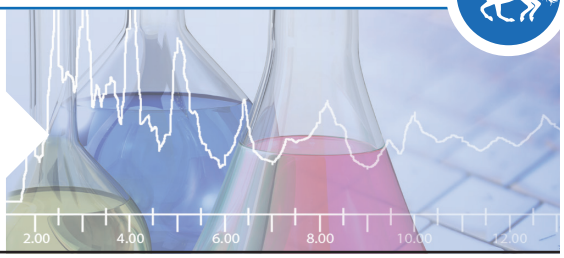
FOCUS ON RESEARCH



Evaluation of TruEquine™ C Postbiotic Supplementation on Immune Function in Senior Horses

***In vivo* and *in vitro* measures of immune function were evaluated in senior horses receiving a dietary TruEquine C postbiotic versus a non-supplemented control group. This study was conducted at the Maxwell H. Gluck Research Center, Department of Veterinary Science at the University of Kentucky.**

Background

Senior horses are a demographic of increasing importance to equine enthusiasts as numbers continue to rise worldwide. Senior horses are frequently ridden for pleasure or kept as companion animals, while some continue engaging in athletic competitions and breeding into their late teens and twenties. The onset of old age is associated with changes in the immune system. Horses, like humans, exhibit the phenomenon of "inflammaging" characterised by physiologic marker responses reflecting chronic, systemic, low-grade inflammation that is associated with advanced age, in addition to exhibiting immunosenescence, a decreased immune response associated with old age. Furthermore, older horses exhibit changes in their gut microflora compared to younger adult horses. Therefore, supporting the gut and immune system could play a vital role in supporting optimal health and vitality during the aging process. TruEquine C postbiotic technology has been shown to affect beneficial effects on digestive and immune balance in horses. The objective of this study was to determine changes in several aspects of immune function as the result of dietary supplementation of TruEquine C postbiotic, a *Saccharomyces cerevisiae* fermentate, as a part of the total daily ration of senior horses.

Overview

- Sixteen senior horses (24.8 ± 3.0 y; BW = 1200 ± 136.1 lb)
- Control (CON; n=8; no supplementation) and TruEquine C postbiotic top-dressed onto a common concentrate (TEC; 21 g/d; n=8).
- The total supplementation period was 56 days.
- Body weight and body condition score (BCS) readings and blood samples were obtained at baseline (day 0) and post supplementation at days 42, 49, and 56.
- Immediately following the day 42 blood sampling all horses were vaccinated with a monovalent influenza vaccine (Fluvacc Innovator®; Zoetis Animal Health, Parsippany, NJ) as an immune system challenge.
- Immune function assays included:
  - Flow Cytometry: In vitro non-specific cell-mediated immune responses (IFN-γ and TNF-α) to determine the percent of IFN-γ and TNF-α producing lymphocytes.
  - Cytokine Gene Expression in whole blood and peripheral blood mononuclear cell (PBMC) fraction expressed as relative quantity (RQ) including IFN-γ, TNF-α, IL-1β, IL-6, IL-10, IL-4, IL-8, IL-13, IL-17, and COX-1 and COX-2.
  - H1 titers were measured to evaluate the response to vaccination.
- Data was analysed to evaluate changes before the vaccine challenge (d 0-42) and after (d 42-56).

TruEquine™ Postbiotic

Results

Pre-Vaccination Challenge Period (d 0-42)

- IL-10 gene expression trended lower ($P < 0.090$) for TEC vs CON horses (Figure 1). IL-10 plays major role in regulating inflammatory balance. A tendency for lower IL-10 gene expression as a marker may suggest immune function that is less suppressed (immunosenescent) and better able to respond appropriately to challenges.
- TNF-α produced per cell trended lower ($P < 0.089$) for TEC vs CON horses (Figure 2). Given the role of TNF-α as a physiological marker of inflammation, this observation may suggest a lower state of inflammation.

Figure 1: IL-10 Whole Blood Gene Expression

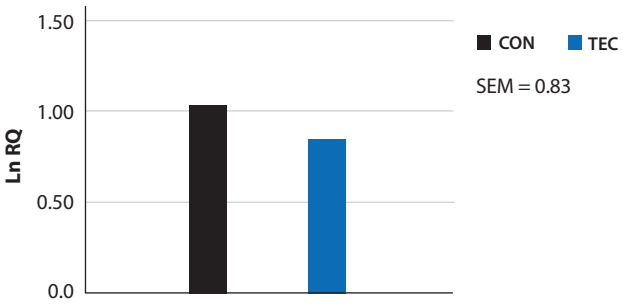
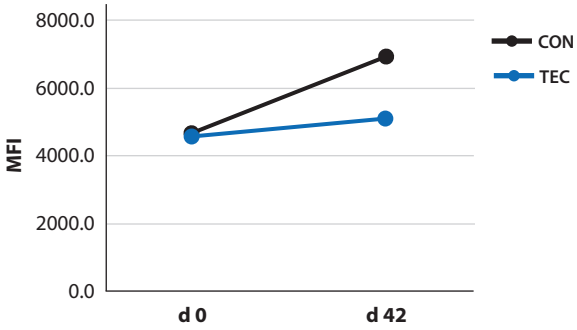


Figure 2: TNF-α MFI



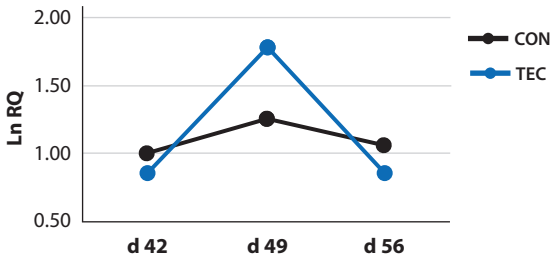
Post-Vaccination Challenge Period (d 42-56)

- The response in IL-13 gene expression tended to be different over time (QUADRATIC, $P < 0.080$) between treatments (Figure 3).
- Horses supplemented with TEC exhibited lower gene expression for several cytokines involved in inflammation signaling compared to CON horses (Table 1).
- The response in IL-13 gene expression tended to be different over time (QUADRATIC, $P < 0.080$) between treatments (Figure 3).
- Serum titers in response to vaccine challenge were similar between treatments (Figure 4).

Table 1: Whole Blood Gene Expression Response, Ln RQ

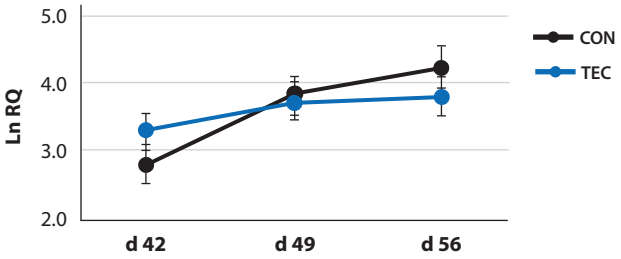
Gene	Whole Blood Gene Expression Response, LnRQ		
	CON	TEC	probF
IFN-γ	1.03	0.79	$P = 0.054$
IL-10	1.04	0.78	$P = 0.030$
IL-6	1.16	0.70	$P = 0.047$

Figure 3: IL-13 Whole Blood Gene Expression



Results - continued

Figure 4: Serum H1 Titers Following Vaccination



Summary

- Under the conditions of this study, TruEquine C tended to modulate pro-inflammatory and anti-inflammatory cytokine gene expression in senior horses to differ from non-supplemented controls under both vaccine-challenged and non-challenged states.

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