

# REVIEW >

< RESEARCH</pre>

Feeding Equine Senior<sup>®</sup> horse feed with ActivAge<sup>™</sup> prebiotic supports immune function and provides nutrition to support senior horses through the aging process.

ALL MILLIN

A SUMMARY OF RESEARCH CONDUCTED BY PURINA ANIMAL NUTRITION OVER A 3-YEAR PERIOD, IN COLLABORATION WITH THE GLUCK EQUINE RESEARCH CENTER AT THE UNIVERSITY OF KENTUCKY, EXAMINED THE EFFECTS OF FEEDING THE ACTIVAGE<sup>™</sup> PREBIOTIC ON IMMUNE FUNCTION IN SENIOR HORSES.

## < INTRODUCTION >

As horses reach their twenties and beyond, they experience a decline in immune function. Immunosenescence in the aged horse is characterized by a decline in overall immune cell function and a reduced antibody response to vaccination. In addition, advanced age is associated with an increased production of pro-inflammatory cytokines and other inflammatory mediators. This phenomenon has been termed "inflamm-aging," and it predicts both increased morbidity and mortality for a variety of chronic diseases. Together, the effects of immunosenescence and inflamm-aging may increase susceptibility to infection and contribute to aged-related health conditions such as arthritis, equine Cushing's disease, and laminitis. ActivAge<sup>™</sup> is a proprietary prebiotic additive derived from a multi-stage fermentation process that produces beneficial metabolites containing vitamins, minerals, amino acids, and antioxidants that work together to support digestive health and immune function.

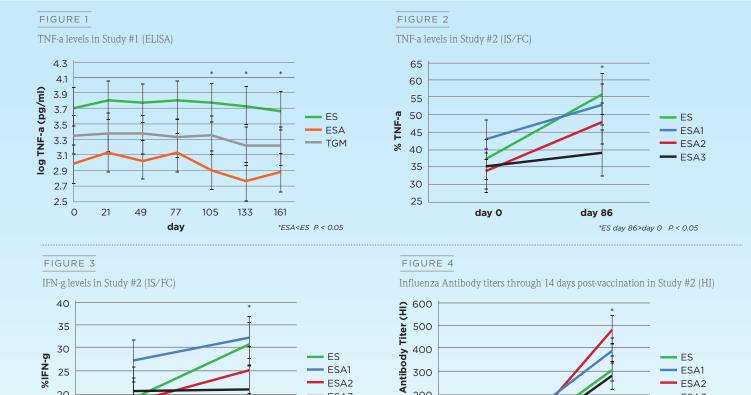
## < MATERIALS AND METHODS >

Two studies were conducted to identify 1) the effects of ActivAge<sup>™</sup> prebiotic on the production of inflammatory cytokines and on the recall immune response to vaccination and 2) to determine the effective dosage level of ActivAge<sup>™</sup> prebiotic. Thirty senior (20-33 years) horses (study #1) and thirty-two senior horses (study #2) of mixed sex and mixed-breed were utilized in these studies. In study #1, horses were assigned to one of three treatment groups for 161 days: TGM (traditional grain mix; n=10), ES (Equine Senior<sup>®</sup>; n=10), and ESA (Equine Senior<sup>®</sup> + ActivAge<sup>™</sup>; n=10). In study #2, horses were assigned to one of four treatment groups for 84 days: ES (Equine Senior<sup>®</sup>; n=8), ESA1 (Equine Senior<sup>®</sup> + ActivAge<sup>™</sup> level 1; n=8), ESA2 (Equine Senior<sup>®</sup> + ActivAge<sup>™</sup> level 3; n=8). Horses were allowed free-choice access to mixed-grass hay while housed on pasture and were fed individually twice daily. Horses were weighed, assigned BCS, and blood samples were taken throughout the study to measure inflammatory cytokines tumor necrosis factor-alpha (TNF-a), interleukin-6 (IL-6), and interferon-gamma (IFN-g) using RT-PCR, intracellular staining/flow cytometry (IS/FC), and ELISA techniques. In study #1, all horses received two separate vaccinations on day 56, an equine influenza vaccine (Calvenza; Boehringer Ingelheim) and a novel antigen (OVA). In study #2, all horses were vaccinated with a novel antigen (KLH) on day 28 and day 42 and with an equine influenza vaccine (Fluvac Innovator; Zoetis) on day 42. Prior to vaccination and two weeks post-vaccination, blood samples were collected to determine influenza and OVA- or KLH-specific immune responses using an antigen-specific ELISA and a hemagglutination-inhibition (HI) assay.

## < RESULTS >

Study #1: All horses gained weight and increased BCS over time. The key inflammatory cytokine TNF-a (Figure 1) was lower in ESA than ES at the end of the study (P<0.05). IL-6 decreased in TGM and increased in ES over time, but did not change in ESA (P<0.05). IFN-g was lower in ESA and ES than TGM at the end of the study (P<0.05), and overall, IFN-g increased over time in TGM and ES but did not change in ESA (P<0.05). There were no effects of diet on vaccination response, but there was a negative correlation between OVA titer and age (R=-0.32; P<0.05).

Study #2: All horses slightly lost weight but increased in BCS over time. A seasonal rise in inflammatory cytokine levels is expected during the winter months (when this study took place), but levels of the key inflammatory cytokines TNF-a (Figure 2) and IFN-g (Figure 3) increased in ES but did not change in ESA1, ESA2, or ESA3 (P<0.05). IL-6 decreased only in ESA2 over time (P<0.05). Flu titers were higher in ESA2 than ES and ESA3 at day 56 (P<0.05; Figure 4), and the increase in flu titer was greatest in ESA1 (P<0.05; Figure 5). There were no effects of diet on KLH-specific vaccination response.



ESA3

### FIGURE 5

20

15

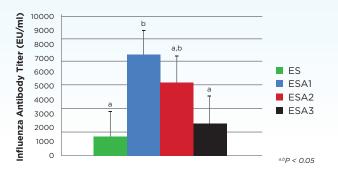
10

day O

Change in Influenza Antibody Titer following Vaccination in Study #2 (ELISA)

day 86

\*ES day 86>day 0 P < 0.05



#### < IMPLICATIONS >

day O

day 35

vaccine administration (day 42)

200

100 0

Influenza

The results of these two studies showed that feeding the ActivAge<sup>™</sup> prebiotic provides nutrition to support horses through the aging process. Based on these results, we have chosen to incorporate two different levels of ActivAge<sup>™</sup> prebiotic into Equine Senior<sup>®</sup> (ESA2) and Equine Senior<sup>®</sup> Active horse feeds (ESA1), as these products have different feeding rates and both levels were found to be efficacious. As horses live longer than ever before, providing nutritional support for immune response serves as an important tool in maintaining the senior horse's health and quality of life.

day 56

< FOR MORE INFORMATION > Please contact your local Purina representative if you would like more information about this study.

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ESA3

\*ESA2 > ES, ESA3 P < 0.05