# *The effect of palaMOUNTAINS MyBeau® on the performance and immune function of dogs*

***Report for:***

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Te Kunenga
ki Pūrehuroa

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## *Introduction*

Working dogs are a vital component of the farming sector in New Zealand, however many animals are subjected to inadequate nutrition that may compromise their short term performance and exercise recovery, and long term usefulness to the farnmer (Jerram, 2005). It is important to note that the health of these dogs is repeated and frequently challenged because of the intensity of work they perform, their higher risk of injuries and accidents and their exposure to the elements and environment. If these factors are compounded by inadequate nutrition, there is a higher risk of skeletal injuries, poor performance and recovery and increased susceptibility to disease. Unfortunately, many working dogs in New Zealand are fed diets that are deficient in essential nutrients, or imbalanced in macronutrient content or poorly digestible so that the nutrients are unavailable and unable to be absorbed.

The majority of farmers feed their dogs meat sourced on the farm itself (homekill). This meat must be boiled or frozen to meet the national legal requirements for *Taenia ovis* control, a process which destroys most of the vitamins within it. Similar vitamin losses have been observed during processing in commercial dog rolls (Somogyi & Muller, 1989). Another option for farmers are dry diets, however there is a frustration in the farming sector that even the apparent "quality" dry diets are insufficient energy-wise as a complete diet to sustain the high work load of the animals.

High energy neutraceutical supplement products appear to have the potential to assist in supplying extra energy to dogs and contain supplemental vitamins to replenish those lacking in the dog's normal diet and cover increased requirements due to heavy workload. PalaMOUNTAINS MyBeau® is a liquid multivitamin supplement containing both fat and water soluble vitamins and minerals suspended in an enriched Omega-3, -6 & -9 fatty acid base.

This study was undertaken to investigate the effect of dietary supplementation of palaMOUNTAINS MyBeau® on the performance of working dogs. Very little information is currently available on the performance effects of dietary supplements in dogs. Previous studies in other countries with Sled dogs (Kronfeld, 1973; Reynolds et al., 1994) and working dogs (Downey et al., 1980) have shown elevated post-exercise free fatty acid and triglyceride levels in dogs fed high fat diets, which appear to convey a performance advantage.

## *Materials and Methods*

### *Animals and Housing*

This study was performed in accordance with the directions and regulations of the Massey University Animal Ethics Committee (Anonymous 2005) who approved its implementation.

Eighteen adult harrier hounds (3-8 years of age) were used in the study. The dogs were housed in pairs in custom built dog runs, consisting of a weatherproof indoor area and an open air outside area at the Canine Unit, IFNHH, Massey University. This facility met both the requirements of the 'Code of Recommendations and Minimum Standards for the Welfare of Dogs (1998)', and the 'Code of Recommendations and Minimum Standards for the Care of Animals in Boarding Establishments (1993)'. The facility was also inspected by an RSPCA officer and the dog control officer for the Palmerston North City Council, and met city council requirements. On arrival at the facility, all dogs were examined by a veterinarian and weighed. All animals were healthy, weighed from 18 to 34 kg (average 25.8 kg) and had body condition scores ranging from 4 - 6. During the trial, each dog was exercised daily. This consisted of either a 20-minute walk or treadmill run. The dog runs were cleaned daily.

### *Acclimatisation and Treadmill Training*

After an initial five-day acclimatisation period following arrival at the facility, the dogs were introduced to a purpose-built equine treadmill (Institute of Veterinary, Animal and Biomedical Sciences, Massey University) and trained in pairs to run on it over a 35 day period. Six dogs failed to adapt to the treadmill during the first 3 weeks of training (Wendy, Bridget, Abbo, Webster, Windsor and Trusty) and were returned to the Hunt kennels. Two replacement animals (Beacon and Bandit) were obtained and adapted quickly to the treadmill. Their shorter acclimatisation period did not appear to affect their performance during the trial.

At the end of the 35 day training period all dogs could run at 3.5 m/s for 1h (a total distance of 12.6 km) with a break of 5 minutes after 30 minutes. The dogs then entered the main study which was conducted over an 8 week period.

### *Diets and Feeding*

The dogs were paired according to performance (as judged by the hunt master), sex and age and randomly assigned to either Test (palaMOUNTAINS MyBeau®) or Control treatment group. Baseline food requirements were calculated using the equation ME = 132 x Weight $(kg)^{0.75}$ (Burger, 1993). In addition, weekly body weights (BW) and body condition scores (BCS) were used to calculate daily meal allowance of ChampMax biscuits (Heinz Wattie's Ltd, Hastings, NZ) so that a constant body weight range was maintained. The body condition scoring was carried out on the weigh days using the Purina Body Condition System described by Laflamme (1993).

Animals in the test group received palaMOUNTAINS MyBeau® (VitaPower, Wanganui, NZ) and animals in the control group received the same amount of calories as gelatinised corn starch (National Starch & Chemical Ltd, Auckland, NZ) which was combined with a ground portion of their diet daily. The supplements were added to the diet according to BW (1.35 g palaMOUNTAINS MyBeau® per kg BW and 3.25 g of starch per kg BW), and animals in both groups received the same amounts of energy per kg BW, with energy source of the supplement being different: carbohydrate for the control group and fat for the treatment group. The meal allowance for each dog was pre-weighed into individual feed bowls, and the supplement was added prior to feeding. Fresh water was available *ad libitum* throughout the trial.

On days 0, 14, 28 and 56 the dogs were transported to the treadmill and participated in an exercise test where the animals ran in pairs at 3.5m/s for 1h on a flat treadmill. The treadmill was stopped for 5 minutes after 30 minutes of exercise. A rectal temperature and heart rate was taken from each dog immediately before, and after 30 minutes and 1 hour’s exercise on the treadmill. A blood sample (7 ml) was taken from each dog’s jugular vein by a veterinarian before and immediately after each run. One drop of blood was used immediately to determine lactate levels while the rest of the sample was collected into a series of vacutainer tubes for analysis.

On the sampling days, the dogs had access to water in their runs before they were transported to the treadmill and immediately after exercise and final sampling. In weeks 1, 3, 5, 6 and 7 in between the subexercise tests, the dogs were exercised on the treadmill twice a week for a total of 45 minutes to maintain fitness. The exercise regime consisted of one 30 minute session on the treadmill at 3.5 m/s and one 15 minute session at the same speed with the treadmill inclined for 5 minutes at 3º, 6º and 9º.

### *Chemical Analyses*

The basal diet sample, a sample of test supplement, a sample of the control supplement were analysed for dry matter (DM), crude protein (CP), and gross energy. Dry matter and residual dry matter were determined by oven drying for 16 hr at 105ºC (AOAC 1995). Crude protein was determined by multiplying nitrogen by 6.25, with nitrogen determined using the Dumas method on a LECO analyser (Dumas 1831).

Following blood sampling, one drop of blood was analysed for blood lactate immediately with an Accutrend Lactate and Accusport meter (Sports Resource Group Inc, New York, USA). The remainder of the 7 ml blood sample was divided between a grey-top vacutainer tube (0.5 ml; potassium oxalate) and sent to Gribbles Laboratory in Palmerston North for glucose analyses, a green top vacutainer tubes (3 and 2 ml; sodium heparin) for immune assays and triglyceride and vitamin E analysis in the Nutrition Laboratory at Massey University, and a red-top vacutainer tube (1.5 ml; no addative), which was centrifuged at 2,500 rpm for 10 minutes before the plasma was separated off and stored prior to analysis for free fatty acids (FFA).

### *Immune Assays*

Several assays to measure immune function were carried out covering a range of natural and acquired immune responses (Table 1).

*Table 1. Immune function assays*

| Response | Tissue sites/cells | Assay (Method) |
|---|---|---|
| "Specific" cellular responses | Peripheral Blood Lymphocytes | Proliferation in response to mitogens T cells -Con A*, PHA# ($^3$H-thymidine incorporation) |
| | Blood/lymphocytes | Immunophenotyping (Flow cytometry) |
| "Natural" cellular responses | Blood phagocytes | Phagocytosis (Flow cytometry) |
| Specific antibody responses | Serum | IgG (ELISA test) |
| | Saliva | IgA (ELISA test) |

* Concanavalin A (Con A) # Phytohaemogglutinin (PHA)

The methods used for these assays have been optimised and standardised by our group for use in dogs and are based on published methods. All of the samples were processed individually.

### *Statistical Analyses*

Analysis of variance (ANOVA) was used to determine significant differences in blood parameters, heart rate and body temperature between the experimental (palaMOUNTAINS MyBeau®-fed) and control groups before and after exercise and in a range of immune assays before exercise only (SAS, 1999). Values of $P<0.05$ were considered significant. Unless otherwise stated, all results are presented as mean ± SEM.

## *Results*

### *Diets, Bodyweight, Body Condition Score and Intakes:*

The nutrient profiles of the 2 dietary treatments are presented in Table 2.

*Table 2. Nutrient Profiles of the Control and Treatment Diets.*

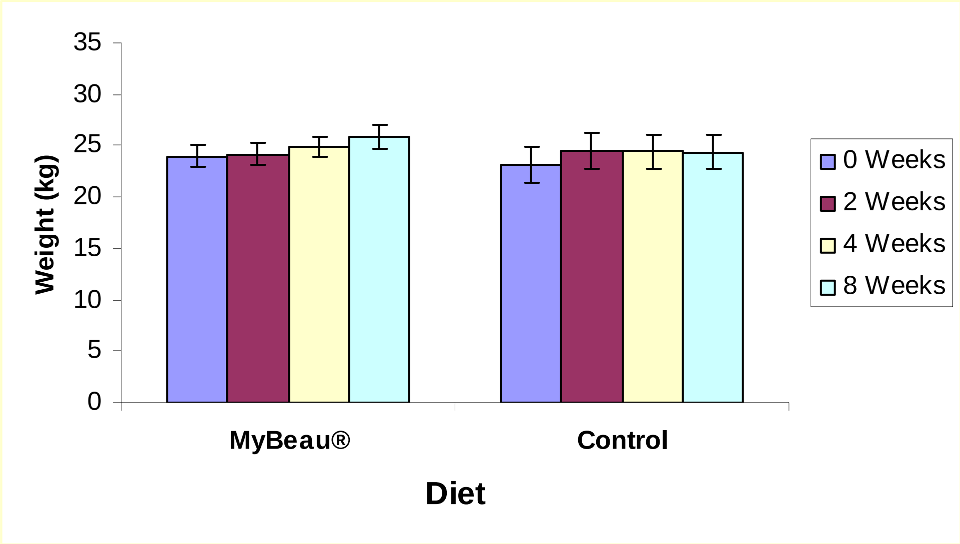
| Dietary treatment | Dry matter | Crude protein | Gross energy |
|---|---|---|---|
| | % | | kcal/g |
| Control Diet (Basal Diet + Starch) | 91.90 | 19.66 | 20.17 |
| Treatment Diet (Basal Diet + MyBeau®) | 91.55 | 21.13 | 21.03 |

During the trial, the dogs were fed between 70% and 130% of their energy needs for maintenance, dependent on body weight and body condition score (BCS). Intake was adjusted at weekly intervals to maintain a consistent body condition score in animals in both groups. There were no differences in BCS between the groups during the trial.

*Table 3. Average Bodyweight (BW), Body Condition Score (BCS) and Daily feed intake per dog during the trial.*

| Group | Name | BW (kg) | BCS | Total intake (g) | Intake per kg BW (g) | Energy Intake (kcal/kg BW) |
|---|---|---|---|---|---|---|
| Control | Astle | 26.3 | 5 | 465.9 | 17.7 | 357.1 |
| | Beacon | 23.2 | 5 | 479.9 | 20.7 | 417.4 |
| | Bella | 23.8 | 4 | 489.1 | 20.6 | 415.3 |
| | Rufus | 31.8 | 6 | 439.2 | 13.8 | 278.5 |
| | Tessa | 20.8 | 5 | 426.3 | 20.5 | 413.1 |
| | Venus | 24.1 | 5 | 399.0 | 16.5 | 333.6 |
| | Wench | 18.4 | 6 | 307.7 | 16.8 | 337.8 |
| | | 24.09 | 5 | 429.58 | 17.86 | 360.22 |
| Treatment | Archer | 24.4 | 5 | 388.5 | 15.9 | 335.2 |
| | Bandit | 24.4 | 5 | 452.8 | 18.6 | 390.7 |
| | Maestro | 27.5 | 5 | 496.8 | 18.1 | 379.9 |
| | Una | 22.1 | 5 | 408.5 | 18.5 | 388.3 |
| | Vagabond | 28.4 | 5 | 508.9 | 17.9 | 377.2 |
| | Wilma | 24.7 | 5 | 457.2 | 18.5 | 389.5 |
| | Wispa | 20.7 | 5 | 399.2 | 19.3 | 405.8 |
| | | 24.72 | 5 | 444.56 | 18.08 | 380.21 |

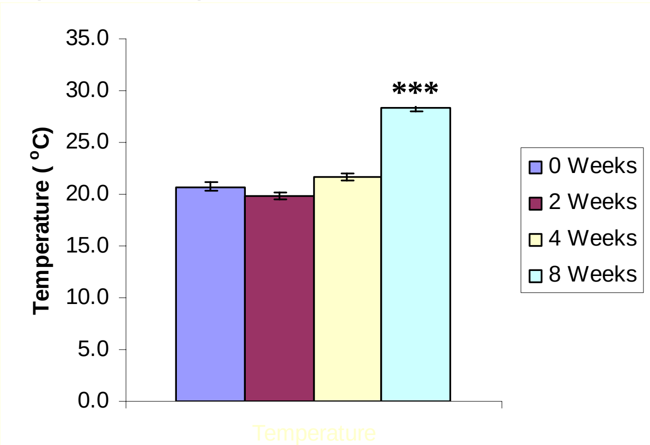
*Fig 1: Average weekly body weights (kg) of the dogs during the trial.*




The average weekly body weights of the dogs on the two treatments during the trial are shown in Figure 1. There were no differences in body weight within each group or between the two groups during the trial.

During the 8 week trial the total amount of exercise each dog received was 165.01 ± 5.62 km (20.63 km/week). This was the same for both groups of animals (Treatment (palaMOUNTAINS MyBeau®-fed) group 169.88 ± 5.52km; Control 160.15 ± 5.50km).

*Fig 2: Ambient temperature in the treadmill room during each exercise test (***P<0.001).*




Each animal was subjected to a 12.4km exercise test at 0, 2, 4 and 8 weeks. One dog (Venus) developed an infection between her toes and could not run on the treadmill on days 14 and 28. Another dog (Vagabond) developed an enlarged testicle and was unable to run on the treadmill on day 28 and for only 30 minutes on day 56. Only data from the first exercise test (Venus) and the first and second tests (Vagabond) only were included in the analysis for these two dogs. Data from both dogs were excluded from the immune analysis. The testing procedure was identical for each test, however the ambient temperature in the treadmill building was significantly higher (P<0.001) during the week 8 test (see Figure 2).

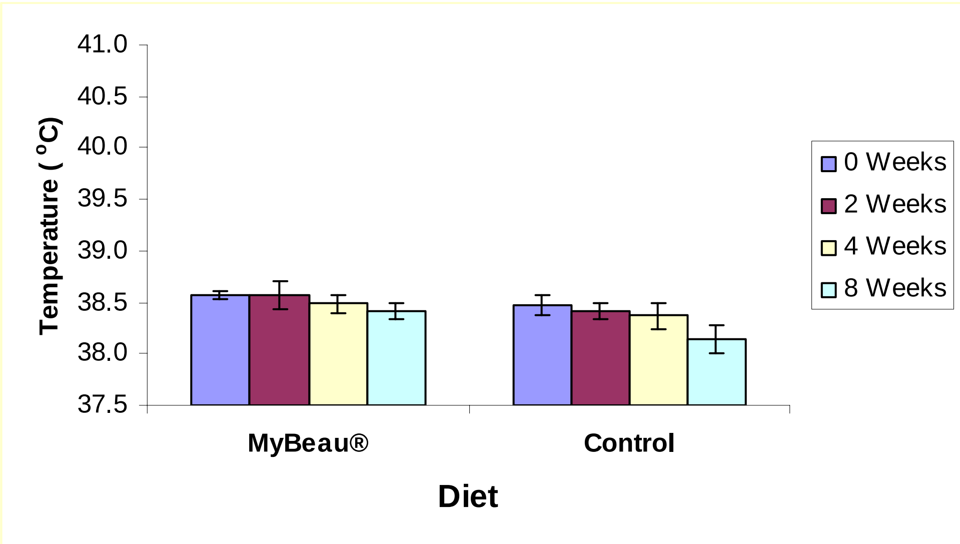
*Environmental Conditions, Body Temperature and Heart rate:*

The rectal temperatures and heart rates of the dogs before and after each of the four exercise exercise tests during the trial are shown in Figures 3 and 4. The mean values and standard errors are summarised in Appendix 1, Tables 1 - 4. The normal resting range of a dog is considered to be between 37.8ºC and 39.2ºC (Carlson & Griffin, 1992). There were no differences in rectal temperature and heart rates before each of the exercise tests in the two groups. After each exercise test, rectal temperature increased significantly ($P<0.001$) in both groups compared to the pre-exercise level. After the 8 week test, the rectal temperature of animals in the treatment (palaMOUNTAINS MyBeau®-fed) group was significantly higher ($P<0.001$) than in the 3 previous tests. The control animals showed a similar increase in rectal temperature after the 8 week test, although when compared to temperature data from the other tests the difference was not significant ($P>0.05$).

Compared to pre-exercise levels, heart rates were significantly elevated in control group animals following each exercise test ($P<0.05$), and in the treatment (palaMOUNTAINS MyBeau®-fed) group following the initial week 0 test only ($P<0.05$). However, when the post-exercise heart rates from animals in each group were analysed no differences were found ($P>0.05$).

*Fig 3. Average rectal temperatures (ºC) of the dogs in the treatment (palaMOUNTAINS MyBeau®-fed) and control groups (a) before and (b) after exercise test (**P<0.01).*

(a)



(b)

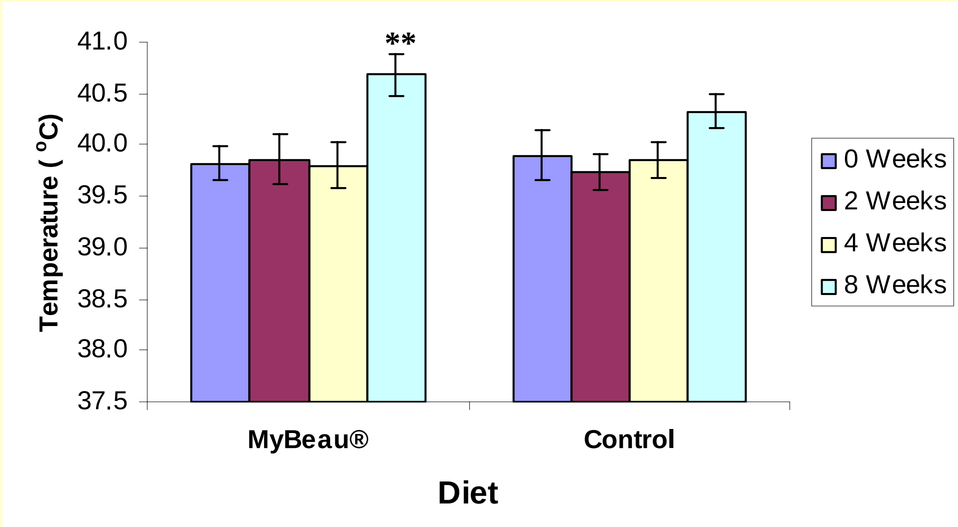
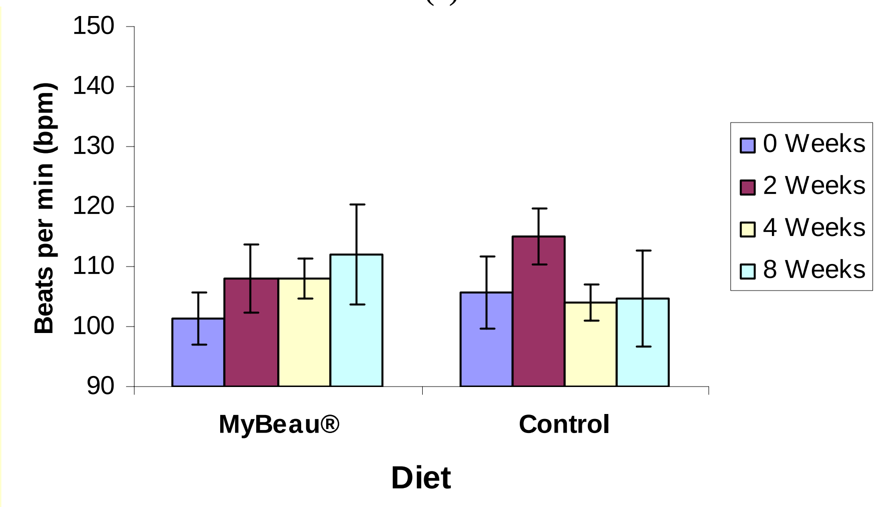
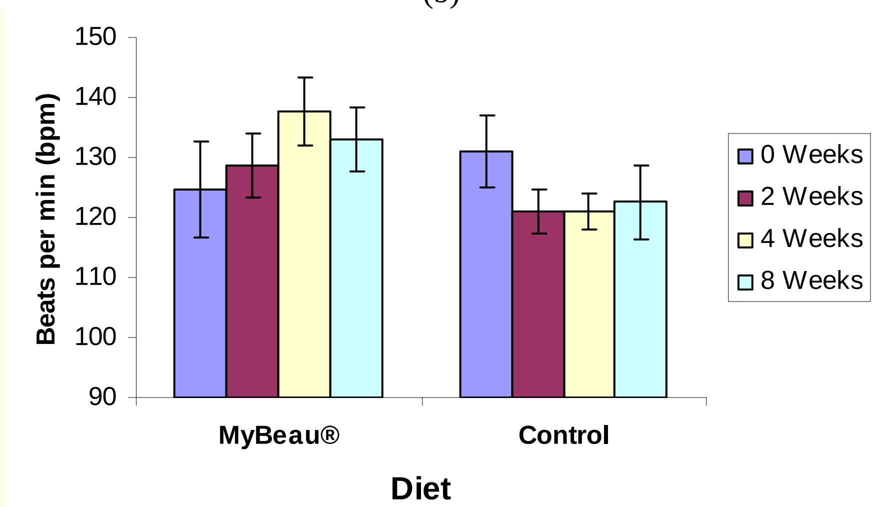



*Fig 4. Heartrate (bpm) of the dogs in the treatment (palaMOUNTAINS MyBeau®-fed) and control groups (a) before and (b) after exercise test.*

(a)




(b)



*Blood Parameters:*

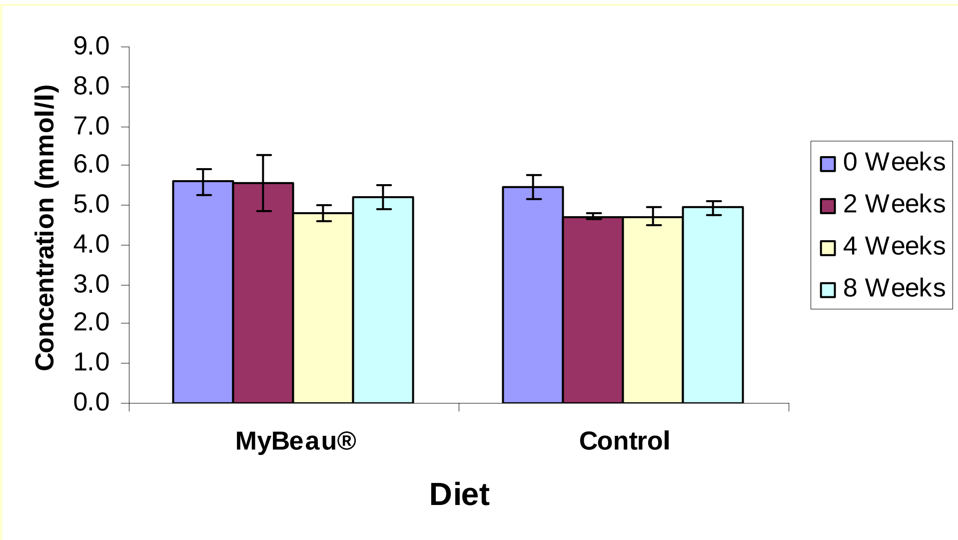
The effects of dietary palaMOUNTAINS MyBeau®on a number of blood parameters before and after exercise are shown in Figures 5-9. The mean values and standard errors are summarised in Appendix 1, Tables 5 - 14.

Blood parameters were selected to investigate the relative contribution of carbohydrate and fat metabolism as an energy source in dogs. Glucose, which is stored in muscles as glycogen, is an immediate energy source in a range of species and normally increases in response to exercise. Blood lactate is a product of glucose metabolism and also increases after exercise.

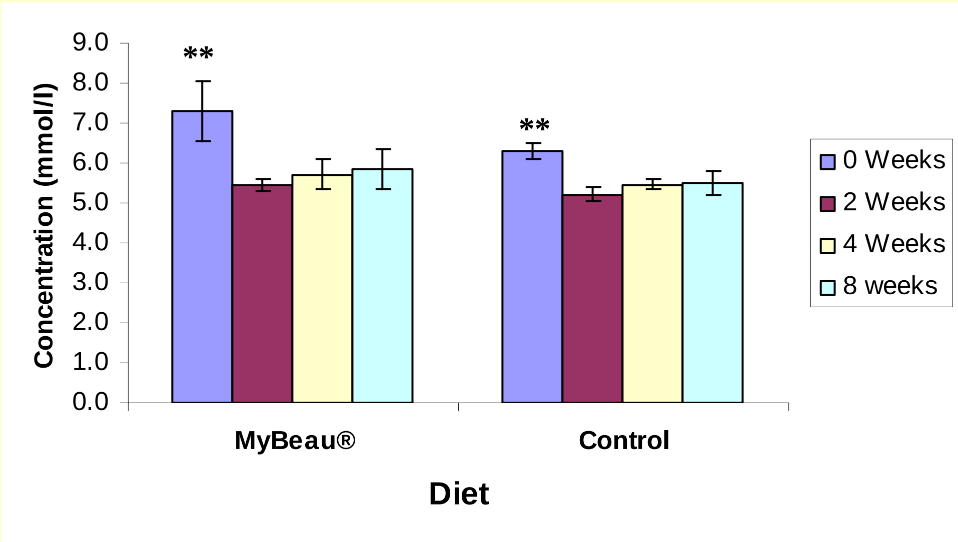
Endurance training on the other hand has been shown to improve the use of fats and fatty acids as an energy source by enhancing free fatty acid (FFA) oxidation. This increased reliance on FFA oxidation in the trained state slows muscle glycogen utilisation and promotes endurance and delays exhaustion keeping reserves for later in the race. Serum triglyceride (Terjung et al., 1982; Reynolds et al., 1994) and FFA (Paul & Issekutz, 1967; Reynolds et al., 1994) are suspected to play important glycogen-sparing roles by replenishing intramuscular fat which is an important fuel for muscles during endurance work. Vitamin E is a fat soluble vitamin that works with other antioxidants in the body to prevent oxidative damage of cell membranes. If vitamin E is present in appropriate levels, it helps maintain cellular integrity and defence mechanisms that are important against disease and environmental insults. Vitamin E deficiency can result in damage to red blood cells, immune cells, muscle, nerve cells and other cells in the body which are more pronounced if other antioxidant nutrients are also deficient or if the oxidative insult is large or sustained.

*Fig 5: Glucose levels of the dogs in the treatment (palaMOUNTAINS MyBeau®-fed) and control groups (a) before and (b) after exercise test (**P<0.01within groups after exercise).*

(a)



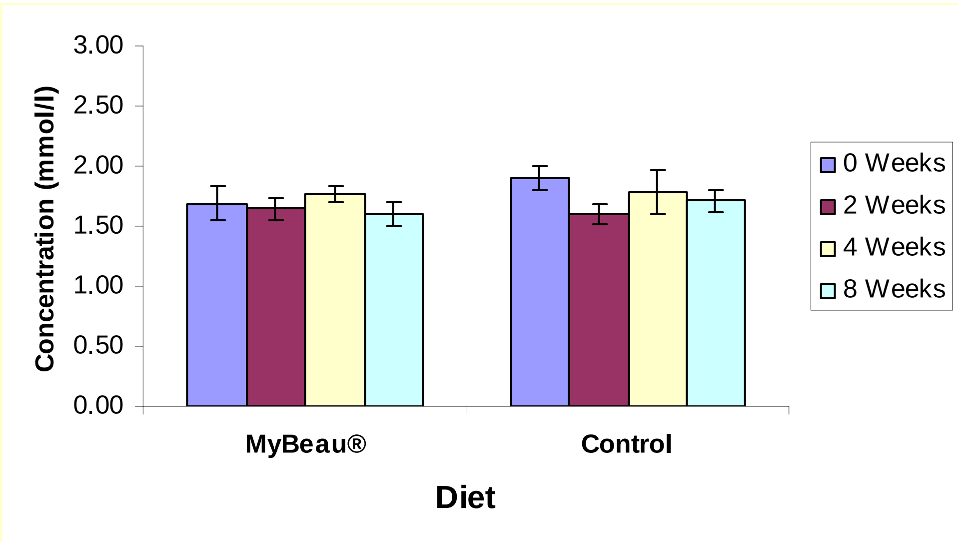
(b)



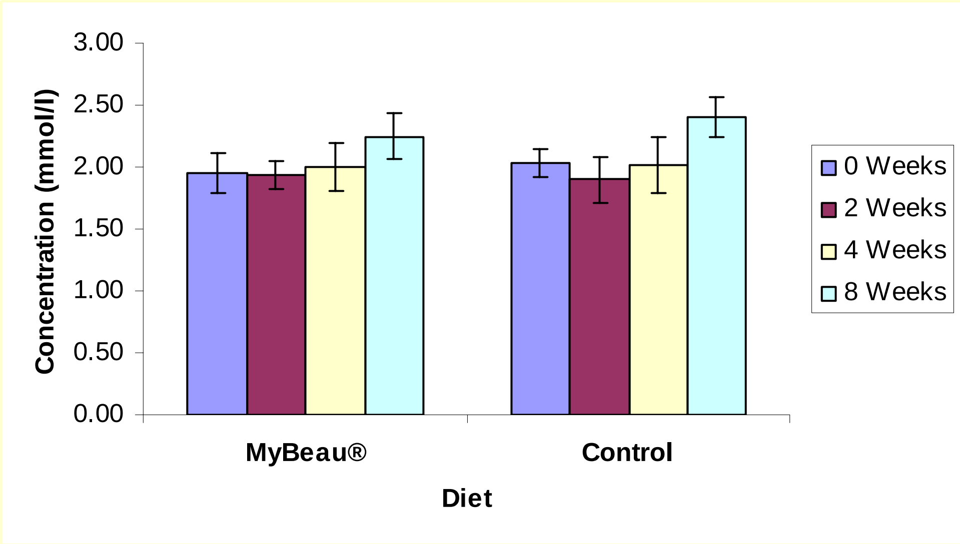

Blood glucose levels of the dogs before and after each exercise test are shown in Figure 5. As expected, no differences in glucose levels were detected before each of the exercise tests in the two groups. However, after each exercise test glucose levels increased significantly in both treatment (palaMOUNTAINS MyBeau®-fed; P<0.05) and control group animals (P<0.01). When post-exercise glucose levels from both groups of animals were analysed, levels following the week 0 test were significantly higher then those after the week 2, 4 and 8 tests (P>0.01). This may indicate a stress response associated with the animal's first experience of an exercise test.

*Fig 6: Blood lactate levels of the dogs in the treatment (palaMOUNTAINS MyBeau®-fed) and control groups (a) before and (b) after exercise test.*

(a)



(b)

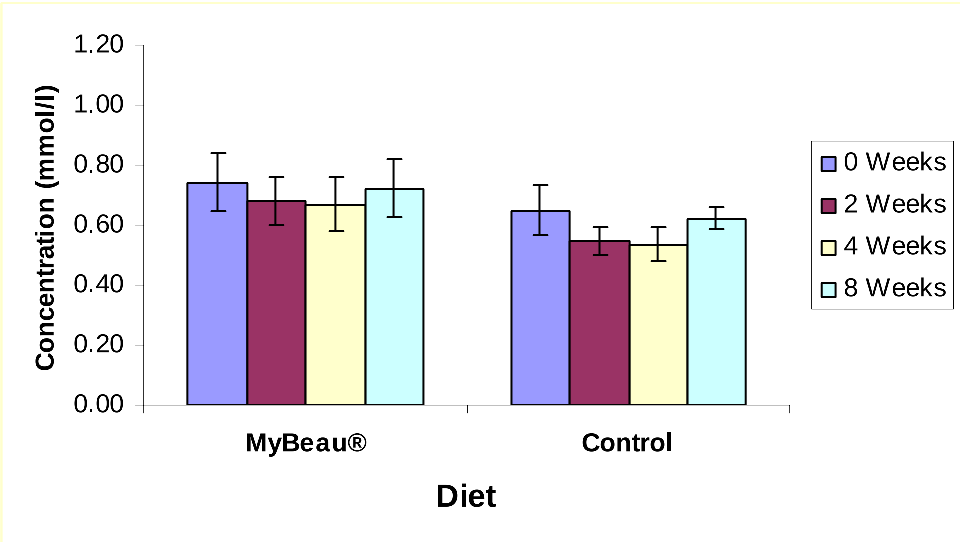



Blood lactate levels in the dogs before and after each of the exercise tests are shown in Figure 6. Again, there were no differences in lactate levels before each of the tests both within and between the two groups. Post-exercise levels were generally higher in both groups but only achieved significance after the week 8 test ($P<0.01$) when compared to pre-exercise levels. Post-exercise lactate levels in both groups of animals following the week 8 test were numerically higher than those after the week 0, 2 and 4 tests but this difference was not statistically different ($P>0.05$).

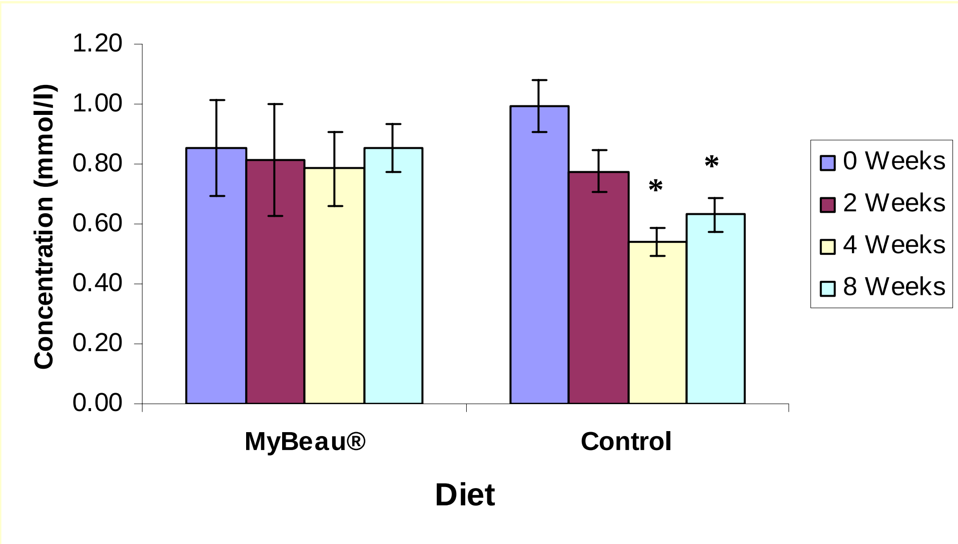
Triglyceride levels of the dogs before and after each of the exercise tests are shown in Figure 7.

*Fig 7: Triglyceride levels of the dogs in the treatment (palaMOUNTAINS MyBeau®-fed) and control groups (a) before and (b) after exercise test (*P<0.05 within the control group after exercise).*

(a)



(b)

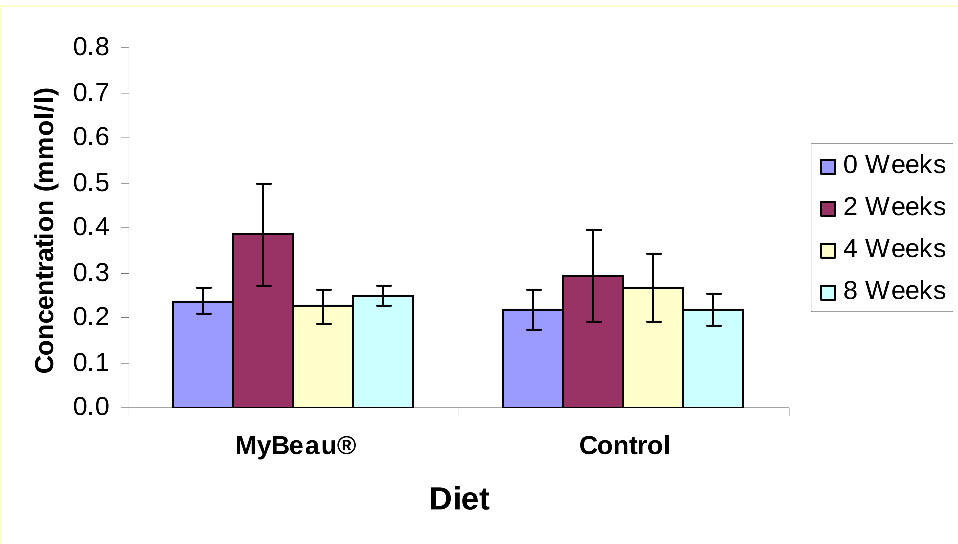



As previously observed in the other blood parameters, there were no differences in pre-exercise triglyceride levels both within and between the two groups. Post-exercise levels were generally higher in both groups when compared to pre-exercise levels. Post-exercise triglyceride levels remained constant in the treatment (palaMOUNTAINS MyBeau®-fed) group after each exercise test. In contrast, post-exercise triglyceride levels declined in the control group after the week 4 and 8 tests, and were significantly lower (P<0.05) than levels after the initial week 0 test.

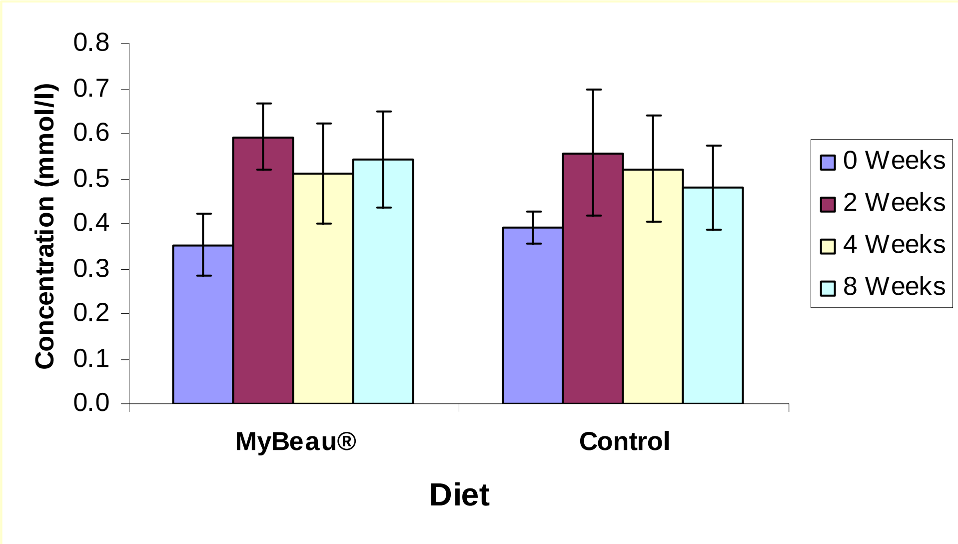
Free fatty acid (FFA) levels of the dogs before and after each of the exercise tests are shown in Figure 8.

*Fig 8: Free fatty acid (FFA) levels of the dogs in the treatment (palaMOUNTAINS MyBeau®-fed) and control groups (a) before and (b) after exercise test.*

(a)



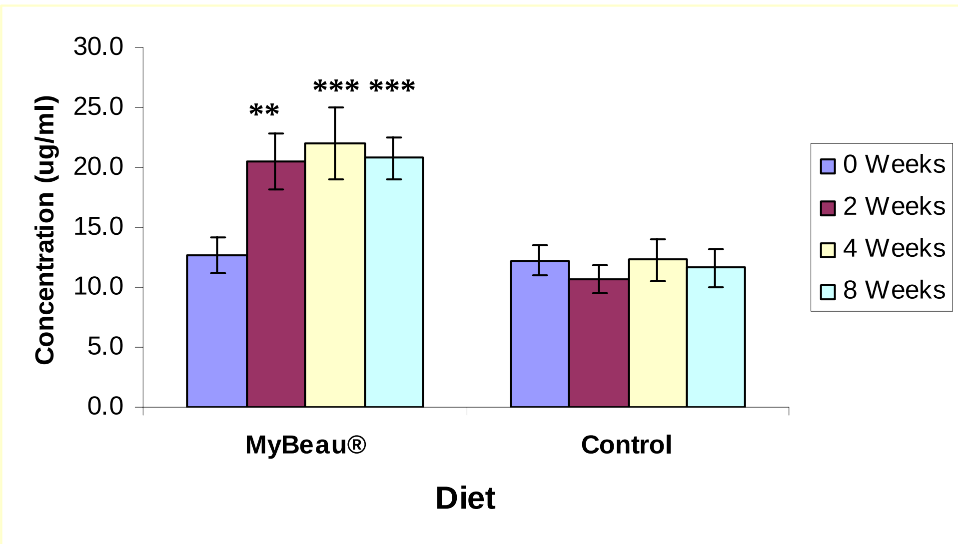

(b)



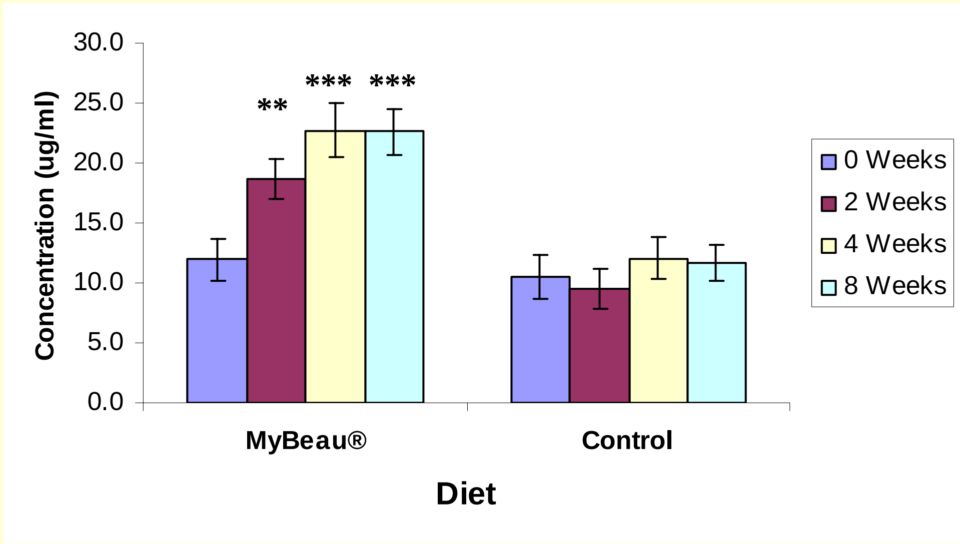

As previously observed in the other blood parameters, there were no differences in pre-exercise levels of FFA both within and between the two groups. Post-exercise levels were significantly higher (P<0.05) in both groups when compared to pre-exercise levels after the week 4 and 8 tests. Post-exercise FFA levels peaked in the treatment (palaMOUNTAINS MyBeau®-fed) group after the week 2 test, and remained constant after each of the remaining exercise tests. A similar post-exercise peak in FFA level after the week 2 test was observed in the control group, but levels appeared to decline after each of the subsequent exercise tests. This decline was not as marked as previously observed with triglyceride levels and the data is more variable.

*Fig 9: Vitamin E levels of the dogs in the treatment (palaMOUNTAINS MyBeau®-fed) and control groups (a) before and (b) after exercise test (**P<0.01; ***P<0.001 within the treatment group before and after exercise).*

(a)



(b)




Vitamin E levels of the dogs before and after each of the exercise tests are shown in Figure 8. In contrast to the other blood parameters, the data clearly shows a time dependent increase in vitamin E level in the treatment (palaMOUNTAINS MyBeau®-fed) animals independent of exercise. The increase in concentration was statistically significant after 2 weeks of feeding (P=0.009), and highly significant after 4 weeks of feeding (P=0.0002). In contrast, vitamin E levels in control animals remained unchanged during the trial period.

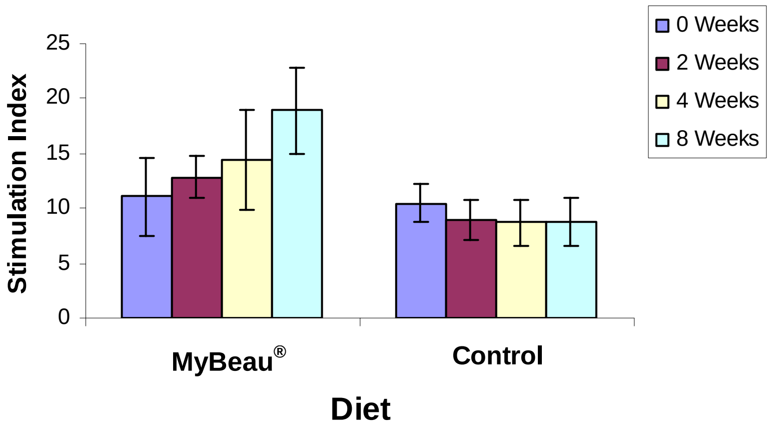
### *Specific Immune responses*

### *Lymphocyte Proliferation*

Lymphocyte proliferation measures the ability of the cells to respond to mitogenic stimulation hence giving a measure of the readiness of the cells to respond to and fight infection or disease. Two mitogens were used: Concanavalin A (Con A) and Phytohaemagglutinin (PHA), which selectively stimulate the activation and proliferation of specific lymphocyte populations.

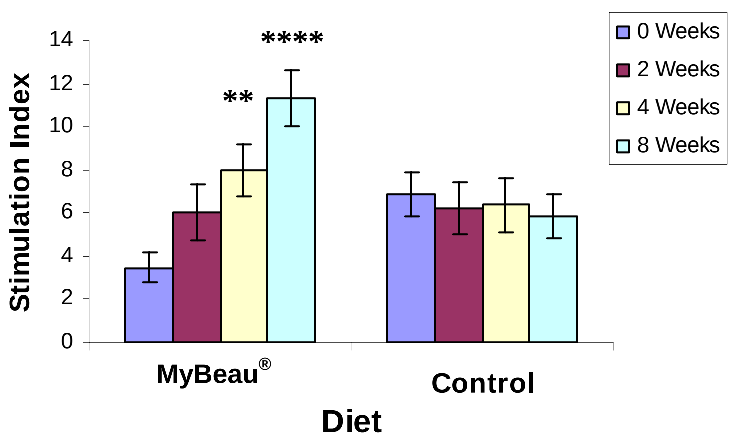
The effects of the dietary palaMOUNTAINS MyBeau® on lymphocyte proliferative responses to Concanavalin A (Con A) in the dogs prior to exercise are shown in Figure 10. The mean values and standard errors are summarised in Appendix 2, Table 1. The treatment (palaMOUNTAINS MyBeau®-fed) group showed a trend of increasing proliferative responses to Con A over time (P=0.066 at 8 weeks), while the control group showed no significant changes over the time period tested. At 8 weeks the treatment (palaMOUNTAINS MyBeau®-fed) group showed significantly higher proliferative responses to Con A than the control group (P=0.02).

*Fig. 10: Effect of dietary palaMOUNTAINS MyBeau® on lymphocyte proliferative responses to Concanavalin A in dogs prior to exercise*




The effects of dietary palaMOUNTAINS MyBeau® on lymphocyte proliferative responses to Phytohaemagglutinin (PHA) in dogs prior to exercise are shown in Figure 11. The mean values and standard errors are summarised in Appendix 2, Table 2. The treatment (palaMOUNTAINS MyBeau®-fed) group showed highly significant increases (P= 0.007 and P=0.0001) in lymphocyte proliferative response to PHA after 4 and 8 weeks of feeding. The value after 2 weeks of feeding was numerically higher than at time 0 although the difference was not statistically significant (P=0.12). The kinetics of the results obtained however, does suggest that the enhancement observed is a time dependent effect, and that a feeding period of approximately 4 weeks is required to observe a significant increase. In contrast, proliferative responses of the control group did not change significantly over the period tested. Note that the control group at time 0 had significantly higher baseline proliferative responses compared to the test group (P=0.047), which represents the animal to animal variation which is frequently observed in any outbred animal population.

*Fig. 11: Effect of dietary palaMOUNTAINS MyBeau® on lymphocyte proliferative responses to Phytohaemagglutinin (PHA) (**P<0.01, ****P<0.0001) in dogs prior to exercise*



### Immunophenotyping

It is possible to use the presence of various cell surface markers (cluster differentiation (CD) antigens) to identify the relative amounts of different types of cells present in blood and tissue samples. These values can be used to determine amongst other things immunodeficiency. In the blood, the lymphocyte population is made up of T-helper cells ($CD4^+$), cytotoxic T-cells ($CD8^+$) and B-cells. Large changes in either T- or B-cell numbers can lead to a predisposition to certain types of infection or disease. Therefore such changes are not desirable. The list of CD markers used in this study, the cells which they specifically label and their roles in the immune response are shown in Table 4.

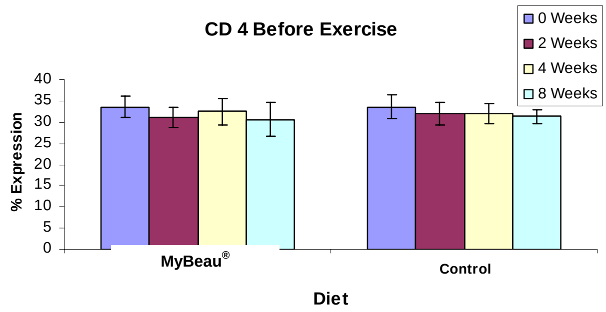
*Table 4. Specificity of CD markers and role of the labelled cell type*

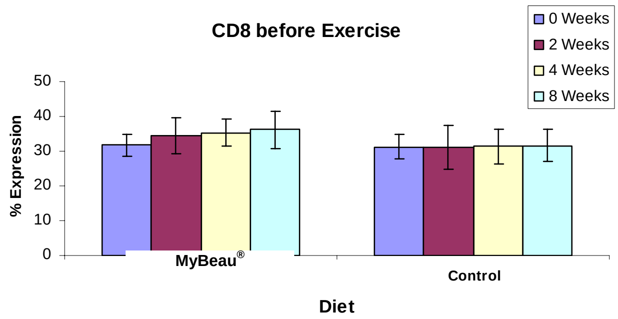
| CD Marker | Type of Cell | Role |
|---|---|---|
| CD 4 | T-helper cell | Secrete specialized factors that activate other white blood cells to help fight off infection |
| CD 8 | Cytotoxic T-cell | Directly kill certain tumour cells, viral-infected cells and sometimes parasites |
| B cell | B cell | Cells which differentiate into plasma cells which produce antibodies to foreign proteins of bacteria, viruses, and tumour cells |
| CD 14 | Monocyte | Phagocytes which circulate in the blood |
| CD 11b | Neutrophil Activation Marker | Measure of phagocytic polymorphonuclear cell function |

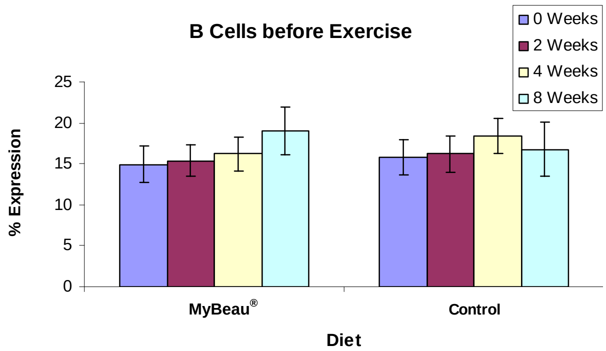
The effects of the dietary palaMOUNTAINS MyBeau® on the expression of the various cell surface markers in dogs prior to exercise are shown in Figure 12. The mean values and standard errors are summarised in Appendix 2, Tables 3-7.

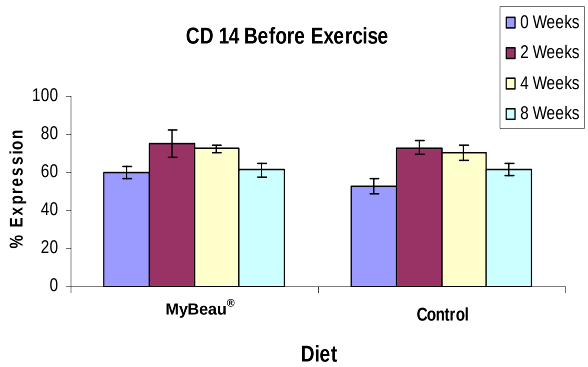
As can be seen from the figures below, dietary intake of palaMOUNTAINS MyBeau® over an 8 week period did not significantly change the level of expression of CD4, CD8, B cells or CD11b. For CD14, although the values at 2 weeks for the treatment (palaMOUNTAINS MyBeau®-fed) group were significantly higher (P=0.011) than the same animals at time 0, the control group also showed significant increase at both 2 (P=0.0009) and 4 weeks (P=0.004), and there were no significant differences between the treatment group and the control group at any of the time points. It is likely therefore that the increases observed were transient increases and not a direct effect of the dietary supplementation.

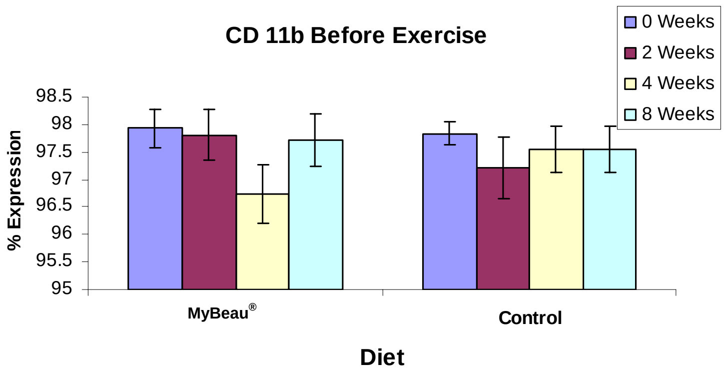
*Fig. 12: Expression of cell surface markers*



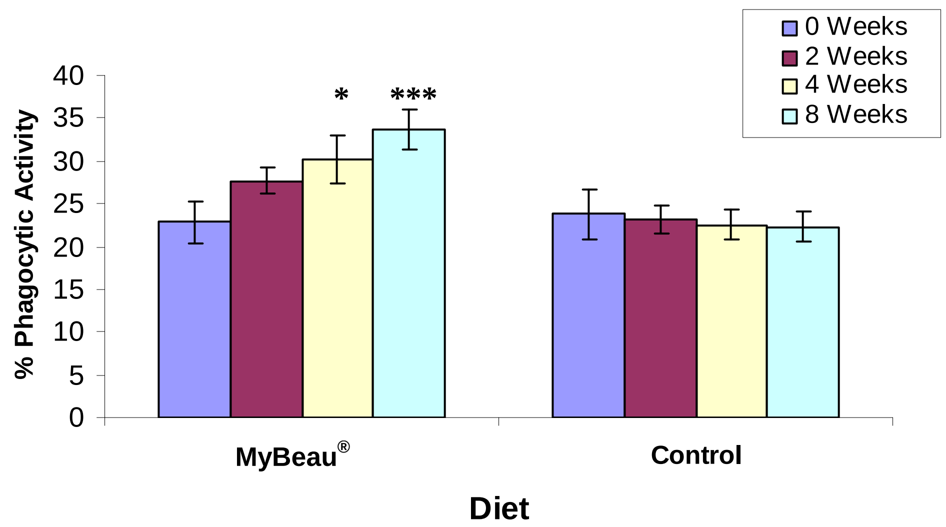
*Innate responses*

*Phagocytosis*

Phagocyte function assays measure the ability of cells to ingest and destroy foreign particles such as bacteria, and as such give a measure of the cells' ability to fight infection and disease. The assay used here specifically measures the ability of cells to ingest foreign particles.

The effect of dietary palaMOUNTAINS MyBeau® on peripheral blood phagocytosis is shown in Figure 13. The mean values and standard errors are summarised in Appendix 2, Table 8. Figure 13 clearly shows a time dependent increase in phagocytic activity in the treatment (PalaMOUNTAINS MyBeau®-fed) animals, with the enhancement being statistically significant after 4 weeks of feeding (P=0.025), and highly significant after 8 weeks of feeding (P=0.0012). In contrast again, the phagocytic activity of the control group did not change significantly during the trial period.

*Fig. 13: Effect of dietary palaMOUNTAINS MyBeau® on peripheral blood phagocytosis in dogs prior to exercise (* P=0.025, ***P=0.0012)*



## *Discussion*

This study was designed to investigate the effect of palaMOUNTAINS MyBeau®, a liquid multivitamin supplement, on both performance and immune parameters of a group of harrier hounds.

This study clearly showed that after an adequate period of adaptation, palaMOUNTAINS MyBeau® with its free fatty acid base enhanced the availability of local and peripheral lipid stores during exercise. The elevated post-exercise levels of serum triglyceride (and to a lesser extent FFA) in the dogs receiving the palaMOUNTAINS MyBeau® suggests that there could well have been a glycogen-sparing effect occurring in the dogs, and the rapid replenishment of this muscle fuel source was promoted. This rapid replenishment and resulting performance advantage has previously been demonstrated in two dog studies (Kronfeld, 1973 and Downey et al., 1980).

In this initial study, we had limited knowledge of the physical capacity for controlled exercise of the dogs. In addition, the Massey University Animal Ethics Committee restricted the level of exercise testing we could carry out. However, it is clear that in further studies, more prolonged and intense exercise using the current group of dogs is required to definitely demonstrate a performance advantage in terms of endurance.

However, the present study indicated that potentially palaMOUNTAINS MyBeau® can produce a metabolic advantage that may lead to a much higher level of performance. The body temperatures measured post-exercise were within the normal resting range quoted for dogs (Carlson & Griffin, 1992). It was only during the final test when the ambient temperature in the treadmill building was significantly higher that the dogs showed increased body temperature and lactate levels. Consequently, it is logical to conclude that the elevated ambient temperature increased the work required for heat loss and advanced the onset of exhaustion leading to the increase in body temperature.

PalaMOUNTAINS MyBeau® consumption caused a significant increase in vitamin E levels, indicating the high availability of this ingredient from the supplement and increasing antioxidant capacity in these dogs. Low vitamin E status has been suggested as a risk factor in sled dogs developing "tying-up syndrome" (exertional rhabdomyolysis) and muscle degeneration (Reinhardt et al., 2006), and has been shown to decrease markedly in racing Greyhounds under stress conditions (Kronfeld, 1989). Current recommended maintenance dietary allowances for vitamin E in dogs are 20 IU per kg of diet. This is suspected to be inadequate for working dogs and recommendations for exercising sled dogs are in excess of 400IU per day (Reinhardt et al., 2006).

Results from the immune assays showed that palaMOUNTAINS MyBeau® consumption caused a significant enhancement of lymphocyte proliferative responses to the T-cell mitogen Phytohaemagglutinin (P=0.07, P=0.0001), indicating that in these cells activity was upregulated by palaMOUNTAINS MyBeau®, and are thus ready to proliferate in response to an appropriate antigenic challenge such as a bacterial infection. Proliferative responses to Concanavalin A showed a trend for increased proliferation as well.

There were no significant changes in the level of expression of the lymphocyte markers CD4, CD8 or B cells. This is doubly advantageous, first there is no obvious undesirable effects on the cellular (T cells) or humoral (B cells) arm of the immune system. Second, there is also no selective loss of T cell function as reduced numbers of CD4 or CD8 lymphocytes can potentially increase susceptibility to disease and infection

There were also no biologically significant increases in the level of expression of the monocyte marker (CD14) or the neutrophil activation marker (CD11b).

There was a highly significant increase in the phagocytic activity of peripheral blood leukocytes from dogs fed the palaMOUNTAINS MyBeau® diet (P=0.025 at 4 weeks, P=0.0012 at 8 weeks), with the increase in activity appearing to increase over time with the consumption of the product. This increase in phagocytic activity is considered an indicator of greater ability to fight infection and disease.

The fact that the phagocytic activity of the palaMOUNTAINS MyBeau®-fed animals increased, but the level of expression of the CD14 and CD11b cells did not, indicated that the mechanism for the increase in phagocytic activity was not via an increase in either the expression of monocytic cells or via increased neutrophil activation.

*Conclusions*

- PalaMOUNTAINS MyBeau® consumption significantly enhanced post-exercise levels of serum triglyceride
- PalaMOUNTAINS MyBeau® consumption significantly enhanced levels of vitamin E
- PalaMOUNTAINS MyBeau® consumption significantly enhanced lymphocyte proliferation to PHA
- PalaMOUNTAINS MyBeau® consumption significantly enhanced peripheral blood leukocyte phagocytic activity
- PalaMOUNTAINS MyBeau®consumption had no negative effects on the level of expression of cell surface markers
- PalaMOUNTAINS MyBeau®consumption significantly improved immune parameters that may enhance resistance, the ability to fight disease and infection as well as providing an efficient energy source for working dogs

## *References*

Anonymous (2005) Code of Ethical Conduct for the use of Live Animals for Teaching and Research. Rev. Ed., Massey University, Palmerston North, New Zealand.

AOAC (1995) AOAC 923.03 Official Methods of Analysis, AOAC Official Method 923.03.

Burger, I. (1993) A basic guide to nutrient requirements. In: The Waltham book of Companion Animal Nutrition. Pergamon Press, Oxford UK. pp5-24.

Code of Recommendations and Minimum Standards for the Care of Animals in Boarding Establishments (1993). Code of Animal Welfare No. 9. Animal Welfare Advisory Committee, Wellington, New Zealand.

Code of Recommendations and Minimum Standards for the Welfare of Dogs (1998). Code of Animal Welfare No. 20. Animal Welfare Advisory Committee. PO Box 2526, Wellington, New Zealand.

Carlson, D.G. and Griffin, J.M. (1992) Dog Owner's Home Veterinary Handbook. Howell, New York, USA. 407pp.

Dumas JBA (1831) Ann. Chim. Phys. **2** 47: 198-213.

Downey, R.L., Kronfeld, D.S. & Banta, C.A. (1980) Diet of beagles affects stamina. J. Am. Vet. Med. Assoc. 1980: 273-277.

Jerram, P. (2005) Caring for New Zealand's farm dogs. Masterpet Commercial Report 5pp.

Kronfeld, D.S. (1973) Diet and performance in racing sled dogs. J. Am. Vet. Med. Assoc. 162: 470-474.

Kronfeld D.S., Adkins T.O. and Downey R.L. (1989) Nutrition, anaerobic and aerobic exercise and stress. In: Nutrition of the Dog and Cat (eds. Burger I.H. and Rivers, J.P.W.) Cambridge University Press, Cambridge, UK. p133-142.

Laflamme D.P. (1993) Body condition scoring and weight maintenance. Proc. N. Am. Vet. Conf. Jan 16-21, Orlando, FL, USA. pp 290-291.

Paul, P. and Issekutz, B. (1967) Role of extramuscular energy sources in the metabolism of the exercising dog. J. Appl. Physiol. 22, 615-622.

Reinhardt, G., Hinchcliff, K., Reynolds, A. and Hayek, M. (2006) Supplementing vitamin E to Alaskan sled dogs. IAMS commercial report 3pp.

Reynolds, A.J., Fuhrer, L., Dunlap, H.L., Finke, M.D. and Kallfelz, F.A. (1994) Lipid metabolite responses to diet and training in sled dogs. J. Nutr 124, 2754S-2759S.

SAS Institute Inc (1999) The SAS system for windows, release 6.12. SAS Institute Inc, Cary, NC, USA

Somogyi, J.C. and Muller, H.R. (1989) Nutritional Impact of Food Processing. Karger Publishing, London, UK.

Terjung, R.L., Budohoski, L., Nazar, K., Kobryn, A. and Kaciuba-Vacilko, H. (1982) Chylomicron triglyceride metabolism in resting and exercising fed dogs. J. Appl. Physiol. 52, 815-820.

*APPENDIX 1.*

Table 1 *Effect of dietary palaMOUNTAINS MyBeau® on the rectal temperature of dogs prior to exercise*

| | Temperature (ºC) ± SE | | | |
|---|---|---|---|---|
| Diet | 0 weeks | 2 weeks | 4 weeks | 8 weeks |
| MyBeau® | 38.57 ± 0.04 | 38.57 ± 0.14 | 38.48 ± 0.08 | 38.41 ± 0.09 |
| Control | 38.47 ± 0.10 | 38.42 ± 0.07 | 38.37 ± 0.13 | 38.14 ± 0.14 |

Table 2 *Effect of dietary palaMOUNTAINS MyBeau® on the rectal temperature of dogs after exercise*

| | Temperature (ºC) ± SE | | | |
|---|---|---|---|---|
| Diet | 0 weeks | 2 weeks | 4 weeks | 8 weeks |
| MyBeau® | 39.82 ± 0.17 | 39.86 ± 0.24 | 39.80 ± 0.22 | 40.68 ± 0.21 |
| Control | 39.90 ± 0.25 | 39.73 ± 0.18 | 39.85 ± 0.18 | 40.33 ± 0.16 |

Table 3 *Effect of dietary palaMOUNTAINS MyBeau® on the heart rate of dogs prior to exercise*

| | Beats per minute (bpm) ± SE | | | |
|---|---|---|---|---|
| Diet | 0 weeks | 2 weeks | 4 weeks | 8 weeks |
| MyBeau® | 101.43 ± 4.36 | 108.00 ± 5.74 | 108.00 ± 3.48 | 112.00 ± 8.32 |
| Control | 105.71 ± 6.08 | 115.00 ± 4.75 | 104.00 ± 2.97 | 104.57 ± 7.95 |

Table 4 *Effect of dietary palaMOUNTAINS MyBeau® on the heart rate of dogs after exercise*

| | Beats per minute (bpm) ± SE | | | |
|---|---|---|---|---|
| Diet | 0 weeks | 2 weeks | 4 weeks | 8 weeks |
| MyBeau® | 124.67 ± 8.11 | 128.57 ± 5.31 | 137.67 ± 5.74 | 133.00 ± 5.23 |
| Control | 131.00 ± 6.08 | 121.00 ± 3.61 | 121.00 ± 2.86 | 122.57 ± 6.26 |

Table 5 *Effect of dietary palaMOUNTAINS MyBeau® on glucose levels in dogs prior to exercise*

| Diet | Concentration (mmol/l) ± SE | | | |
|---|---|---|---|---|
| | 0 weeks | 2 weeks | 4 weeks | 8 weeks |
| MyBeau® | 5.59 ± 0.35 | 5.55 ± 0.71 | 4.81 ± 0.19 | 5.21 ± 0.32 |
| Control | 5.46 ± 0.30 | 4.72 ± 0.08 | 4.71 ± 0.23 | 4.93 ± 0.17 |

Table 6 *Effect of dietary palaMOUNTAINS MyBeau® on glucose levels in dogs after exercise*

| Diet | Concentration (mmol/l) ± SE | | | |
|---|---|---|---|---|
| | 0 weeks | 2 weeks | 4 weeks | 8 weeks |
| MyBeau® | 7.30 ± 0.75 | 5.46 ± 0.16 | 5.72 ± 0.38 | 5.83 ± 0.49 |
| Control | 6.29 ± 0.20 | 5.22 ± 0.16 | 5.46 ± 0.12 | 5.49 ± 0.30 |

Table 7 *Effect of dietary palaMOUNTAINS MyBeau® on lactate levels in dogs prior to exercise*

| Diet | Concentration (mmol/l) ± SE | | | |
|---|---|---|---|---|
| | 0 weeks | 2 weeks | 4 weeks | 8 weeks |
| MyBeau® | 1.69 ± 0.14 | 1.64 ± 0.10 | 1.77 ± 0.06 | 1.60 ± 0.11 |
| Control | 1.90 ± 0.10 | 1.60 ± 0.08 | 1.78 ± 0.18 | 1.71 ± 0.09 |

Table 8 *Effect of dietary palaMOUNTAINS MyBeau® on lactate levels in dogs after exercise*

| Diet | Concentration (mmol/l) ± SE | | | |
|---|---|---|---|---|
| | 0 weeks | 2 weeks | 4 weeks | 8 weeks |
| MyBeau® | 1.95 ± 0.16 | 1.94 ± 0.11 | 2.00 ± 0.19 | 2.25 ± 0.18 |
| Control | 2.03 ± 0.11 | 1.90 ± 0.19 | 2.02 ± 0.22 | 2.40 ± 0.16 |

Table 9 *Effect of dietary palaMOUNTAINS MyBeau® on triglyceride levels in dogs prior to exercise*

| Diet | Concentration (mmol/l) ± SE | | | |
|---|---|---|---|---|
| | 0 weeks | 2 weeks | 4 weeks | 8 weeks |
| MyBeau® | 0.74 ± 0.10 | 0.68 ± 0.08 | 0.67 ± 0.09 | 0.72 ± 0.10 |
| Control | 0.65 ± 0.08 | 0.55 ± 0.05 | 0.54 ± 0.06 | 0.62 ± 0.04 |

Table 10 *Effect of dietary palaMOUNTAINS MyBeau® on triglyceride levels in dogs after exercise*

| Diet | Concentration (mmol/l) ± SE | | | |
|---|---|---|---|---|
| | 0 weeks | 2 weeks | 4 weeks | 8 weeks |
| MyBeau® | 0.86 ± 0.16 | 0.81 ± 0.18 | 0.78 ± 0.12 | 0.85 ± 0.08 |
| Control | 0.99 ± 0.09 | 0.78 ± 0.07 | 0.54 ± 0.05 | 0.63 ± 0.06 |

Table 11 *Effect of dietary palaMOUNTAINS MyBeau® on free fatty acid (FFA) levels in dogs prior to exercise*

| Diet | Concentration (mmol/l) ± SE | | | |
|---|---|---|---|---|
| | 0 weeks | 2 weeks | 4 weeks | 8 weeks |
| MyBeau® | 0.24 ± 0.03 | 0.39 ± 0.11 | 0.22 ± 0.04 | 0.25 ± 0.02 |
| Control | 0.22 ± 0.04 | 0.29 ± 0.10 | 0.27 ± 0.07 | 0.22 ± 0.03 |

Table 12 *Effect of dietary palaMOUNTAINS MyBeau® on free fatty acid (FFA) levels in dogs after exercise*

| Diet | Concentration (mmol/l) ± SE | | | |
|---|---|---|---|---|
| | 0 weeks | 2 weeks | 4 weeks | 8 weeks |
| MyBeau® | 0.35 ± 0.07 | 0.59 ± 0.07 | 0.51 ± 0.11 | 0.54 ± 0.11 |
| Control | 0.39 ± 0.04 | 0.56 ± 0.14 | 0.52 ± 0.12 | 0.48 ± 0.09 |

Table 13 *Effect of dietary palaMOUNTAINS MyBeau® on vitamin E levels in dogs prior to exercise*

| Diet | Concentration (µg/ml) ± SE | | | |
|---|---|---|---|---|
| | 0 weeks | 2 weeks | 4 weeks | 8 weeks |
| MyBeau® | 12.66 ± 1.51 | 20.54 ± 2.32 | 21.99 ± 2.99 | 20.76 ± 1.72 |
| Control | 12.18 ± 1.25 | 10.68 ± 1.21 | 12.30 ± 1.76 | 11.61 ± 1.55 |

Table 14 *Effect of dietary palaMOUNTAINS MyBeau® on vitamin E levels in dogs after exercise*

| Diet | Concentration (µg/ml) ± SE | | | |
|---|---|---|---|---|
| | 0 weeks | 2 weeks | 4 weeks | 8 weeks |
| MyBeau® | 11.96 ± 1.77 | 18.69 ± 1.68 | 22.75 ± 2.17 | 22.60 ± 1.87 |
| Control | 10.52 ± 1.81 | 9.49 ± 1.61 | 12.06 ± 1.74 | 11.61 ± 1.51 |

*APPENDIX 2.*

Table 1 *Effect of dietary palaMOUNTAINS MyBeau® on lymphocyte proliferative responses to Concanavalin A in dogs prior to exercise*

| | Stimulation Index ± SE | | | |
|---|---|---|---|---|
| Diet | 0 weeks | 2 weeks | 4 weeks | 8 weeks |
| MyBeau® | 11.111 ± 3.567 | 12.864 ± 1.810 | 14.432 ± 4.619 | 18.933 ± 3.900 |
| Control | 10.490 ± 1.942 | 8.909 ± 1.780 | 8.730 ± 2.077 | 8.785 ± 2.204 |

Table 2 *Effect of dietary palaMOUNTAINS MyBeau® on lymphocyte proliferative responses to Phytohaemagglutinin in dogs prior to exercise*

| | Stimulation Index ± SE | | | |
|---|---|---|---|---|
| Diet | 0 weeks | 2 weeks | 4 weeks | 8 weeks |
| MyBeau® | 3.464 ± 0.676 | 6.012 ± 1.041 | 7.978 ± 1.202 | 11.325 ± 1.301 |
| Control | 6.878 ± 1.325 | 6.187 ± 1.190 | 6.374 ± 1.238 | 5.850 ± 1.021 |

Table 3 *Effect of dietary palaMOUNTAINS MyBeau® on levels of $CD4^+$ cells in dogs prior to exercise*

| | % Expression ± SE | | | |
|---|---|---|---|---|
| Diet | 0 weeks | 2 weeks | 4 weeks | 8 weeks |
| MyBeau® | 33.615 ± 2.587 | 31.112 ± 2.730 | 33.447 ± 3.099 | 30.566 ± 3.986 |
| Control | 33.575 ± 2.513 | 32.045 ± 2.767 | 31.993 ± 2.241 | 31.390 ± 1.642 |

Table 4 *Effect of dietary palaMOUNTAINS MyBeau® on levels of $CD8^+$ cells in dogs prior to exercise*

| | % Expression ± SE | | | |
|---|---|---|---|---|
| Diet | 0 weeks | 2 weeks | 4 weeks | 8 weeks |
| MyBeau® | 31.738± 3.169 | 34.413 ± 3.401 | 35.363 ± 3.815 | 36.168 ± 5.269 |
| Control | 31.260 ± 5.077 | 31.097 ± 6.199 | 31.332 ± 5.012 | 31.560 ± 4.612 |

Table 5 *Effect of dietary palaMOUNTAINS MyBeau® on levels of B cells in dogs prior to exercise*

| Diet | % Expression ± SE | | | |
|---|---|---|---|---|
| | 0 weeks | 2 weeks | 4 weeks | 8 weeks |
| MyBeau® | 14.892 ± 2.223 | 15.358 ± 2.125 | 16.197 ± 2.081 | 19.042 ± 2.876 |
| Control | 15.842 ± 1.909 | 16.197 ± 2.214 | 18.373 ± 2.163 | 16.768 ± 3.324 |

Table 6 *Effect of dietary palaMOUNTAINS MyBeau® on levels of $CD14^+$ cells in dogs prior to exercise*

| Diet | % Expression ± SE | | | |
|---|---|---|---|---|
| | 0 weeks | 2 weeks | 4 weeks | 8 weeks |
| MyBeau® | 59.938 ± 2.998 | 74.982 ± 3.865 | 72.573 ± 2.188 | 61.370 ± 3.461 |
| Control | 53.002 ± 7.126 | 73.123 ± 3.397 | 70.238 ± 3.899 | 61.790 ± 3.027 |

Table 7 *Effect of dietary palaMOUNTAINS MyBeau® on levels of $CD11b^+$ cells in dogs prior to exercise*

| Diet | % Expression ± SE | | | |
|---|---|---|---|---|
| | 0 weeks | 2 weeks | 4 weeks | 8 weeks |
| MyBeau® | 97.933± 0.346 | 97.810 ± 0.209 | 96.740 ± 0.524 | 97.722 ± 0.476 |
| Control | 97.842 ± 0.457 | 97.208 ± 0.565 | 97.553 ± 0.413 | 97.545 ± 0.427 |

Table 8 *Effect of dietary palaMOUNTAINS MyBeau® on peripheral blood phagocytosis in dogs prior to exercise*

| Diet | % Expression ± SE | | | |
|---|---|---|---|---|
| | 0 weeks | 2 weeks | 4 weeks | 8 weeks |
| MyBeau® | 22.843 ± 2.405 | 27.657 ± 2.973 | 30.128 ± 2.798 | 33.757 ± 2.357 |
| Control | 23.751 ± 1.529 | 23.165 ± 1.610 | 22.565 ± 1.788 | 22.327 ± 1.803 |

*Disclaimer*

The Institute for Food, Nutrition and Human Health, Massey University has taken every care to ensure that the contents of this report provide a correct reflection of its current understanding of these results and that the information presented is accurate. The Institute for Food, Nutrition and Human Health cannot, however, accept responsibility for any inaccuracies or errors in the information presented. Similarly, no responsibility is accepted for any interpretations made from the information provided.