TECHNICAL RESEARCH NOTE

A Technical Services report from Arm & Hammer Animal and Food Production

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Effect of WELLMAX Yeast culture on inflammation, stress, and recovery in exercised Labrador Retrievers

STUDY OVERVIEW

A study[1] was conducted to evaluate the effect of WELLMAX yeast culture supplementation on stool quality, fecal microbiota, inflammation, stress, and recovery in exercised Labrador Retrievers. Thirty-six healthy working Labrador Retrievers (18 male and 18 female) were enrolled in this 9-week study. All dogs were maintained on the standard kennel diet and randomly assigned to 1 of 3 treatments: A) 15 g of ground corn germ (Control, n = 12), or B) 7.5 g of WellMax™ postbiotic (n = 12), or C) 15 g of WellMax™ postbiotic (n = 12). Treatments were top dressed and fed for 9 weeks. Dogs had a 7d acclimation period, followed by an exercise regimen of twice weekly 3 mile runs for 7 weeks, and then a transportation stress test in the final week of study. Fecal samples were collected to analyze microbiota dysbiosis, gut inflammation and immune function. On day 0 before acclimation phase, blood serum was collected for baseline for blood chemistry, hematology, and inflammatory cytokines. Exercise related Total Gait Inflammation Index was analyzed before and after the runs. Saliva cortisol levels were measured before and after a trailer transportation stress test. Basic digestibility of diets was assessed at the end of the study using the indicator method with titanium dioxide. Data was analyzed statistically with significance set at $P \leq 0.05$ and tendencies were set at $0.05 < P \leq 0.08$.

RESULTS

No treatment effects were noted for digestibility (Table 1) and stool quality. Administration of WELLMAX did not induce negative changes in blood hematology or chemistries. Dogs on treatment A had a heightened immune response, indicated by tendencies for higher white blood cells ($P = 0.08$), and lymphocyte counts ($P = 0.08$) over the duration of the study and higher Eosinophils at d 63 ($P<0.01$), which was mitigated in dogs on WELLMAX treatments (Table 2). No other treatment effects were noted on hematology parameters and on any blood chemistry parameters.

Table 1. Apparent digestibility coefficients by treatment, on a dry-matter basis.

	A	B	C	*P* – Value
Dry Matter, %	83.00 ± 2.03	87.31 ± 1.85	87.04 ± 1.85	0.24
Protein, %	58.64 ± 5.15	65.86 ± 4.71	67.13 ± 4.71	0.44
Fat, %	89.79 ± 1.36	90.80 ± 1.24	91.81 ± 1.24	0.55
Carbohydrate, %	59.17 ± 4.82	68.90 ± 4.40	72.25 ± 4.40	0.14

Table 2. Hematology results by time point and treatment

	Treatment						*P*-Value		
Parameter	**Baseline (D0)**	**SEM**	**A (D63)**	**B (D63)**	**C (D 63)**	**SEM**	**Day**	**Sex**	**Trt*Day**
WBC, 10^9/L	8.54	0.3	9.52	8.19	8.73	0.42	0.36	0.62	0.08
Lymphocytes, 10^9/L	1.42	0.08	1.59	1.26	1.38	0.1	0.91	0.43	0.08
Eosinophils, 10^9/L	0.07[a]	0.01	0.13[b]	0.07[a]	0.08[a]	0.01	0.05	0.88	0.01

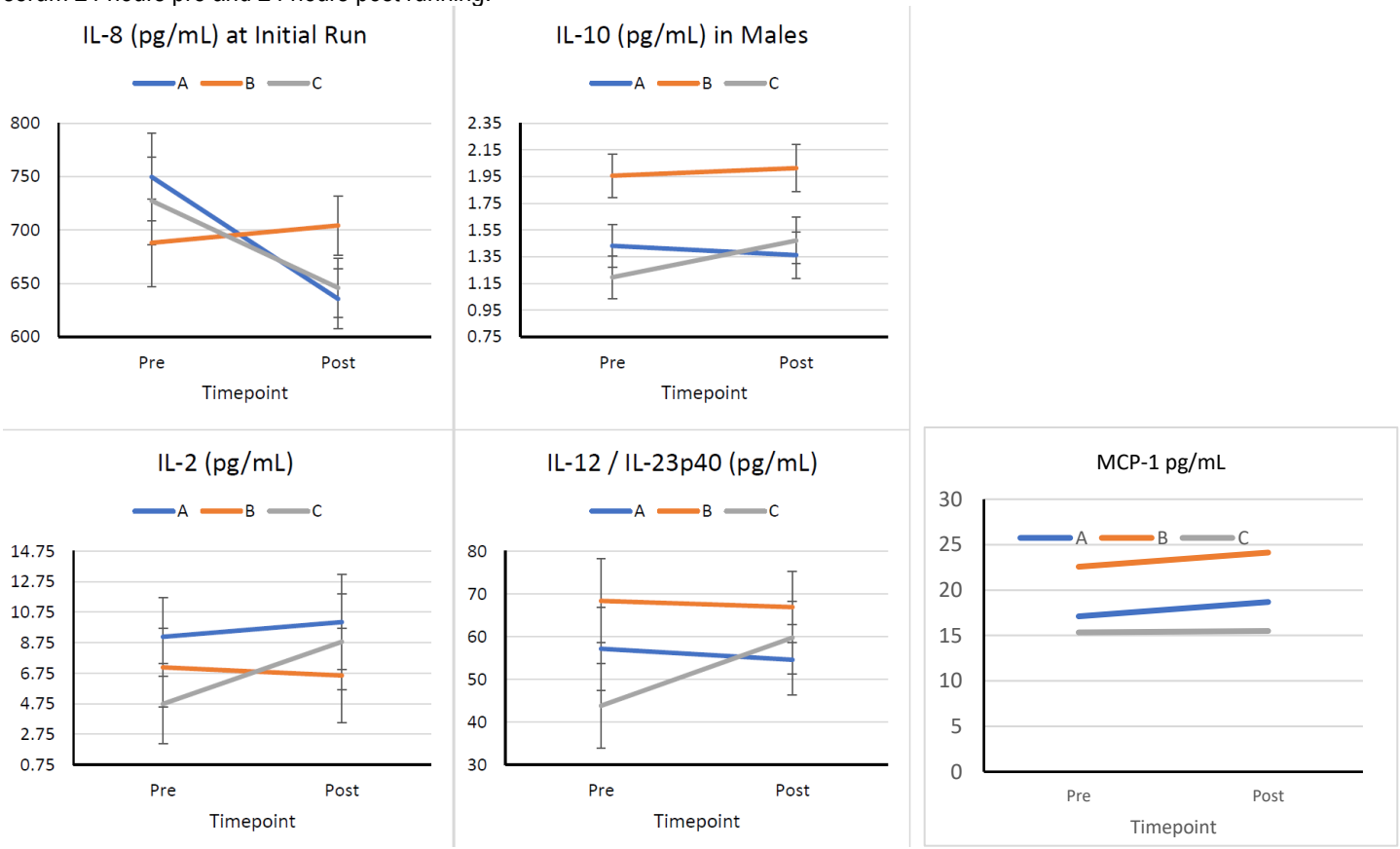
Exercise Recovery:

There is evidence that exercise can both cause and attenuate inflammation. It was hypothesized that the postbiotic WELLMAX might reduce inflammation. In this study, from pre to post run, we observed a decrease in circulating NGFβ, decrease in IL-8 (anti-inflammatory) in first run but an increase in the second run, and an increase in IL-10 (anti-inflammatory) cytokines.

Treatment effects were noted for muscle regeneration chemokine, Monocyte chemoattractant protein (MCP-1), anti-inflammatory cytokines, IL-8 (initial run) and IL-10, and proinflammatory cytokines, IL-2, and IL-12. Treatment B had overall increased IL-8, IL10 and MCP-1 and reduced IL2 and IL12 shortly after exercise, which may be related to improved or more robust muscle recovery (Figure 1).

IL-10 levels in males, on treatment B, were significantly elevated compared to both A and C (P = 02). Except for IL-10, cytokines did not exhibit any sex*treatment interactions. Females did not show a significant treatment effect but tended to have decreased circulating IL-10 in treatment B compared to treatment A, with C intermediate (P = 0.06). Based on human studies, females have faster recovery and are more fatigue resistant[1], so IL-10 levels may have dropped more quickly or may not have changed compared to those observed in males.

Figure 1. Select cytokines by treatment and timepoint. A) IL-8 in males at the initial run of the regimen, B) IL-10 in males for both runs, C) IL-2 for all dogs at both runs, D) IL-12 for all dogs at both runs, E) MCP-1 for all dogs at both runs. Cytokines were measured in serum 24 hours pre and 24 hours post running.



Treatment B had overall increased anti-inflammatory (IL10) and reduced pro-inflammatory cytokines (IL2 and IL12) shortly after exercise, which may be related to improved or more robust muscle recovery. An increase in circulating Monocyte chemoattractant protein (MCP-1) (P < 0.01) level was noted in treatment B compared to C, and tending to differ compared to treatment A, while A and C did not differ. The primary role of MCP-1 is to regulate the migration and infiltration of macrophages and monocytes, but it is also important in myoblast proliferation and muscle regeneration[2]. As there were no increases in macrophages or monocytes in groups B or C, it's likely the increased MCP-1 is related to increased myoblast proliferation and muscle regeneration.

No treatment effects were noted for Gait inflammation index score between runs (Figure 3). Dogs on treatment C had significantly faster average moving speed (P = 0.04) than treatment A, with treatment B intermediate (Figure 4). Saliva cortisol levels increased following transport stress but not treatment effects were noted.

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Figure 3. FRK Total Gait Inflammation Index Score

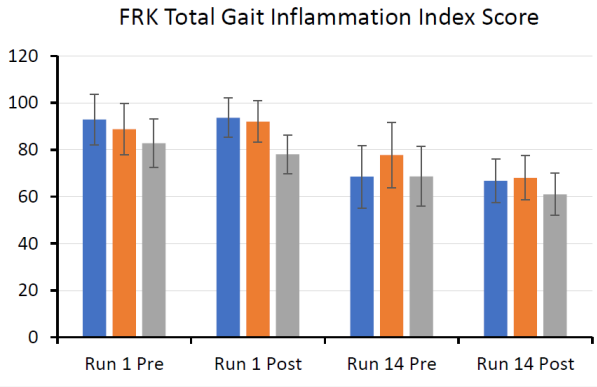
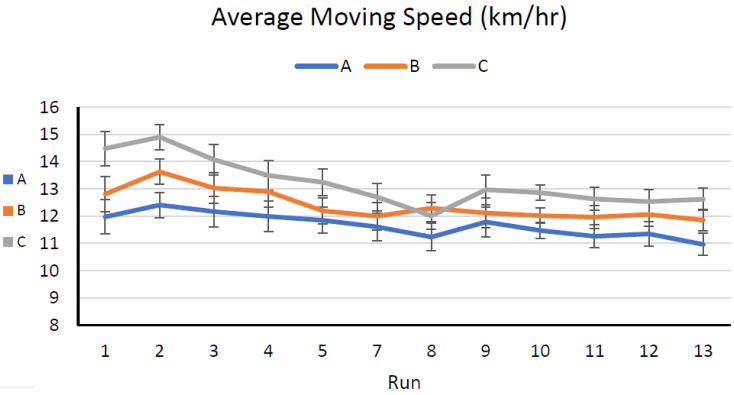


Figure 4. Average Moving Speed (Km/hr.)



Fecal Biomarkers and Gut Microbiota Dysbiosis:

No meaningful changes in fecal biomarkers were noted for S100A12, Alpha-1 proteinase inhibitor, fecal IgA, and calprotectin, indicating healthy gut environment in all dogs on the study.

Abundance of seven genera reported to indicate chronic inflammatory enteropathies were measured and Gut Microbiota dysbiosis index was calculated using a scale of; < 0, normal index with no shifts in the overall diversity; 0-2, mildly increased dysbiosis; > 2 significantly increased dysbiosis. Dogs on all treatments had microbiota dysbiosis index score below 0 indicating animals did not experience significant dysbiosis[3] (Figure 2). Of the seven genera tested, treatment C lowered *Blautia* abundance. Treatment B was able to best maintain or even increase populations of *Clostridium hiran*onis and *Fusobacterium* compared to treatments C and A and sustain a more favorable microbiota diversity[3] (Table 3).

Figure 2. Microbiota Dysbiosis Index Score

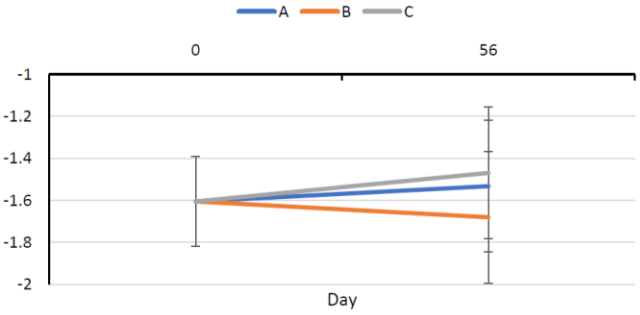


Table 3. Log abundance of identified bacterial genera by timepoint in the microbiota dysbiosis index analysis.

	Baseline	SEM	A	B	C	SEM	Day	Sex	Trt*Day
Faecalibacterium	6.45	0.10	6.53	6.38	6.30	0.14	0.60	0.05	0.44
Turicibacter	8.20	0.08	8.15	8.30	8.24	0.08	0.62	0.37	0.33
Streptococcous	7.36	0.15	7.50	7.39	7.27	0.21	0.87	0.19	0.72
E. coli	5.05	0.17	4.74	4.92	4.88	0.27	0.27	0.21	0.88
Blautia	10.29^{a}	0.02	10.22^{ab}	10.22^{ab}	10.03^{b}	0.06	< 0.01	0.04	0.04
Fusobacterium	9.18^{xy}	0.07	9.30^{x}	9.78^{x}	8.97^{y}	0.11	0.94	0.19	0.07
Clostridium hiranonis	6.87^{x}	0.03	6.66^{y}	6.84^{x}	6.74^{xy}	0.05	< 0.01	0.03	0.07

Conclusion: WELLMAX, at inclusion level of 7.5 g per day per dog, reduced inflammation and helped muscle recovery shortly after exercise and positively changed diversity of the gut microbiome.

REFERENCE:

1. Glenmark, B. *et al.* Difference in skeletal muscle function in males vs. females: Role of estrogen receptor-β. *Am J Physiol Endocrinol Metab* **287**, (2004).
2. Peake, J. M., Neubauer, X. O., Della Gatta, P. A. & Nosaka, X. K. REVIEW Recovery from Exercise Muscle damage and inflammation during recovery from exercise. *J Appl Physiol* **122**, 559–570 (2017).
3. Canine and Feline Microbiota Dysbiosis Index - Gastrointestinal Laboratory. https://vetmed.tamu.edu/gilab/service/assays/canine-microbiota-dysbiosis-index/.