Immune Enhancing Potential of MyBeau™

Confidential Report to Vita Power Ltd

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INTRODUCTION

Dietary components are able to impact on the health of an animal in two ways; firstly in a purely nutritive sense, supplying the necessary energy and amino acids to the animal, and secondly by acting as bioactive molecules and influencing a number of functions within the animal, including immune health. There is currently little information available in the scientific literature on the immune enhancing effects of various dietary ingredients in cats.

Dietary supplementation with specific ratios of omega-3 and omega-6 fatty acids and other oil products have been shown to modulate a range of immune functions in species such as humans, dogs and mice[1-9]. However, little data exists for cats. Results from studies in other animals indicate that possible benefits of dietary supplementation with specific oil fractions may be in the prevention or reduction of arrhythmias and other heart problems[1-4], reduction in wound inflammation[5-8], and modulation of T- and B-cell mitogenic responses[7,9]. Little investigation of the effects on other immune parameters such as phagocytic activity and immunoglobulin levels has occurred.

In this study, using cats from the Centre for Felinine Nutrition (Massey University), we investigated the effects of dietary supplementation with MyBeau™ on a range of immune parameters including: lymphocyte proliferation, phagocytic activity, immunoglobulin levels, and the expression of a number of cell surface markers (e.g. T helper cell, cytotoxic T-cell markers). The cats were fed the MyBeau™ supplemented commercial AAFCO formulated diet for a period of 4 weeks, with immune testing being carried out prior to dietary supplementation and after 2 and 4 weeks of feeding. Results from a test group of animals were compared to a control group receiving the same, but unsupplemented diet.

Methods

Animals and Diet

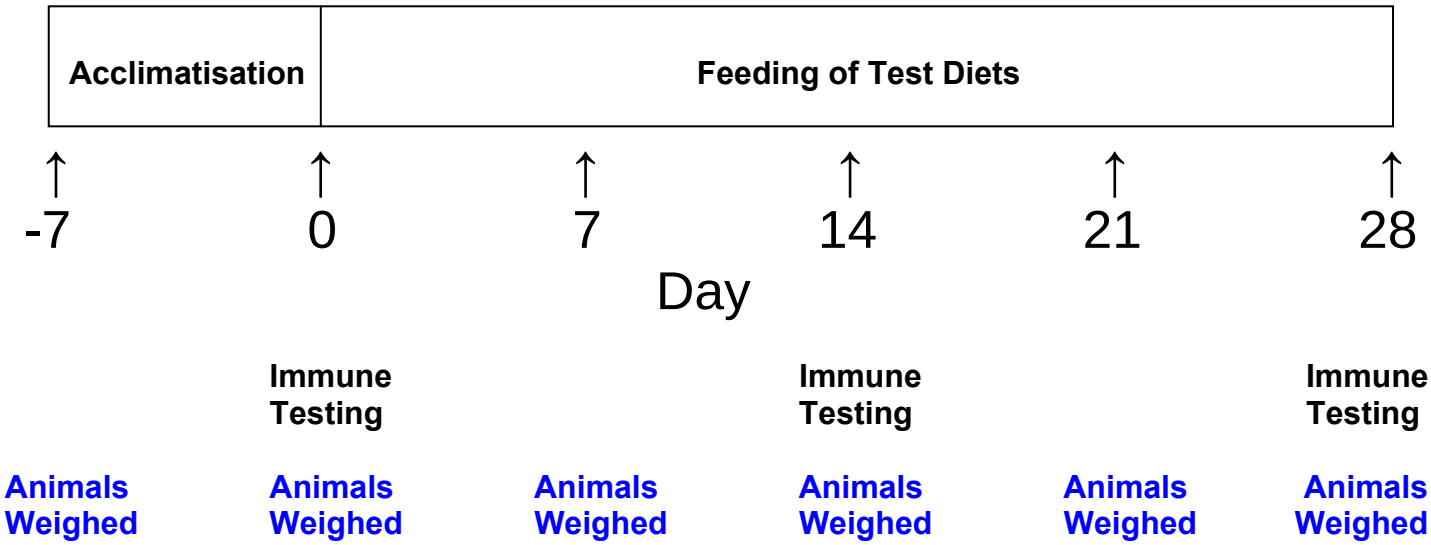
Sixteen animals were used for the trial. The animals were divided into 2 groups of 8 cats (equal numbers of castrated males and females, average age 6.8 years) and allowed to acclimatize to their environment for one week prior to the commencement of the trial. During the acclimatisation period, the cats were fed a commercial AAFCO formulated jellimeat diet. The cats had access to food and water *ad libitum* during both the acclimatisation and test periods. Fresh food was supplied daily, and the amount of food consumed by each group was calculated on a daily basis. The cats were weighed before the acclimatisation period began (Day -7), immediately prior to commencing the trial (Day 0), and weekly for the duration of the trial (Days 7, 14, 21, 28).

On day 0 the animals were blood sampled and saliva was collected and the 4 week feeding of the test diet began. The control diet consisted of a commercial AAFCO formulated Jellimeat diet, and the test diet consisted of the same Jellimeat with MyBeau™ added at a rate of 18.95ml/kg diet, which equates to the recommended dose rate of 10ml/cat/day.

Blood and saliva samples were collected at 0, 2 and 4 weeks of the feeding regime and analysed for immune responses as indicated in Table 1. A diagrammatic representation of the trial design is shown in Figure 1.

The study reported here was approved by and conformed to the requirements of the Massey University Animal Ethics Committee.

Figure 1: Experimental trial design



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)
# Phytohemogglutinin (PHA)

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

Statistical analysis

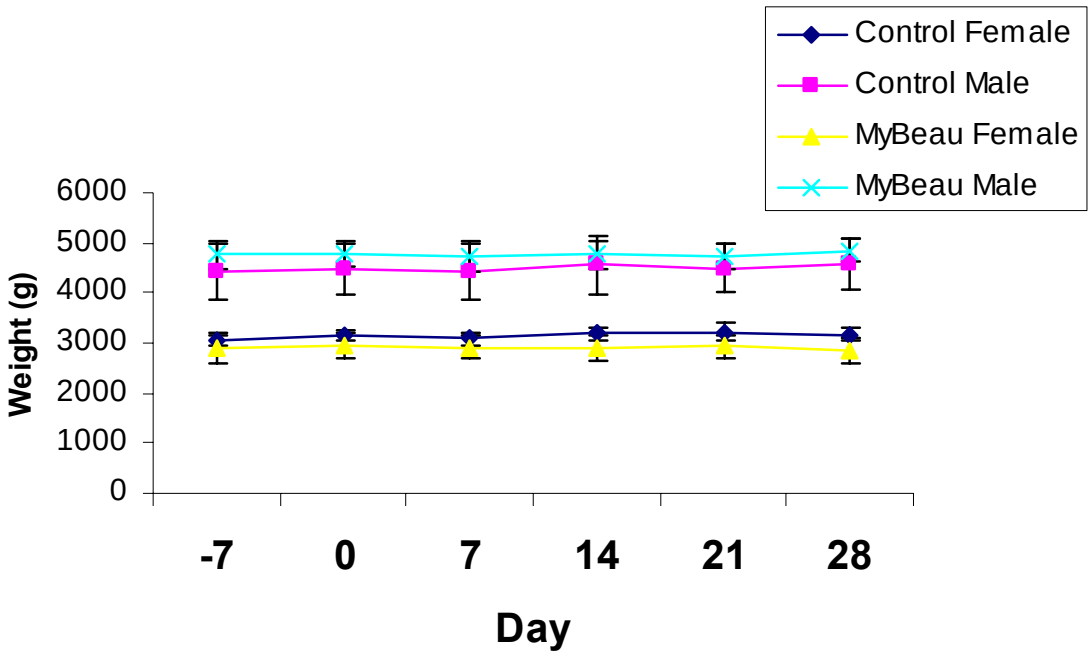
Significant differences between the experimental and control groups on log transformed or untransformed data were determined using ANOVA (the SAS programme). Values of $P<0.05$ were considered significant.

Results

Weight Gain

The effects of daily MyBeau™ consumption on feline body weights over the 4 week feeding period of the trial can be seen in Fig 2. The values have been split into male and female groups in order to make a more appropriate comparison. Clearly there were no significant changes in body weight in either the control or the MyBeau™-fed group over the experimental period.

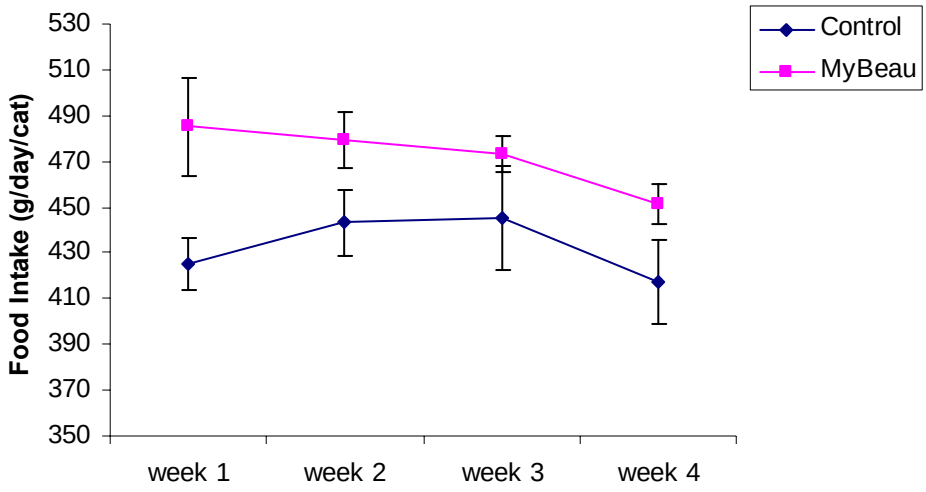
Fig. 2: Effect of dietary MyBeau™ on feline body weight



Feed Intake

The average daily feed intake per day per cat is shown in Fig 3. As can be seen the MyBeau™ fed animals showed a trend of declining feed intake over the 4 week period. However this decline was not statistically significant. Both groups showed a reasonably large decline in feed intake over the final week of the trial, which may indicate a seasonal influence.

Fig. 3: Effect of dietary MyBeau™ intake on average daily feed intake per cat expressed on a weekly basis



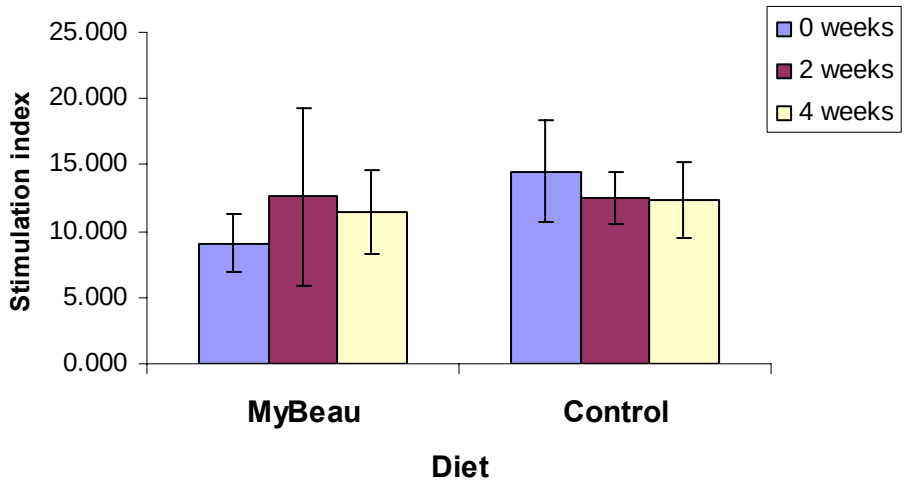
Specific responses

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 Phytohemagglutinin (PHA), which selectively stimulate the activation and proliferation of specific lymphocyte populations.

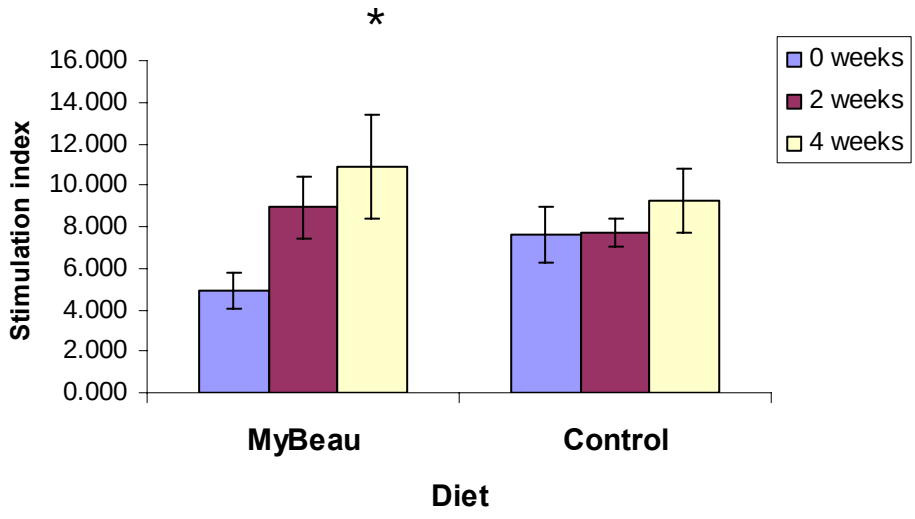
The effects of the dietary MyBeau™ on lymphocyte proliferative responses to concanavalin A (Con A) are shown in Figure 4. The mean values and standard errors are summarised in Appendix 2, Table 1. Neither the MyBeau™ fed group or the control group showed any significant change in lymphocyte proliferative responses to Con A over the time period tested.

Fig. 4: Effect of dietary MyBeau™ on lymphocyte proliferative responses to concanavalin A



The effects of dietary MyBeau™ on lymphocyte proliferative responses to phytohemagglutinin (PHA) are shown in Figure 5. The mean values and standard errors are summarised in Appendix 2, Table 2. The MyBeau™-fed group showed a significant increase (P= 0.026) in lymphocyte proliferative response to PHA after 4 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=0.15). 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 to required to observe a significant increase. In contrast, proliferative responses of the control group did not change significantly over the period tested.

Fig. 5: Effect of dietary MyBeau™ on lymphocyte proliferative responses to phytohemogglutinin (PHA) (*P<0.05)



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 is Table 2.

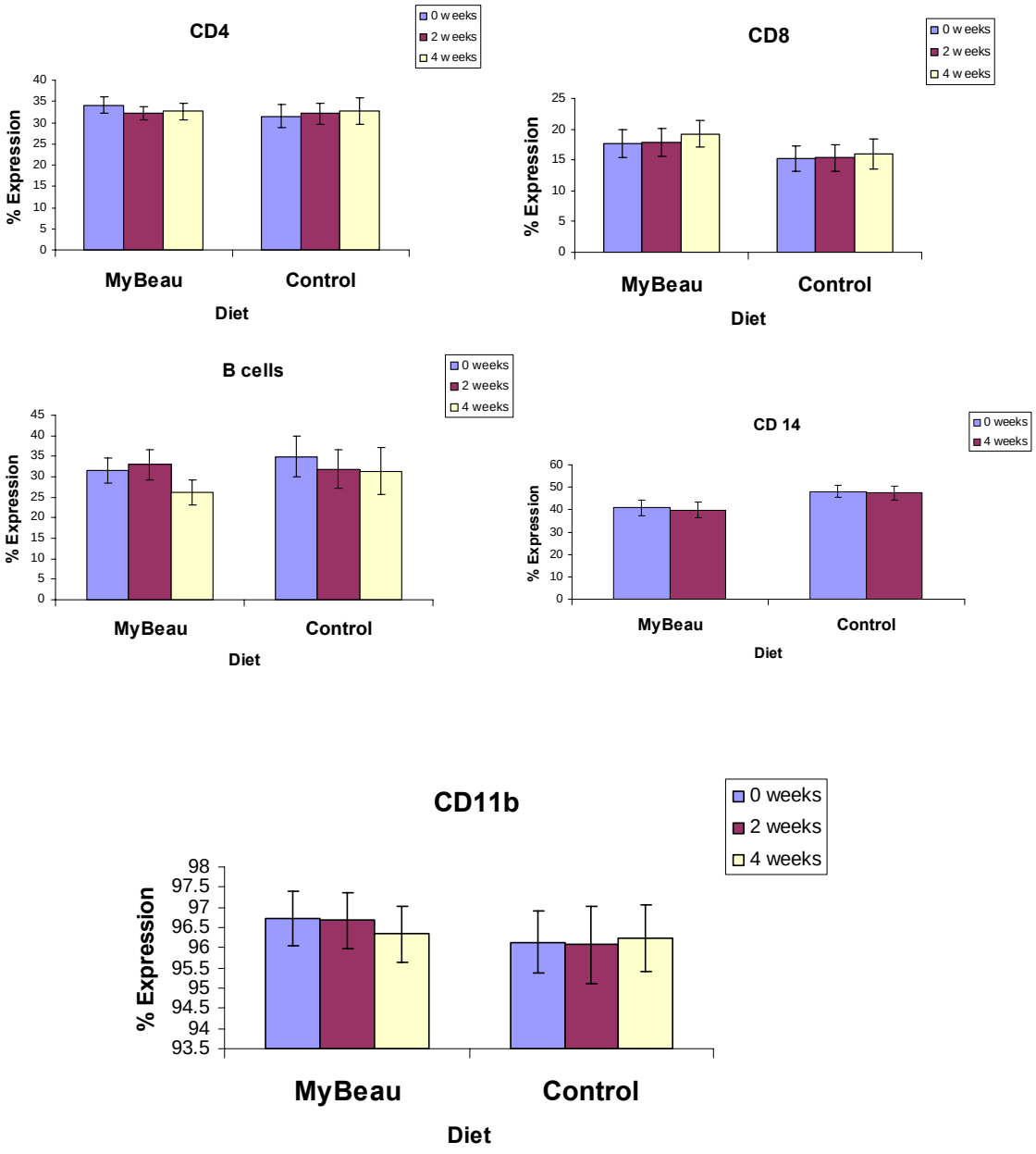
Table 2 ***Specificity of CD markers and role of the labelled cell type***

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 MyBeau™ on the expression of the various cell surface markers are shown in Figure 6. 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 MyBeau™ over a 4 week period did not significantly change the level of expression of any of the cell surface markers tested.

Fig 6 ***Expression of cell surface markers***



N.B. CD14 expression was only measured at 0 and 4 weeks

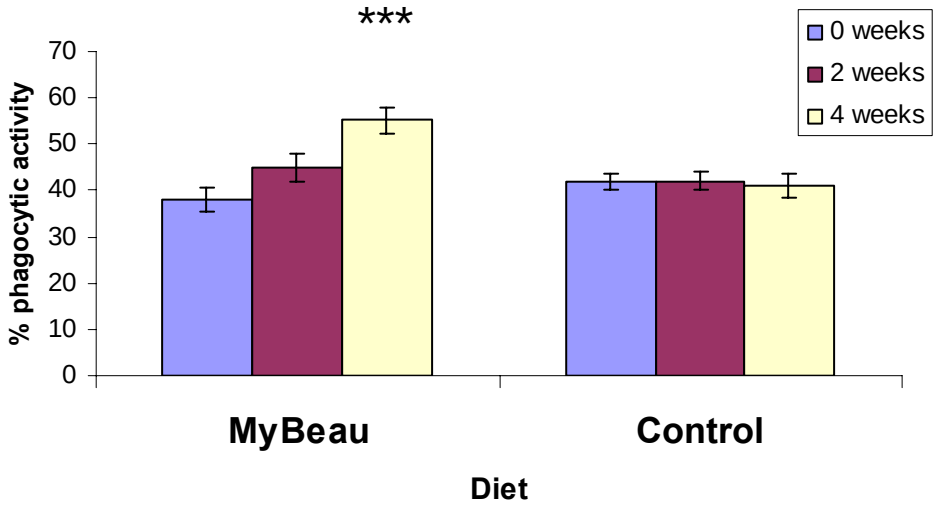
Innate responses

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 MyBeau™ on peripheral blood phagocytosis is shown in Figure 7. The mean values and standard errors are summarised in Appendix 2, Table 8. Fig 7 clearly shows a time dependent increase in phagocytic activity in the MyBeau™-fed animals, with the enhancement being highly statistically significant after 4 weeks of feeding (P=0.0003). In contrast again, the phagocytic activity of the control group did not change significantly during the trial period.

Fig. 7: Effect of dietary MyBeau™ on peripheral blood phagocytosis (P<0.001)***



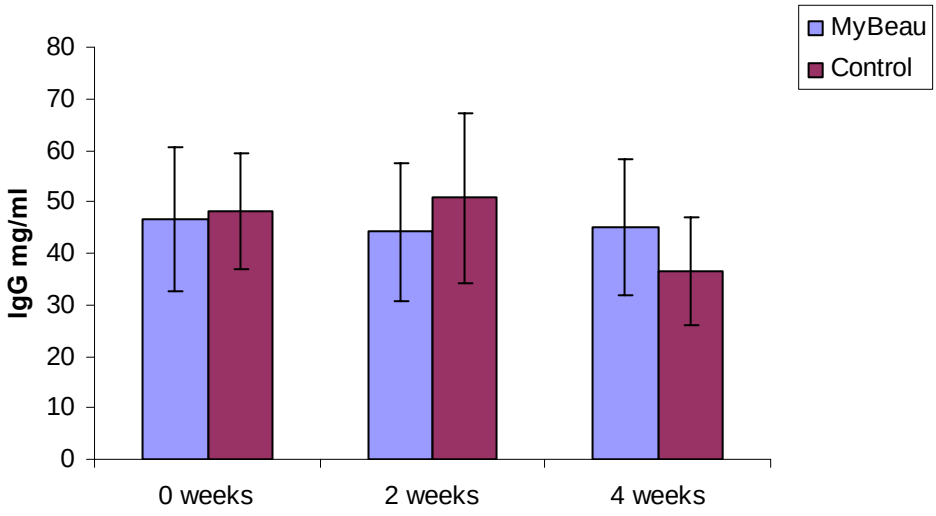
Specific antibody responses

IgG

IgG is a secreted protein which contributes specifically to secondary antibody responses (immunologic memory) and has a major role in host defence against infection. IgG are involved in complement fixation, opsonization and fixation to macrophages. Therefore their role is not solely limited to an antigen binding function, they also activate other molecules and cells which together neutralize, kill or destroy the target. IgG is the most common type of immunoglobulin in the serum, accounting for greater than 75% of the total serum immunoglobulins.

The effect of dietary MyBeau™ on serum IgG levels is shown in Figure 8. The mean values and standard errors are summarised in Appendix 2, Table 9. As can be seen, dietary MyBeau™ consumption had no significant effect on serum IgG levels.

Fig. 8: Effect of dietary MyBeau™ on serum IgG levels



Discussion

The aim of the work described in this report was to investigate the immune enhancing potential of dietary MyBeau™ in cats.

Consumption of MyBeau™, did not cause any significant change in body weight of the animals over the 4 week feeding period. This is interesting, as the feed intake of the MyBeau™ fed animals did decrease by 6.8% over time, from an average of 485g/cat/day over the first week, down to 452g/cat/day during the final week of feeding, however this decrease was not statistically significant ($P>0.05$) as there was a large variation in day to day feed intake. A large proportion of the apparent decrease (4.4%), occurred in the final week. However, intake in the control group also fell in the last week of the study by 6.3% (445g/cat over week 3 vs 417g/cat over week 4). Therefore the decline observed in the final week of the study may have been due to a seasonal influence.

Results from the immune assays showed that MyBeau™ consumption caused a significant enhancement of lymphocyte proliferative responses to the T-cell mitogen phytohemagglutinin ($P=0.026$), indicating that these cells were upregulated by MyBeau™, and are thus primed to proliferate in response to an appropriate antigenic challenge such as a bacterial infection. Proliferative responses to concanavalin A remained unchanged.

There were no significant changes in the level of expression of the lymphocyte markers CD4, CD8 or B cells. This is advantageous, as significant changes in the level of expression of the T cell markers (CD4, CD8), results in the opposing effect in B cells. For example, if T cell expression increases, then B cell expression will decrease. Such changes are undesirable as they can predispose the individual to certain types of disease and infection.

There were also no 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 cats fed the MyBeau™ diet ($P=0.003$ at 4 weeks), with the increase in activity appearing to increase over time with the consumption of the

product. This increase in phagocytic activity is an indicator of greater ability to fight infection and disease.

The fact that the phagocytic activity of the 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.

There was no significant change in serum IgG levels over the course of the study.

Conclusions

- *MyBeau™ consumption significantly enhanced lymphocyte proliferation to PHA*
- *MyBeau™ consumption significantly enhanced peripheral blood leukocyte phagocytic activity*
- *MyBeau™ consumption had no negative effects on the level of expression of cell surface markers*
- *MyBeau™ consumption could significantly improve resistance and ability to fight disease and infection in cats*

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APPENDIX 1.

Table 1 ***Effect of dietary MyBeau™ on feline body weight (g ± SEM)***

Day	MyBeau™	Control
-7	3826.13 ± 399.27	3650.14 ± 351.40
0	3843.38 ± 382.49	3721.43 ± 331.69
7	3813.38 ± 375.01	3670.00 ± 354.59
14	3832.13 ± 391.60	3777.57 ± 359.33
21	3837.50 ± 377.62	3767.71 ± 328.32
28	3848.50 ± 407.27	3766.71 ± 349.55

Table 2 ***Effect of dietary MyBeau™ intake on average daily feed intake per cat (g ± SEM)***

Day	MyBeau™	Control
Week 1	485 ± 21	425 ± 12
Week 2	479 ± 12	443 ± 15
Week 3	473± 8	445 ± 22
Week 4	452 ± 9	417 ± 18

APPENDIX 2.

Table 1 ***Effect of dietary MyBeau™ on lymphocyte proliferative responses to concanavalin A***

	Stimulation Index ± SE		
Diet	0 weeks	2 weeks	4 weeks
MyBeau™	9.077 ± 2.203	12.615 ± 6.704	11.411 ± 3.140
Control	14.508 ± 3.873	12.506 ± 1.996	12.383 ± 2.871

Table 2 ***Effect of dietary MyBeau™ on lymphocyte proliferative responses to phytohemagglutinin***

	Stimulation Index ± SE		
Diet	0 weeks	2 weeks	4 weeks
MyBeau™	4.929 ± 0.889	8.947 ± 1.506	10.899 ± 2.517
Control	7.635 ± 1.362	7.676 ± 0.674	9.266 ± 1.576

Table 3 ***Effect of dietary MyBeau™ on levels of $CD4^+$ cells***

	% Expression ± SE		
Diet	0 weeks	2 weeks	4 weeks
MyBeau™	34.08 ± 1.97	32.13 ± 1.57	32.63 ± 1.86
Control	31.55 ± 2.63	32.12 ± 2.53	32.76 ± 3.05

Table 4 ***Effect of dietary MyBeau™ on levels of CD8$^+$ cells***

Diet	% Expression ± SE		
	0 weeks	2 weeks	4 weeks
MyBeau™	17.66 ± 3.00	17.77 ± 2.25	19.27 ± 2.09
Control	15.24 ± 2.13	15.34 ± 2.16	15.97 ± 2.40

Table 5 ***Effect of dietary MyBeau™ on levels of B cells***

Diet	% Expression ± SE		
	0 weeks	2 weeks	4 weeks
MyBeau™	31.54 ± 3.10	32.92 ± 3.71	26.15 ± 3.07
Control	34.90 ± 4.95	31.81 ± 4.70	31.32 ± 5.76

Table 6 ***Effect of dietary MyBeau™ on levels of CD14$^+$ cells***

Diet	% Expression ± SE	
	0 weeks	4 weeks
MyBeau™	40.80 ± 3.41	39.84 ± 3.48
Control	48.19 ± 2.79	47.50 ± 3.09

Table 7 ***Effect of dietary MyBeau™ on levels of CD11b^{+} cells***

Diet	% Expression ± SE		
	0 weeks	2 weeks	4 weeks
MyBeau™	96.73 ± 0.68	96.68 ± 0.69	96.35 ± 0.69
Control	96.14 ± 0.77	96.07 ± 0.96	96.24 ± 0.83

Table 8 ***Effect of dietary MyBeau™ on peripheral blood phagocytosis***

Diet	% Phagocytic Activity ± SE		
	0 weeks	2 weeks	4 weeks
MyBeau™	37.87 ± 2.63	44.79 ± 3.02	55.18 ± 2.93
Control	41.97 ± 1.77	42.07 ± 2.05	41.03 ± 2.79

Table 9 ***Effect of dietary MyBeau™ on serum levels of IgG***

Diet	mg/ml ± SE		
	0 weeks	2 weeks	4 weeks
MyBeau™	46.52 ± 13.91	44.08 ± 13.43	44.93 ± 13.2
Control	48.29 ± 11.31	50.69 ± 16.61	36.58 ± 10.58

Disclaimer

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