synovial collection. No differences were observed for the following markers between horses consuming Purina® Equine Senior® with or without ActivAge®: MMP, PGE2, substance P, IL-1b, and IL-8. However, there were differences in both IL-6 and TNF- $\alpha$ . The figures below highlight the responses of both the control and the challenged joints of the horses during the CON and TRT periods. For each graph, the data is represented as follows:

- 1) AA-Control: Represents the control joint during the TRT period.
- 2) Con-Control: Represents the control joint during the CON period.
- 3) AA-LPS: Represents the challenged joint during the TRT period.
- 4) Con-LPS: Represents the challenged joint during the CON period.

All data are displayed below as mean  $\pm$  SEM. **Figure 1** displays the effects of the challenged and synovial collection on IL-6, an inflammatory cytokine. At all timepoints PIH, IL-6 remained lower in the TRT-fed group compared to the CON group (AUC: TRT < CON;  $P=0.02$ ). **Figure 2** highlights the effects of treatment on TNF- $\alpha$  content in synovial fluid with a treatment effect at PIH 5 ( $P=0.08$ ) and an overall reduced TNF- $\alpha$  level in the TRT group (AUC: TRT < CON;  $P=0.01$ ).

## < CONCLUSIONS AND IMPLICATIONS >

The objective of this trial was to evaluate the effects of feeding Purina® Equine Senior® with and without ActivAge® on markers of joint inflammation in senior horses following an inflammatory challenge. Data from this study indicate that horses fed Purina® Equine Senior® with ActivAge® responded more favorably to an inflammatory challenge. A hallmark of the aging process in horses is a phenomenon known as “Inflamm-aging” whereby the senior horse experiences increased levels of inflammation systemically as well as locally in joints and other anatomical locations. The ability to support the horses aging process via nutritional modification such as the inclusion of ActivAge® into Purina® Equine Senior® Horse Feed represents a unique opportunity.

FIGURE 1

Interleukin-6 (IL6) levels measured in synovial fluid collected from both challenged (LPS) and control radiocarpal joints from horses consuming Purina® Equine Senior® Horse Feed with and without ActivAge®.

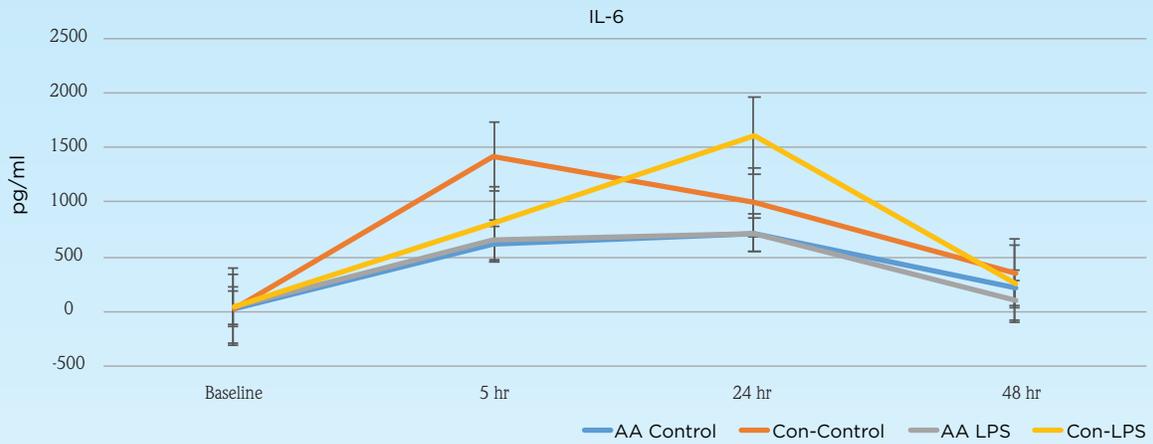
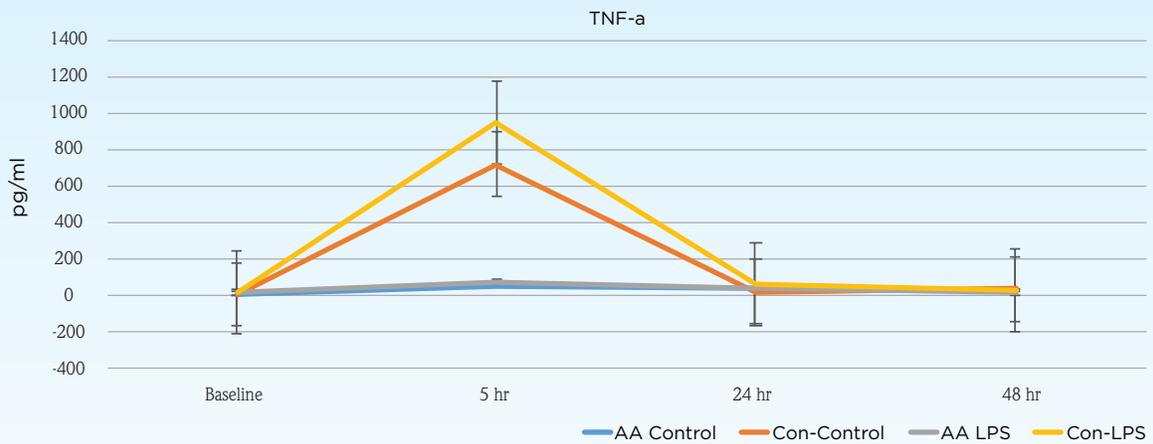


FIGURE 2

Tumor necrosis factor alpha (TNFa) levels measured in synovial fluid collected from both challenged (LPS) and control radiocarpal joints from horses consuming Purina® Equine Senior® Horse Feed with and without ActivAge®.



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