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ORIGINAL ARTICLE

# Bee pollen product supplementation to horses in training seems to improve feed intake: a pilot study

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## Summary

The objective of this study was to determine the efficacy of supplementation of Dynamic Trio 50/50, a bee pollen-based product, to improve physical fitness, blood leukocyte profiles, and nutritional variables in exercised horses. Ten Arabian horses underwent a standardised exercise test (SET), then were pair-matched by sex and fitness and randomly assigned to BP (receiving 118 g of Dynamic Trio 50/50 daily) or CO (receiving 73 g of a placebo) for a period of 42 days. A total collection was conducted from days 18 to 21 on six geldings to determine nutrient retention and neutral detergent fibre (NDF) and acid detergent fibre (ADF) digestibility. Horses were exercise conditioned and completed another SET on day 42. $V_{160}$ and $V_{200}$ were calculated from SET heart rates (HR). Lactate, glucose, haematocrit (HT) and haemoglobin (HB) concentrations were determined from SET blood samples. Total leukocyte count, and circulating numbers of various leukocytes and IgG, IgM and IgA concentrations were determined in rest and recovery blood samples from both SETs. Geldings on BP ($n = 3$) ate more feed than CO. BP had less phosphorus excretion, and tended to retain more nitrogen. BP tended to digest more NDF and ADF while having lower NDF digestibility and tending to have lower ADF digestibility. No treatment differences existed for $V_{160}$ and $V_{200}$, HR, lactate, HT and HB. There was a trend for lymphocyte counts to be lower in BP than CO on day 42. Dynamic Trio 50/50 supplementation may have a positive effect on performance by helping horses in training meet their potentially increased nutrient demands by increasing feed intake and thus nutrient retention.

## Introduction

Bee pollen is used widely as a dietary supplement in the equine industry, with many trainers reporting anecdotally numerous beneficial effects of pollen on their horses. However, few studies have been conducted to determine the efficacy of bee pollen supplementation in horses. A popular press article reported findings from a study in which horses supplemented with bee pollen had improved oxygen utilisation capabilities, lower heart rates (HR), and firmer muscle tone (Speedhorse, 1992). A search through scientific literature yielded no results of the original study.

Studies with bee pollen have been conducted on other species. Rats fed only pollen and water for 12 weeks survived and remained healthy, drawing the conclusion that bee pollen is a complete food and contains all the essential nutrients to sustain life (Liebelt and Calcagnetti, 1999). Additionally, rats fed pollen and/or propolis (another bee product similar to pollen) had increased weight gains, improved utilisation of iron, increased calcium and phosphorus absorption, and improved regeneration efficiency of haemoglobin (HB; Haro et al., 2000). Similar results were seen in pregnant rats, in which greater weight gains and higher total protein, HB, serum iron, and albumin were recorded in dams fed either 10 or 20 g/kg bee pollen daily (Xie et al., 1994). In human beings, the effects on immunomodulation of feeding healthy volunteers 500 mg propolis for 13 days was investigated (Bratter et al., 1999). They reported no changes in plasma cytokine concentrations during the study. However, they did report increases in spontaneous and lipopolysaccaride-induced cytokine secretion capacity following short-term *ex vivo* culture of peripheral blood leukocytes. In this study, control subjects receiving a placebo were not tested against the subjects receiving the propolis. Thus, it cannot be conclusively determined that the cytokine increases were due to propolis. However, the humoral immune response of rats was stimulated by bee pollen (Dudov et al., 1994). Though the number of well-controlled studies examining the effects of bee pollen is limited, there is evidence to suggest that feeding bee pollen has beneficial effects. Therefore, our objective was to determine the efficacy of a bee pollen-based supplement to horses in training. As bee pollen products appear to have wide ranging effects, from changes in immunal response to changes in exercise response, a pilot study was conducted to narrow the focus for future studies. It was hypothesised that supplementation with a bee pollen-based product would positively influence physical fitness, immunological status, and nutritional variables such as digestibility and nutrient retention.

## Materials and methods

### Horses

Ten mature unfit Arabian horses (6 geldings, 4 mares) were used in the 42-day study. The horses were used in the collegiate horsemanship classes. Prior to the commencement of the study, the horses were adapted to a free-flow exerciser for 3 days in which the exercise tests would take place.

### Standard exercise tests and sample collection

On day 0, horses performed a standard exercise test (SET) on a free-flow exerciser consisting of 5 min each at 3, 4, and 5 m/s followed by 7.5 min at 6 m/s or until the horse could no longer maintain the pace. Between each step, horses came to a complete halt for 90 s to allow for blood collection. This SET was used to determine pre-trial fitness of the horses. A second SET identical to the day 0 SET was conducted on day 42.

Blood samples were taken at rest, at the end of each speed completion, and 2, 5, 10, 15, 20, 30, 45, and 60 min post-recovery. To prepare the horse for blood sampling during the SET, a 14 gauge × 14 cm indwelling catheter was placed in the jugular vein and attached to a 51-cm extension. The catheter was flushed with physiological heparinised saline both before and between samples to prevent blood clotting in the catheter. Blood was collected into a 30-ml syringe and then separated into labelled vacutainer tubes (Becton Dickinson and Company, Franklin Lakes, NJ, USA) containing EDTA [for HB, haematocrit (HT) and immunological variables] or potassium oxide and sodium fluoride (for glucose and lactate). The tubes were immediately stored on ice until further processing. Within 4 h of collection, the chilled blood samples for lactate, glucose and immunological analysis were centrifuged at 4 °C for 15 min at 2 500 × *g*, then the plasma was transferred to microcentrifuge tubes and frozen at −80 °C until further analysis. Vacutainers for HT and HB analysis were not centrifuged. Instead, they were kept refrigerated as whole blood for a period of time not longer than 48 h until analysis.

Heart rate was monitored using an equine HR monitor (EQB, Unionville, PA, USA) with an electrode placed under the girth on the left side of the horse. Continuous HR could be measured and recorded when blood samples were taken.

### Treatments

The horses were pair-matched by gender and pretrial fitness level (maximum HR and ability to complete SET) and placed into one of the two groups. The treatment group (BP) received 118 g of Dynamic Trio$^{TM}$ 50/50 (Winners Bee Pollen Company, Phoenix, AZ, USA) consisting of 55% bee pollen. The control group (CO) received 73 g of placebo, which contained 50% red wheat bran, 25% evaporated cane juice crystal sugar, 17% baking flour, and 8% powdered apple peels. This mixture of ingredients was selected to mimic the colour and consistency of Dynamic Trio 50/50 so that the researchers could be blinded to treatment. The treatment and placebo were fed in equal volume according to the manufacturer's instructions, thus accounting for the differences in weight. The horses were group-housed on pasture at all times and were also allowed *ad libitum* access to hay in hay feeders in the pasture. They were individually fed their respective treatments in 0.2 kg of sweet feed once daily.

### Training protocol

During the 42-day study, horses continued to participate in horsemanship classes and were ridden several times a week. To account for variations in exercise intensity and frequency, as well as to test the effects of supplementation in horses in training, the horses were also placed on a conditioning program. Twice a week the horses exercised on the free-flow exerciser. The horses began the first week by exercising for 3 m/s for 20 min and 4 m/s for 10 min. Gradually the exercise program increased in intensity so that during the final week horses exercised for 20 min at 3 m/s, 20 min at 4 m/s, 5 m/s for 10 min, and 6 m/s for 5 min. Additionally, horses exercised on a treadmill once per week, with the same exercise program each week consisting of 2 min at 1.8 m/s, 6 min at 5 m/s, 4 min at 8 m/s, 6 min at 5 m/s, 4 min at 8 m/s, 6 min at 5 m/s, and 1 min at 1.8 m/s.

### Blood sample analysis and HR

Hematocrit was analysed from whole blood within 24 h of collection. Whole blood HB was analysed using a commercial kit (Stanbio Hemoglobin, Stanbio Laboratory, Boerne, TX, USA). A commercially available kit was also used to analyse plasma glucose (Glucose C2, Wako Chemicals, Richmond, VA, USA). Plasma lactate concentrations were determined using a YSI 1500 Sport (John Morris Scientific, Australia). Duplicate samples were run for all assays.

Total leukocyte counts were obtained using an electronic cell counter (Z1 Coulter Particle Counter, Beckman-Coulter, Palo Alto, CA, USA)and leukocyte differentials on a flow cytometer (FACSCalibur, Becton Dickinson) using characteristic forward and side scattering properties of lymphocytes, monocytes, neutrophil, and eosinophils to determine the percentages of each in the total leukocyte population present. Samples were run in duplicate. By multiplying the percent of a given leukocyte type by the total leukocyte count, the blood concentrations of each leukocyte type were determined.

Plasma immunoglobulins A, G, and M were assayed in duplicate by single radial immunodiffusion kits (VMRD, Pullman, WA, USA).

From the HR data collected during the SET, HR vs. speed was plotted. A best-fit line was created and from that line, approximate $V_{160}$ and $V_{200}$ values were generated. $V_{160}$ and $V_{200}$ represent the projected velocities at HR of 160 and 200 bpm.

### Nutritional variables

From day 18 to 21, the six geldings were housed in stalls for a 3-day total collection period. Uncontaminated faecal and urine samples are difficult to simultaneously collect in mares due to anatomical reasons; hence they were excluded from this portion of the study. The horses were given their respective treatments in a mixture of 0.2 kg sweet feed and oats fed at 0.25% BW on an as-fed basis. Hay was provided to the horses at 2.25% BW on an as-fed basis. The hay and grain intakes were recorded during the 3 days. The geldings were fitted with total collection devices or nappies (Equisan, Melbourne, Vic., Australia), which allowed complete separation of urine and faeces. Every 8 h, the nappies were emptied and faecal weight and urine volume were recorded. Ten percent of the faeces and urine was then saved and placed in a freezer until further analysis. Total faeces and urine were collected for the 3-day period.

The faeces were pooled into one collective sample per horse, and analysed for nitrogen retention, neutral detergent fibre (NDF) and acid detergent fibre (ADF) digestibility as well as Ca, P, and Fe. After thawing, urine was acidified with 1 ml HCl per 50 ml urine, pooled into one sample per horse, and analysed for N, Ca, P, and Fe. NDF and ADF were analysed using an approved protocol (Goering and Van Soest, 1970). Nitrogen was determined by a Leco-FP-2000 (LECO, St. Joseph, MO, USA) using AOAC method 990.03. Faeces, hay and supplement (Dynamic Trio 50/50 or placebo) was digested through a microwave digest procedure and analysed for mineral content. Urine was not digested, but simply diluted with double deionised water to determine mineral content. Calcium and iron were measured using a Unicam 989 atomic absorption spectrophotometer (Thermoelemental, Waltham, MA, USA). Inorganic phosphate was determined using a spectrophotometer (Beckman DU 7400, Beckman-Coulter, Palo Alto, CA, USA). All samples were run in duplicate.

Nutrient retention was calculated as the difference between nutrient intake and nutrient output (in urine and faeces). Nutrient digestibility was calculated as the difference between nutrient intake and output divided by intake.

### Statistical analysis

Differences between treatments, day of sample, time of sample, and various interactions were analysed using Proc Mixed of SAS. When starting values at day 0 differed between treatments, starting values were used as a covariate to account for such differences. Differences between treatments were considered significant at $p < 0.05$. As this was a pilot study using minimal animal numbers, trends were investigated at $p < 0.1$.

## Results

### Physical fitness

$V_{160}$ increased from day 0 to day 42 for both BP (7.4 ± 1.0 to 9.2 ± 0.9 m/s; $p < 0.05$) and CO (6.7 ± 0.9 to 9.4 ± 0.9 m/s; $p < 0.05$). $V_{200}$ also increased from day 0 to day 42 for both BP (9.9 ± 1.0 to 12.5 ± 0.9 m/s; $p < 0.05$) and CO (9.3 ± 0.9 to 12.8 ± 0.9 m/s; $p < 0.05$). There was a test difference with HR, HT, HB, and lactate concentrations being lower on day 42 than on day 0 ($p < 0.0003$; Table 1). Likewise, there were no treatment differences for HR, HT, HB, or lactate concentrations ($p > 0.10$; Table 1) as well as $V_{160}$ and $V_{200}$. No differences in glucose concentrations were seen between treatments in their response from day 0 to day 42 ($p = 0.52$).

### Immune status

On day 42, there was a trend for lymphocyte numbers to be lower in BP than CO ($p = 0.08$). There was an overall change from rest to recovery in total leukocytes, lymphocyte counts, percent lymphocytes, and neutrophil counts on both days 0 and 42 ($p < 0.05$), percent neutrophils on day 0 only ($p < 0.05$) and monocyte counts on day 42 only ($p < 0.05$). There was an overall change from day 0 to day 42 in total leukocyte counts, lymphocyte counts, percent lymphocytes, and percent neutrophils ($p < 0.05$). There were no treatment differences in immunological variables (Table 2), except for the concentration of lymphocytes.

**Table 1** Physical fitness variables at rest and recovery (1 hr post-exercise).

| | Day 0 | | Day 42 | |
|---|---|---|---|---|
| Variable | Rest | Recovery | Rest | Recovery |
| Heart rate (bpm)* | | | | |
| BP | 40.2 ± 2.1 | 59.0 ± 2.7 | 45.4 ± 1.3 | 39.2 ± 2.0 |
| CO | 41.6 ± 1.6 | 54.8 ± 1.8 | 43.0 ± 2.2 | 43.6 ± 1.9 |
| Haematocrit* | | | | |
| BP | 0.35 ± 0.01 | 0.38 ± 0.006 | 0.36 ± 0.006 | 0.30 ± 0.003 |
| CO | 0.37 ± 0.004 | 0.36 ± 0.01 | 0.38 ± 0.004 | 0.33 ± 0.008 |
| Haemoglobin (g/dl)* | | | | |
| BP | 12.8 ± 0.3 | 13.5 ± 0.1 | 13.5 ± 0.3 | 12.3 ± 0.3 |
| CO | 14.3 ± 0.2 | 13.8 ± 0.3 | 15.1 ± 0.2 | 11.1 ± 0.1 |
| Lactate (mmol/l)* | | | | |
| BP | 1.3 ± 0.2 | 3.3 ± 0.4 | 0.89 ± 0.08 | 0.67 ± 0.04 |
| CO | 1.2 ± 0.1 | 3.5 ± 0.3 | 0.59 ± 0.02 | 0.62 ± 0.02 |

*Overall decrease from day 0 to day 42 (p ≤ 0.0003).

**Table 2** Immunological variables in rest and recovery samples on day 0 and day 42

| | Day 0 | | Day 42 | |
|---|---|---|---|---|
| Variable | Rest | Recovery | Rest | Recovery |
| Total leukocytes (×10[6] cells/ml) | | | | |
| BP | 5.5 ± 0.5 | 5.8 ± 0.5* | 6.1 ± 0.4 | 4.7 ± 0.4*,† |
| CO | 6.2 ± 0.5 | 7.1 ± 0.5* | 6.2 ± 0.5 | 5.2 ± 0.4*,† |
| Lymphocytes (% in sample) | | | | |
| BP | 30.8 ± 2.8 | 23.3 ± 2.8* | 22.7 ± 2.0 | 16.8 ± 1.9*,† |
| CO | 31.6 ± 2.8 | 20.9 ± 2.8* | 22.3 ± 2.0 | 19.3 ± 2.0*,† |
| Lymphocytes (×10[6] cells/ml) | | | | |
| BP | 169 ± 16 | 136 ± 16* | 123 ± 16 | 77 ± 16*,†,‡ |
| CO | 195 ± 16 | 146 ± 16* | 145 ± 16 | 118 ± 16*,† |
| Eosinophils (% in sample) | | | | |
| BP | 2.6 ± 0.8 | 3.2 ± 0.8 | 1.2 ± 0.8 | 1.6 ± 0.8 |
| CO | 0.9 ± 0.8 | 1.2 ± 0.8 | 0.8 ± 0.8 | 1.2 ± 0.8 |
| Eosinophils (×10[6] cells/ml) | | | | |
| BP | 15.5 ± 4.6 | 18.9 ± 4.6 | 5.9 ± 4.6 | 5.7 ± 4.6 |
| CO | 5.1 ± 4.6 | 8.6 ± 4.6 | 5.1 ± 4.6 | 6.9 ± 4.6 |
| Monocytes (% in sample) | | | | |
| BP | 6.1 ± 0.5 | 5.2 ± 0.5 | 5.5 ± 0.5 | 4.8 ± 0.5 |
| CO | 6.0 ± 0.5 | 5.4 ± 0.5 | 5.5 ± 0.5 | 6.1 ± 0.5 |
| Monocytes (×10[6] cells/ml) | | | | |
| BP | 34.4 ± 3.8 | 30.9 ± 3.8 | 30.9 ± 3.8 | 21.1 ± 3.8* |
| CO | 37.4 ± 3.8 | 37.8 ± 3.8 | 37.3 ± 3.8 | 35.1 ± 3.8* |
| Neutrophils (% in sample) | | | | |
| BP | 56.9 ± 2.5 | 63.4 ± 2.5* | 66.5 ± 2.5 | 71.1 ± 2.5† |
| CO | 57.4 ± 2.5 | 69.4 ± 2.5* | 66.3 ± 2.5 | 66.4 ± 2.5† |
| Neutrophils (×10[6] cells/ml) | | | | |
| BP | 314 ± 44 | 369 ± 44* | 379 ± 44 | 317 ± 44* |
| CO | 357 ± 44 | 499 ± 44* | 463 ± 44 | 395 ± 44* |
| Immunoglobulin A (ml/dl) | | | | |
| BP | 203 ± 34 | 218 ± 38 | 230 ± 34 | 195 ± 38 |
| CO | 170 ± 34 | 194 ± 44 | 183 ± 34 | 195 ± 44 |
| Immunoglobulin G (ml/dl) | | | | |
| BP | 999 ± 114 | 1 147 ± 135 | 1 246 ± 114 | 1 248 ± 135 |
| CO | 983 ± 114 | 749 ± 164 | 1 152 ± 114 | 1 164 ± 164 |
| Immunoglobulin M (ml/dl) | | | | |
| BP | 47.6 ± 8.2 | 48.9 ± 8.7 | 59.7 ± 8.2 | 61.5 ± 8.7 |
| CO | 46.5 ± 8.2 | 47.6 ± 9.4 | 55.9 ± 8.2 | 56.4 ± 9.4 |

*Overall change from rest to recovery (p ≤ 0.05).
†Overall change from day 0 to day 42 (p ≤ 0.05).
‡ Trend for BP to be lower than CO on day 42 (p = 0.08).

**Nutrient retention and digestibility**

There was no difference ($p = 0.73$) in the geldings' body weights at the time of total collections between BP (497 ± 22 kg) and CO (505 ± 9 kg). Hay intake was greater in BP than CO (9.4 ± 0.3 kg vs. 6.30 ± 0.5 kg; $p < 0.0001$). When adjusted for body weight of the horse, hay intake was still greater in BP than CO ($p < 0.0001$). Total NDF digested tended to be higher in BP than CO [2 476 ± 154 g/day (BP) vs. 1 896 ± 154 (CO); $p = 0.06$] as did total ADF digested [1 220 ± 85 g/day (BP) vs. 958 ± 58 (CO); $p = 0.09$]. However, the percent NDF digested was lower in BP than CO (41.6 ± 0.4% (BP) vs. 45.3 ± 0.4% (CO); $p = 0.002$) and the percent ADF digested tended to be lower in BP than CO (35.4 ± 1.0% (BP) vs. 38.7 ± 1.0% (CO); $p = 0.07$). There was a trend for nitrogen retention to be greater in BP than CO (63.9 ± 9.6 g/day (BP) vs. 33.7 ± 8.7 g/day (CO); $p = 0.08$). Phosphorus retention was greater in BP than CO (−2.5 ± 0.7 g/day (BP) vs. −12.2 ± 1.4 g/day (CO); $p = 0.03$). There were no treatment differences in iron retention (−1.5 ± 0.4 g/day (BP) vs. −1.3 ± 0.4 g/day (CO); $p = 0.77$) or calcium retention (0.15 ± 2.2 g/day (BP) vs. −4.6 ± 2.0 g/day (CO); $p = 0.33$).

## Discussion

The horses were more fit on day 42 than on day 0, as evidenced by the increased $V_{160}$ and $V_{200}$, and decreased HR, HT, HB, and lactate concentrations. $V_{200}$ is considered an estimate of maximal aerobic power in the horse (Physick-Sheard, 1985). Increases in $V_{200}$ have been found in Thoroughbreds (Eaton et al., 1999) and decreases in HR, HB and HT were seen in Andalusions in response to training (Munoz et al., 1998).

There were no treatment differences detected in the fitness variables. This may be due to several factors, one of which is the horses' responses to the exercise tests. On the first day, not all the horses could complete the SET. However, after training, the day 42 SET was no longer a challenge, as evidenced by the physical fitness data. The horses may not have been stressed enough physically to elicit treatment differences.

Some immunological variables were affected by the SETs. As expected, there was an increase in total leukocyte count after exercise on day 0, which concurs with previous literature in human beings (McCarthy and Dale, 1988). Although immune responses to exercise can be quite variable, leukocytosis is one of the most consistent changes (Hines et al., 1996). There was a decrease in total leukocyte count after exercise on day 42, and leukocytes were decreased on day 42 compared to day 0. This would suggest a training effect. In a study conducted with three horses, endurance exercise reduced resting leukocyte numbers after 5 months (Fan et al., 2002). However, in studies with larger numbers of both humans and horses, resting leukocyte numbers were not shown to be affected by training (Soppi et al., 1982; Snow et al., 1983). Changes in leukocytes are most likely due in part to changes in neutrophils. Both total and percent neutrophils were increased after exercise on day 0, which is in accordance with a previous study in horses that found enhanced neutrophil numbers after moderate exercise (Raidal et al., 2000). There was also an increase in percent neutrophils from day 0 to day 42, indicating an increase due to training. As with the total leukocyte count, there was a decrease in neutrophils after exercise on day 42. This conflicts with studies that show while there is a decrease in neutrophil function, the actual number of neutrophils does not change with training (Rose and Hodgson, 1982; Snow et al., 1983). Both total and percent lymphocytes were decreased post-exercise on both days 0 and 42. This effect has also been found in endurance horses (Carlson et al., 1976). The occurrence of conflicting results between this study and other studies is a common finding when comparing studies with exercise and immune function.

Although there were some effects of exercise, few treatment differences were observed in the immunological variables. There was a trend for the Dynamic Trio 50/50 supplemented horses to have decreased lymphocyte numbers compared to the control horses. It is difficult to conclude that such modest effects on lymphocyte numbers altered the horses' overall health or well-being. To make such a claim, one would need to test the functional capacity of the cells and expose the animals to a disease challenge. Therefore, it was not possible to draw definitive conclusions about the horses' health based on this study.

In contrast, differences in nutrient utilisation proved to be interesting. All 10 horses were used in the physical fitness and immunological analyses. For the detection of differences in nutrient utilisation, only the geldings ($n = 6$) were used. The difficulty of obtaining clean urine and faecal samples from mares limited the digestion trial to the geldings. The differences in feed intake were not anticipated; therefore, using the mares for feed intake data only was not planned. With such limited numbers, strong treatment differences were not expected.

During the total collection period, the horses were given a small set amount of grain (0.2 kg) to administer the Dynamic Trio 50/50 or placebo, and this was consumed entirely by all horses on all days. However, horses had access to a larger amount of hay that they did not entirely consume. There was a dramatic difference in the amount of hay the horses consumed, with BP consuming an average of 9.4 kg/day, while CO ate an average of 6.3 kg/day. In fact, the range of hay intake on each of the three collection dates varied from 8.4 to 11.1 kg/day for BP while it ranged from 3.4 to 7.9 kg/day for CO. This increase in hay intake explains why NDF and ADF digestion was lower in BP than CO. Equids consuming less dry matter intake per day have slower rates of passage through the digestive tract and higher fibre digestibilities (Pearson and Merritt, 1991). Additionally, the increased hay intake also can help explain the increased retention of some of the other nutrients such as N and P. As more nutrients are presented to the animal, and if a demand for the nutrient is present, greater retention is likely. Hence, the stimulatory effect of Dynamic Trio 50/50 on feed intake may be viewed as advantageous in situations in which a horse is not consuming enough to meet nutrient needs not because of gut fill but because of some other reason such as apathy.

Our theory on the increase in hay intake in BP horses is that Dynamic Trio 50/50, as a bee pollen-based product, contained a higher concentration of B vitamins, specifically thiamin, than the placebo. As thiamin is needed as a cofactor in energy metabolism, the ability of heavily exercising horses to synthesize adequate amounts of the vitamin may become limited, causing the horse to become thiamin deficient. The thiamin requirement established by the 1978 NRC has been challenged as being too low for the exercising horse (Topliff et al., 1981). Although the Dynamic Trio 50/50 and the placebo were not analysed for nutrient content, calculations of bee pollen from Winners Bee Pollen based on previous analyses and individual ingredient nutrient analyses (http://www.nutritiondata.com) of the placebo were used to obtain very rough estimates of thiamin and other B vitamins. Dynamic Trio 50/50 was roughly estimated to provide more than double the amount of thiamin than the placebo. At the time of our digestion trial, midway through the study, the horses were still relatively unfit and thus, in heavy exercise. It is possible that they became thiamin deficient, and thus would have a depressed appetite, as this is a common result of thiamin deficiency (Carroll et al., 1949). Dynamic Trio 50/50 possibly contained more thiamin and other B vitamins than the placebo, and the BP horses likely were able to overcome the deficiency, thus stimulating their appetite. Supplemental thiamin has been shown to increase feed intake in chickens (Ali and Bartlet, 1982). There is wide belief in the equine industry that thiamin and other B vitamins stimulate appetite, but there is a lack of scientific evidence. Future studies should investigate the effects of thiamin supplementation on feed intake in horses. Unfortunately, as we were not expecting the differences in feed intake and the differences between treatments were not analysed until the completion of the study, body weights were only taken on the six geldings prior to the digestion trial to calculate the amount of hay to be offered. Although they were not different between treatment groups at the study midpoint, conclusions about the effects of the Dynamic Trio 50/50 supplementation and subsequent feed intake on body weights cannot be made as day 0 and day 42 weights were not available.

In conclusion, supplementation of Dynamic Trio 50/50 did not appear to affect the measured physical fitness and immunological variables. Supplementation did appear to increase feed intake and nutrient retention. Horses in training have increased nutrient and metabolic requirements. Therefore, Dynamic Trio 50/50 supplementation may help the horse meet those needs through increased feed intake, and thereby increased nutrient retention. However, as this is a pilot study, additional studies should be conducted to confirm the feed intake results.

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