Enhancing Cognitive Function Through Diet in Cats

Yuanlong Pan, PhD,[1] and Norton W. Milgram, PhD [2,3]
[1]Nestlé Purina Research
St. Louis, MO
[2]CanCog Technologies
Toronto, Canada
[3]University of Toronto
Department of Pharmacology
Toronto, Canada
Email: yuanlong.pan@rdmo.nestle.com

In this study, we selected a blend of nutrients based on their ability to either minimize or eliminate risk factors associated with brain aging and dementia, and we tested the effects of the blend on cognitive functions in middle-aged and senior cats. The cats were tested on four cognitive test protocols during the one-year study. The cats fed the test diet showed significantly better performance on three of the four tests. The results support our hypothesis that brain function of cats can be improved by the nutrient blend that minimizes or eliminates the risk factors associated with brain aging and dementia.

Glossary of Abbreviations
AAFCO: Association of American Feed Control Officials
AD: Alzheimer's Disease
ANOVA: Analysis of Variance
BPB: Brain Protection Blend
CDS: Cognitive Dysfunction Syndrome
DHA: Docosahexaenoic Acid
DNMP: Delayed-Non-Matching-to-Position Test
EPA: Eicosapentaenoic Acid
FGTA: Feline General Test Apparatus
Land-0: Landmark 0 Task
Land-1: Landmark 1 Task
NO: Nitric Oxide
RBC: Red Blood Cells

Cognitive dysfunction syndrome (CDS) is a major disease in geriatric cats and is clinically associated with disorientation, altered social interactions, sleep-wake cycle disturbance, loss of housetraining, and altered activity levels and patterns.[1,2] It has been estimated that CDS affects 28% of 11- to 14-year-old cats and 50% of cats over 15 years of age.[1,2] Severe and irreversible loss of brain cells and synapses results in dementia in people and CDS in pets over the course of aging.[3-6]

The brains of aged cats, like both dogs and humans, develop beta-amyloid pathology,[7,8] including plaques immunopositive for AB42, but lack senile plaques observed in the human brains.[9] Although aged cats have also been reported to develop hyperphosphorylated tau protein, they don't develop neurofibrillary tangles.[10] We have recently reported that neuropsychological test performance in cats shows age-dependent changes that parallel those seen in dogs and in humans.[11]

Dementia in humans and CDS in pets are not curable diseases because brain cells and synapses that die cannot be replaced in sufficient quantities to provide normal brain functions.[3-5, 12,13] Therefore, a more promising strategy is disease prevention focusing on retarding the loss of brain cells and synapses in both humans and pets. We have hypothesized that the best option to promote healthy brain aging is to retard brain atrophy by reducing or eliminating risk factors associated with brain aging and dementia.[3] Accelerated brain aging and dementia have been associated with many risk factors, including increased oxidative stress, chronic inflammation, DHA deficiency, high homocysteine, low status of vitamin B6, vitamin B12 and folic acid, high blood pressure, and cerebral vascular lesions.[14-22]

Since multiple factors are linked to accelerated brain aging and dementia risk, we would not expect any single nutrient or bioactive compound to retard brain aging and reduce the risk of dementia. Many risk factors have been identified for higher risk of dementia, including Alzheimer's disease (AD) in people.[14-22] Limited data suggests that the same risk factors associated with higher risk of AD in people (age, gender, sex hormone deficiency, oxidative stress) are also linked to higher risk for CDS in pets.[23] Therefore, we formulated a blend of nutrients, referred to as the Brain Protection Blend (BPB), based on their ability to minimize or eliminate the risk factors associated with accelerated brain aging and dementia. The BPB includes fish oil, arginine, B vitamins, and selected antioxidants. Fish oil containing DHA and EPA prevents and corrects DHA deficiency and offers anti-inflammatory benefits.[24,25] Arginine enhances nitric oxide (NO) synthesis, which is involved in circulation, blood pressure control and cognition.[26,27] B vitamins prevent and correct any B vitamin deficiency and minimize the risk of hyperhomocysteinemia.[28-30] Antioxidants, including vitamins E, C and selenium, protect both brain tissue and blood vessels against oxidation and inflammation-induced damage.[31-36]

The objective of this study was to determine the effect of BPB on cognitive functions in middle-aged and senior cats. The cognitive evaluation included protocols for assessing landmark discrimination,

From the Purina Companion Animal Nutrition Summit: *Nutrition for Life*; Austin, TX; March 27-29, 2014.

Table 1. Dietary Compositions*

	Control	Test (BPB)**
Nutrient Composition (% as fed)		
Moisture	8.31	8.19
Ash	5.75	6.08
Crude Protein	40.50	40.90
Crude Fat	17.9	18.6
Crude Fiber	1.08	0.87
Linoleic Acid (% of total fat)	14.0	13.7
Energy Content		
Calculated ME§(kJ/g)	16.20	16.33

* This table is modified from the original published in a previous paper[42]
§ Calculated based on the predictive equation for metabolizable energy in cat foods[61]
** BPB = Brain Protection Blend including the addition of DHA, EPA, vitamin C, and elevated levels of arginine, B vitamins, selenium, and alpha tocopherol

Table 2. Levels of BPB Ingredients in Diets§

	Control	Test (BPB)*
Eicosapentaenoic Acid (EPA)**	0.04	0.28
Docosahexaenoic Acid (DHA)**	0.04	0.27
Arginine**	1.48	2.30
Alpha Tocopherol Acetate(mg/kg)	73.6	550
Vitamin C (mg/kg)	0	80
Selenium (mg/kg)	0.72	1.00
Thiamine (mg/kg)	37.3	55.0
Riboflavin (mg/kg)	17.2	30.9
Pantothenic Acid (mg/kg)	26.1	55.4
Pyridoxine (mg/kg)	15.4	18.0
Cyanocobalamin (mg/kg)	0.05	0.09
Folic Acid (mg/kg)	1.6	4.25

§ This table is modified from the original published in a previous paper[42]
* BPB = Brain Protection Blend including the addition of DHA, EPA, vitamin C, and elevated levels of arginine, B vitamins, selenium, and alpha tocopherol
** % as fed

Table 3. Cognitive Test Schedule§

Cognitive Tests	Days
Baseline DNMP Test	-12 to -1
Egocentric Protocol	31 to 74
Landmark Protocol	82 to177
Size Discrimination Protocol	201 to 281
Retest of DNMP	304 to 345

§ This table is modified from the original published in a previous paper[42]

egocentric discrimination, size discrimination, and visuospatial memory, or the delayed-non-matching-to-position (DNMP) test. The landmark discrimination learning test was designed to assess visuo-spatial learning and perception, and it had previously been shown to be sensitive to age in dogs.[37,38] The egocentric test protocol was designed to assess egocentric spatial ability as initially reported by Christie et al.[39] The size discrimination protocol was also previously reported for cognitive evaluation of dogs.[40,41] Both the egocentric and size discrimination protocols included reversal learning components, which were intended to provide measures of the executive function.[41] The initial DNMP test was used in group stratification.

Materials and Methods

Cats and Diets

Thirty-two domestic shorthaired cats aged 6.65 ± 0.72 years (range 5.5 to 8.7 years) were enrolled in the study with 16 cats per group. The cats were randomly assigned to either the control or test group based on their baseline DNMP test results. The study protocol, which was approved by the CanCog Technologies Institutional Animal Care Committee, complied with the guidelines of the Ontario Ministry of Agriculture and the Nestlé Purina Animal Welfare Guidelines. The cats were group-housed based on compatibility in rooms with environmental enrichment consisting of toys, beds and opportunities to play outside daily.

The control diet was based on a commercial super-premium product for adult cats. Both diets, manufactured by Nestlé Purina PetCare (St. Louis, MO), were isoenergetic and contained the same levels of protein, fat and carbohydrates, but they differed regarding the ingredients from the BPB (Tables 1 & 2). Cats were individually fed their assigned diet in sufficient amounts to maintain body weight.

Jugular blood samples were collected at baseline and after 200 or 345 days of treatment to measure selected folic acid, B12 and homocysteine concentrations, fatty acid profiles in red blood cells (RBC), and total antioxidant status.

Cognitive Testing Apparatus

All cognitive tests were conducted with the Feline General Test Apparatus (FGTA),[43] which has an enclosure with a front consisting of three adjustable gates and a movable food tray. The cats stayed in the enclosure for 5 to 15 minutes, depending on the cognitive tests. The technician was separated from the cat by a one-way mirror and a hinged door, which could be opened for presentation of the food tray. A food reward, consisting of approximately 1 gm of canned cat food that was selected based on the individual preference of the cat, was used to motivate the cat to participate in the tests.

All cognitive tests were administered by trained behavioral technologists who were blinded with respect to diets. All cognitive testing was performed in the morning or early afternoon, and each cat was tested at about the same time each day.

Baseline Cognitive Testing and Randomization

All the cats enrolled in this study had previous cognitive testing that included, at a minimum, testing on object discrimination and reversal learning and on a two-component version of the DNMP task.[44] Twelve cats had been extensively trained on a

From the Purina Companion Animal Nutrition Summit: *Nutrition for Life*; Austin, TX; March 27-29, 2014.

variety of cognitive tasks and were characterized as an experienced group. The remaining 20 cats had the minimal possible experience and were, therefore, classified as an inexperienced group. The cats were divided into two equal groups based on: (1) baseline cognitive performance on the DNMP task over five consecutive sessions, and (2) extent of previous cognitive experience. After striation, the two treatment groups were equivalent in body weight (4.26±0.27 kg for control and 4.13±0.20 kg for the BPB group), age and cognitive performance. The groups were then randomly assigned to dietary treatment. After a 30-day wash-in period, the cats were evaluated with four cognitive test protocols (Table 3).

Cognitive Test Protocols

The delayed-non-matching-to-position task evaluates both visuo-spatial learning and short-term visuo-spatial memory, and the test procedure was based on a protocol originally developed for dogs.[44] The cats were presented with a series of trials, in which each contained both a sample phase and a test phase. During the sample phase, the cat was presented with a single object covering a food reward over either the left or right food well. During the test phase, the cat was presented with two identical objects covering both the left and right food wells. To obtain the food reward, the cat had to respond to the well that was not covered during the sample phase. All cats had been trained on the DNMP prior to the study. At baseline, the cats were all given five test sessions, and their performance was used in group randomization. During the test phase, the cats were tested for relearning of the DNMP task with the delay between the sample and test phase set at 5 seconds.

In the egocentric test, cats were required to locate the food reward by selecting an object based on the proximity to either its left or right side.[39] The egocentric test protocol consisted of three phases: a one-day preference test phase, an egocentric discrimination phase and an egocentric reversal phase. The reversal task is designed to assess executive function including the ability to switch response sets, adapt to new situations, and reason. During the preference test phase, cats received 10 presentations of identical objects to both the left and right food wells, with both objects covering a food reward. Thus, the cats were rewarded for selecting either object (well). The side that the cat selected more frequently was designated the preferred side. If both sides were selected equally, the preferred side was decided by a coin toss. The preferred side was used as the correct choice during the egocentric discrimination phase. For example, if the preferred side was the left side, then during the egocentric discrimination phase, the cat was rewarded for selecting the left food well covered by an identical object.

During the egocentric discrimination phase, the cats received 12 trials per day using two wells for each trial with three possible configurations: left versus center, left versus right, or right versus center. Each configuration was given to the cat for four trials. Cats were given the tests daily until they successfully achieved a two-stage learning criterion. The cats completed the first stage when they either made 11 or more correct choices on one day, 20/24 over two consecutive days, or 29/36 over three consecutive days. To complete the second stage, the cats had to select correctly on at least 26 of 36 trials over three successive sessions. After completion of the egocentric discrimination phase, the location of reward was switched to the opposite side. Thus, if the cats were initially rewarded for approaching the object closest to their left side, they were now rewarded for approaching the object closest to their right side. After completing the first reversal task, the cats were given additional reversal training until they completed a total of 40 sessions following the egocentric discrimination. For the first and second reversal tasks, a two-stage learning criterion was used. Completion of these stages used the same criteria as completion of the egocentric discrimination phase. For subsequent reversals, a one-stage criterion was used; the cats completed the stage if they either selected correctly on 90% or more of the trials on a single day or if they responded correctly on 80% of the trials over two successive days.

The landmark discrimination task evaluated allocentric spatial learning ability, which entailed utilization of an external landmark to locate a food reward. The protocol consisted of two parts: Land-0 and Land-1. During Land-0, the landmark (a yellow rod) was attached to the middle of one of two coasters and the cats had to respond directly to the landmark to obtain the reward. The cats were given 10 trials per day until they either completed a two-stage learning criterion or failed to complete the criterion within 30 training days. To complete the first stage, the cats had to first select correctly on at least 9/10 trials on a single day or on 16/20 trials over a two-day period. To complete the second stage, the cats had to respond correctly on 70% of the trials over three consecutive days. Thus, it took a minimum of four days for the cats to complete the two-stage criterion. Cats that failed to complete the task within the 30 days were given a remedial training. If a cat failed to pass Land-0 after remedial training, it was not tested further on the subsequent Land-1 task. During the Land-1 test, the landmark was placed 2.5 mm from the edge of the coaster and the cats had to select the coaster closer to the landmark.

The size discrimination task measures visual discrimination learning ability, which depends on associative learning. The reversal task assesses executive function. The cats were first trained to respond to one of two identical objects that differed in size. The test protocol started with a one-day preference test, in which the cats were allowed to respond to either of two objects over 10 trials, with both responses associated with reward. The object chosen more frequently was considered the preferred object and was used as the correct object associated with reward during the discrimination learning phase. After the preference test, the cats received up to 40 sessions to complete a two-stage learning criterion on the size discrimination task. After completion of the size discrimination task, the cats were trained on the reversal learning

task. The protocol was identical to that of the size discrimination learning test, except that the correct object associated with the reward was reversed. The cats were given a maximum of 40 sessions to pass the reversal task. For both phases of the tests, the passing criterion was the same as that used for the landmark test.

Statistical Analysis

We originally planned to use an error measure as evidence of cognitive performance, with a failure to respond counted as 0.5 error. However, a number of cats did not achieve the *a priori* learning criterion due to frequent response failures, which artificially elevated the total number of errors in individual cats that normally performed very accurately. Accordingly, we analyzed the data with a second target variable, which was the percent of correct responses of the total attempted responses, ignoring trials with response failures. For instance, if an animal responded correctly on five trials out of five attempted responses but failed to respond on the other five trials, its score would be 100%. In addition, it became necessary to remove individual cats from specific tasks because of too frequent inconsistent responses. For the BPB group, two cats were removed from the landmark test, two from the size test, and one from the DNMP.

The data were analyzed using either analysis of variance or two-tail t tests. The results were considered statistically significant if the significance level was ≤0.05. Repeated measures analysis of variance was used for determining differences between groups in body weight, fatty acid profiles of RBCs, vitamin B12, folic acid, and total antioxidant status. Values are expressed as means ± SEM except the cognitive data in the figures.

The data for the initial learning and reversal of the egocentric test were analyzed with a repeated analysis of variance using task as the within subject variable and both treatment (control versus BPB) and experience as the between subject variables. The data of size discrimination tests were analyzed with a repeated measures ANOVA using treatment group and experience as between subject variables and task (size discrimination and reversal) as the within subject variable. The data from landmark tests were analyzed with a repeated measures ANOVA, using treatment group and previous experience as between subject variable and task as a within subject variable. The data of the DNMP test were analyzed with a factorial analysis of variance using diet and previous experience as within subject variables.

Results

Effects on Body Weight, Fatty Acid Profile of RBCs, B Vitamins, and Total Antioxidant Status

The cats in both groups had identical fatty acid profiles at the baseline. By the end of the study, the cats in the BPB group had significantly higher DHA, EPA, total n-3 fatty acids, lower LA, and total n-6 fatty acids than the cats in the control group. In addition, the cats fed the BPB diet had significantly lower omega-6 to omega-3 ratios than the cats fed the control diet.

Table 4. Performance (% correct) on Egocentric Discrimination and Reversal[&§]

	Control		BPB	
	Mean	SE	Mean	SE
Egocentric Discrimination	75.46	1.37	80.39*	1.66
Egocentric Reversal	59.75	1.10	67.02**	0.99

§ This table is prepared from the data published in a previous paper[42]
BPB = Brain Protection Blend
& n = 16
* Control vs. BPB: p=0.042
** Control vs. BPB: p=0.002

The BPB diet did not significantly affect blood vitamin B12, homocysteine or total antioxidant capacity; however, cats fed the BPB diet had significantly ($p<0.05$) higher fasting blood levels of folic acid (10.18±1.41 vs. 15.6 ±1.51ng/ml) 200 days after the feeding started. Body weights did not differ significantly between control and BPB groups at any point.

Effect of Diets on Performance of the Egocentric Test

The results of analysis revealed highly significant main effects of treatment and task. Cats in the BPB group performed significantly more accurately on both tests than the cats in the control group (Table 4).

Effect of Diets on Performance of the Landmark Test

Two of the cats in the BPB group were dropped before completing the land-0 task because of inconsistent responding, and their data were not included in the statistical analysis. The results revealed a significant effect of task (p=0.009) and no other significant effects or interactions. Although cats in the BPB group performed more accurately than the cats in the control group, the difference was not statistically significant (Figure 1).

Effect of Diets on Performance of the Size Discrimination Learning and Reversal Tests

Analysis revealed a significant effect of group and a marginally significant effect of task (Figure 2). The task effect reflects less-accurate performance on the reversal learning phase. The significant group effect was driven by group differences in the discrimination learning phase, in which the cats in the BPB group performed significantly better on the size discrimination task (Tukey multiple comparisons; p=0.010) than the cats in the control group.

Effect of Diets on Performance of the DNMP Test

The results showed a significant effect of diet (p=0.0149), and no other significant effects or interactions (Figure 3). The cats in both groups performed at equivalent levels at baseline; however, cats in the BPB group performed significantly better than cats in the control group at the end of the study.

From the Purina Companion Animal Nutrition Summit: *Nutrition for Life*; Austin, TX; March 27-29, 2014.

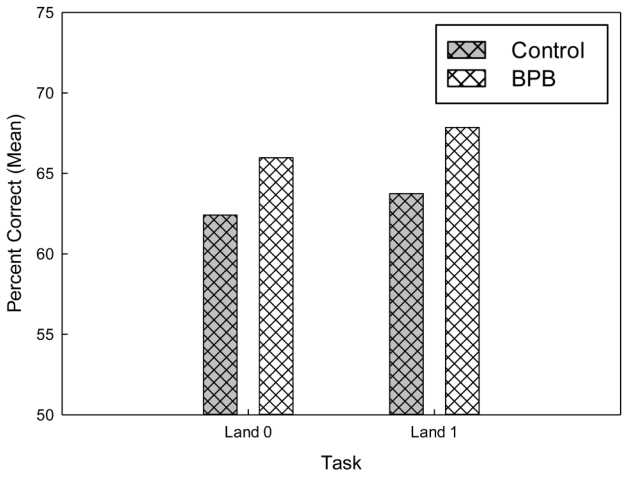

Figure 1. Effects of the BPB supplementation on cats' performance in object discrimination (Land-0) and landmark discrimination (Land-1) learning tasks. The performance was expressed as percent of correct choices. The data are means ± SE, n = 16 for controls and n = 14 for BPB group. This figure is prepared from the data published in a previous paper.[42]

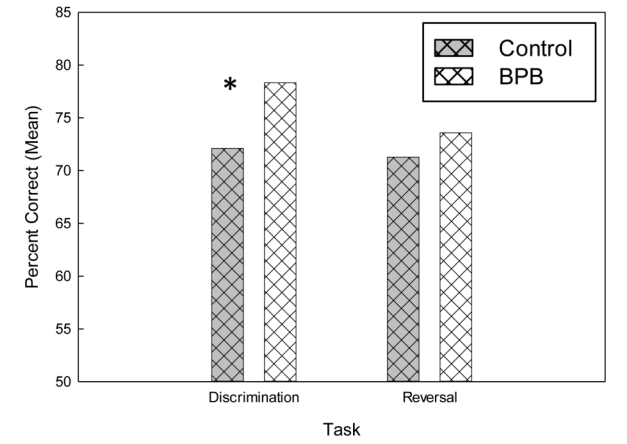

Figure 2. Effects of the BPB supplementation on cats' performance in the size discrimination and reversal learning tasks. The performance was expressed as percent of correct choices. The data are means ± SE, n = 16 for controls, and n = 14 for BPB group. There were statistically significant differences in the size discrimination test. (*p<0.05). This figure is prepared from the data published in a previous paper.[42]

Discussion

The objective of this long-term study was to evaluate the effects of BPB on cognitive performance in middle-aged and old cats. Baseline cognitive performance was used in dividing the cats into two cognitively equivalent groups. Over the course of the one-year study, the cats were tested on four test protocols: (1) landmark discrimination learning, (2) egocentric learning and reversal, (3) size discrimination learning and reversal, and (4) relearning the DNMP task.

With the exception of the landmark discrimination protocol, the cats fed the BPB diet showed significantly better performance than the cats fed the control diet. In the egocentric task, the cats in the BPB group performed significantly better in both discrimination and reversal tasks than the cats in the control group. The cats fed the BPB diet performed significantly more accurately on the size discrimination test. Finally, the cats in the BPB group had significantly better performance in the DNMP than the cats in the control group.

Collectively, these data indicate that the BPB diet has either cognition-enhancing benefits, neuroprotective benefits, or possibly both. The suggestion that the diet may have cognition-enhancing properties is supported by the fact that the cats fed the BPB diet showed significantly better cognitive performance in the egocentric tests, which started within a month of the study yet was too short a timeframe to demonstrate neuroprotective effects. The suggestion that the diet had neuroprotective effects was supported by the results of the DNMP test, in which performance of the cats fed the BPB diet at the end of the one-year study was significantly superior to that of the cats fed the control diet. The lack of any savings effect in the control cats may be due to an age-related cognitive decline over the one-year period of the study. Overall, the results from this study support our hypothesis that brain aging can be managed successfully by targeting risk factors associated with accelerated brain aging and dementia.[3]

Our strategy to test the effects of a nutrient blend was driven by the assumption that no one single nutrient or bioactive compound is sufficient for reducing or minimizing multiple risk factors associated with dementia and AD; we, therefore, selected the ingredients of the BPB based on their ability to reduce or eliminate multiple risk factors associated with brain aging and dementia.[24-36] The

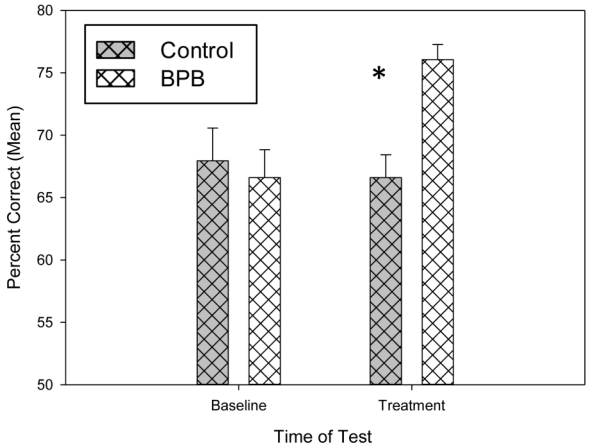

Figure 3. Effects of the BPB supplementation on cats' performance in the DNMP test. The performance was expressed as percent of correct choices. The data are means ± SE, n = 16 for controls, and n = 15 for BPB group. There were statistically significant differences between the control and BPB groups. (*p<0.05). This figure is prepared from the data published in a previous paper.[42]

From the Purina Companion Animal Nutrition Summit: *Nutrition for Life*; Austin, TX; March 27-29, 2014.

levels of B vitamins and antioxidants in the BPB diet are found in commercially available, highly nutrient-dense products for adult cats. With the exception of the inclusion of fish oil and ascorbic acid and elevated levels of arginine, B vitamins and antioxidants in the BPB diet, the control and BPB diets contained the same levels of protein, fat, carbohydrate, fiber, and essential fatty acid (Table 1), calcium, choline, potassium, magnesium, taurine, zinc, and amino acid profile (data not shown). More importantly, all the essential nutrients in the control diet were above the daily nutrient requirement recommended for cats by the Association of American Feed Control Officials (AAFCO).

The observed beneficial effects of the BPB on brain functions in cats are consistent with the beneficial effects of a Mediterranean diet on cognitive function in older adults.[46] In fact, all the ingredients in the BPB are present in the fruits, vegetables, cereals, seeds, legumes, vegetable oils, and fatty fish of the Mediterranean diet. More interestingly, all the ingredients of the BPB are also present in the natural prey of cats,[47-51] indicating that cats naturally obtain those nutrients from their prey.

Low status of DHA has been linked to cognitive decline in both normal elderly subjects and dementia and Alzheimer's disease subjects.[52,53] Fish oil supplementation has been reported to improve cognitive function in people[54] and in aged rodents.[55] In humans, maximal cardiovascular protection of DHA and EPA is achieved with a concentration of 8% erythrocyte fatty acids as EPA+DHA.[56] Coincidentally, the levels of DHA and EPA in the nutrient blend resulted in close to 8% of erythrocyte fatty acids as EPA and DHA in the cats. More studies should be conducted to determine the optimal levels of EPA+DHA in erythrocyte for optimal protection against brain aging and CDS in cats.

Inclusion of elevated levels of B vitamins was further supported by a study indicating that B vitamin supplementation not only reduced blood total homocysteine and slowed down the decline in cognition but also reduced the rate of accelerated brain atrophy in subjects with mild cognitive impairment.[30] Since the B vitamins were contained in many of the food ingredients in both the control and test diets, the levels of all B vitamins in the control diet were higher than the daily requirements for cats. Vitamin premix was added to the BPB diet to further increase the B vitamins. Cats fed the BPB diet had significantly higher levels of blood folic acid, but this did not significantly affect vitamin B12 and homocysteine 200 days after the feeding study began.

Hypertension is a risk factor for both brain aging and AD in people.[15,20] L-arginine is the precursor for the production of nitric oxide. NO is involved in cognition through a variety of pathways, and administration of NO donors in rats protected against the development of cognitive disorders.[58] Peripherally, NO is important in controlling blood pressure; in rats, induced hypertension can be ameliorated by administration of L-arginine.[59] Although blood pressure was not evaluated in the cats in this study, dietary L-arginine supplementation was also able to decrease both systolic and diastolic blood pressure in human subjects with mild hypertension.[60]

Oxidative stress and chronic inflammation are important risk factors for brain aging and dementia.[19,21] The antioxidants and EPA in the blend may reduce oxidative stress-induced damages and low-grade inflammation in the whole body including the brain and cerebrovascular system. Selenium and vitamin E came from the food ingredients and exceeded the daily requirements of adult cats in the control diet; no significant difference in the total blood antioxidant capacity was detected between cats fed the control and test diets.

Although the control diets were not deficient in any nutrients, all the nutrients included in the BPB were present at levels higher in the BPB diet than those in the control diet. For instance, the levels of B vitamins were at least three and half times higher than the daily requirements, and arginine was two times higher than the daily requirement for adult cats in the BPB. Improved cognitive functions in the cats in the BPB group suggest that cats need to consume those nutrients at levels much higher than their daily minimum requirements to protect their brains from aging-induced decline in functions.

In summary, this study confirms that the BPB, containing ingredients with the ability to reduce or eliminate the known risk factors for brain aging and dementia, can significantly enhance brain functions and retard aging-induced decline in brain functions in normal middle-aged and senior cats.

Acknowledgements

The authors wish to thank Wendell Kerr for statistical analysis of the blood chemical data, Drs. Bruce A. Watkins and Yong Li for RBC fatty-acid profile analysis, Ms. Shanon L Byous for the analysis of blood B vitamins and total antioxidant status, and Ms. Rachel Anderson for homocysteine analysis.

References

1. Gunn-Moore DA, Moffat K, Christie LA, et al. Cognitive Dysfunction and the Neurobiology of Aging in Cats. *J Sm An Pract*. 2007;48:546-553.

2. Landsberg GM, Denenberg S, Araujo JA. Cognitive Dysfunction in Cats: A Syndrome We Used to Dismiss as 'Old Age.' *J Feline Med Surg*. 2010;11:837-848.

3. Pan YL. Enhancing Brain Functions in Senior Dogs: A New Nutritional Approach. *Top Comp An Med*. 2011;26:10-16.

4. Head E, Milgram NW, Cotman CW. Neurobiological Models of Aging in the Dog and Other Vertebrate Species. In: *Functional Neurobiology of Aging*. Hof PR, Mobbs CV (eds). Academic Press, San Diego. 2001;457-465.

5. Hof PR, Morrison JH. The Aging Brain: Morphomolecular Senescence of Cortical Circuits. *Trends in Neurosci*. 2004;27: 607-613.

6. Zhang JH, Sampogna S, Morales FR, et al. Age-Related Changes in Cholinergic Neurons in the Laterodorsal and the Pedunculo-Pontine Tegmental Nuclei of Cats: A Combined Light and Electron Microscopic Study. *Brain Res*. 2005;1052: 47-55.

7. Brellou G, Vlemmas I, Lekkas S, et al. Immunohistochemical Investigation of Amyloid Beta-Protein (Abeta) in the Brain of Aged Cats. *Histol Histopathol*. 2005;20:725-731.

8. Head E, Moffat K, Das P, et al. Beta-Amyloid Deposition and Tau Phosphorylation in Clinically Characterized Aged Cats. *Neurobiol Aging*. 2005;26:749-763.

9. Cummings BJ, Pike CJ, Shankle R, et al. Beta-Amyloid Deposition and Other Measures of Neuropathology Predict Cognitive Status in Alzheimer's Disease. *Neurobiol Aging*. 1996;17:921-933.

10. Gunn-Moore DA, McVee J, Bradshaw JM, et al. Aging Changes in Cat Brains Demonstrated by Beta-Amyloid and AT8-Immunoreactive Phosphorylated Tau Deposits. *J Feline Med Surg*. 2006;8:234-242.

11. Milgram NW. Neuropsychological Function and Aging in the Cat. 15th annual Conference on Canine Cognition and Aging. Laguna Beach, CA. 2010.

12. Pugliese M, Gangitano C, Ceccariglia S, et al. Canine Cognitive Dysfunction and the Cerebellum: Acetylcholinesterase Reduction, Neuronal and Glial Changes. *Brain Res*. 2007;1139: 85-94.

13. Siwak-Tapp CT, Head E, Muggenburg BA, et al. Region-Specific Neuron Loss in the Aged Canine Hippocampus Is Reduced by Enrichment. *Neurobiol Aging*. 2008;29:39-50.

14. Cole GM, Ma QL, Frautschy SA. Omega-3 Fatty Acids and Dementia. *Prostaglandins Leukot Essent Fatty Acids*. 2009;81: 213-221.

15. Duron E, Hanon O. Hypertension, Cognitive Decline and Dementia. *Arch Cardiovasc Dis*. 2008;101:181-189.

16. Selhub J, Bagley LC, Miller J, et al. B Vitamins, Homocysteine and Neurocognitive Function in the Elderly. *Am J Clin Nutr*. 2000;71:614S-620S.

17. Jellinger KA, Attems J. Prevalence and Impact of Cerebrovascular Pathology in Alzheimer's Disease and Parkinsonism. *Acta Neurol Scand*. 2006;114:38-46.

18. Miller JW, Green R, Ramos MI, et al. Homocysteine and Cognitive Function in the Sacramento Area: Latino Study on Aging. *Am J Clin Nutr*. 2003;78:441-447.

19. Markesbery WR. Oxidative Stress Hypothesis in Alzheimer's Disease. *Free Radic Biol Med*. 1997;23:134-147.

20. Qiu C, Winblad B, Fratiglioni L. The Age-Dependent Relation of Blood Pressure to Cognitive Function and Dementia. *Lancet Neurol*. 2005;4:487-499.

21. Weninger SC, Yankner BA. Inflammation and Alzheimer's Disease: The Good, the Bad and the Ugly. *Nat Med*. 2001;7: 527-528.

22. Taupin P. A Dual Activity of ROS and Oxidative Stress on Adult Neurogenesis and Alzheimer's Disease. *Cent Nerv Syst Agents Med Chem*. 2010;10:16-21.

23. Pan YL. Cognitive Dysfunction Syndrome in Dogs and Cats. *CAB Rev*. 2013;8:1-10.

24. Ciubotaru I, Lee YS, Wander RC. Dietary Fish Oil Decreases C-Reactive Protein, Interleukin-6, and Triacylglyceride to HDL-Cholesterol Ration in Postmenopausal Women on HRT. *J Nutr Biochem*. 2003;14:513-521.

25. Wall R, Ross RP, Fitzgerald GF, et al. Fatty Acids from Fish: The Anti-Inflammatory Potential of Long-Chain Omega-3 Fatty Acids. *Nutr Rev*. 2010;68:280-289.

26. Dong JY, Qin LQ, Zhang Z, et al. Effect of Oral L-Aarginine Supplementation on Blood Pressure: A Meta-Analysis of Randomized, Double-Blind, Placebo-Controlled Trials. *Am Heart J*. 2011;162:959-965.

27. Yamada K, Noda Y, Nakayama S, et al. Role of Nitric Oxide in Learning and Memory and in Monoamine Metabolism in the Rat Brain. *Br J Pharmacol*. 1995;115:852-858.

28. Bryan J, Calvaresi E, Hughes D. Short-Term Folate, Vitamin B-12, or Vitamin B-6 Supplementation Slightly Affects Memory Performance, But Not Mood in Women of Various Ages. *J Nutr*. 2002;132:1345-1356.

29. Selhub J, Bagley LC, Miller J, et al. B Vitamins, Homocysteine and Neurocognitive Function in the Elderly. *Am J Clin Nutr*. 2000;71:614S-620S.

30. Smith AD, Smith SM, de Jager CA, et al. Homocysteine-Lowering by B Vitamins Slows the Rate of Accelerated Brain Atrophy in Mild Cognitive Impairment: A Randomized Controlled Trial. *PLoS ONE*. 2010;5:e12244.

31. Frank B, Gupta S. A Review of Antioxidants and Alzheimer's Disease. *Ann Clin Psychiatry*. 2005;17:269-286.

32. Loef M, Schrauzer GN, Walach H. Selenium and Alzheimer's Disease: A Systematic Review. *J Alzheimers Dis*. 2011;26:81-104.

33. Lynch SM, Gaziano JM, Frei B. Ascorbic Acid and Atherosclerotic Cardiovascular Disease. *Subcell Biochem*. 1996;25: 331-367.

34. Peluzio Mdo C, Teixeira TF, Oliveira VP, et al. Grape Extract and αTocopherol Effect in Cardiovascular Disease Model of Apo E -/- Mice. *Acta Cir Bras*. 2011;26:253-260.

35. Pocernich CB, Lange ML, Sultana R, et al. Nutritional Approaches to Modulate Oxidative Stress in Alzheimer's Disease. *Curr Alz Res*. 2011;8:452-469.

36. Schwenke DC, Behr SR. Vitamin E Combined with Selenium Inhibits Atherosclerosis in Hypercholesterolemic Rabbits Independently of Effects on Plasma Cholesterol Concentrations. *Circ Res*. 1998;83:366-377.

37. Milgram NW, Adams B, Callahan H, et al. Landmark Discrimination Learning in the Dog. *Learning and Mem*. 1999;6:54-61.

38. Milgram NW, Head E, Muggenburg B, et al. Landmark Discrimination Learning in the Dog: Effects of Age, an Antioxidant Fortified Diet and Cognitive Strategy. *Neurosci Biobehav Rev*. 2002;26:679-695.

39. Christie LA, Studzinski C, Araujo J, et al. A Comparison of Egocentric and Allocentric Age-Dependent Spatial Learning in the Beagle Dog. *Prog Neuro-Psychopharmacol Biol Psychiatry*. 2005;29:361-369.

40. Head E, Callahan H, Cummings BJ, et al. Visual-Discrimination Learning Ability and ß-Amyloid Accumulation in the Dog. *Neurobiol Aging*. 1998;19:415-425.

41. Tapp PD, Siwak CT, Estrada J, et al. Size and Reversal Learning in the Beagle Dog as a Measure of Executive Function and Inhibitory Control in Aging. *Learning Mem*. 2003;10:64-73.

42. Pan YL, Araujo JA, Burrows J, de Rivera C, et al. Cognitive Enhancement in Middle-Aged and Old Cats with Dietary Supplementation with a Nutrient Blend Containing Fish Oil, B Vitamins, Antioxidants and Arginine. *Brit J Nutr*. 2013;110:40-49.

43. Milgram NW, Head E, Weiner E, et al. Cognitive Functions and Aging in the Dog: Acquisition of Nonspatial Visual Tasks. *Behav Neurosci*. 1994;108:57-68.

44. Head E, Mehta R, Hartley J, et al. Spatial Learning and Memory as a Function of Age in the Dog. *Behav Neurosci*. 1995;109:851-858.

45. Watkins BA, Allen KG, Hoffmann WE, et al. Dietary Ratio of (n-6)/(n-3) Polyunsaturated Fatty Acids Alters the Fatty Acid Composition of Bone Compartments and Biomarkers of Bone Formation inRrats. *J Nutr*. 2000;130:2274-2284.

46. Feart C, Samieri C, Barberger-Gateau P. Mediterranean Diet and Cognitive Function in Older Adults. *Curr Opin Clin Nutr Metab Care*. 2010;13:14-18.

47. Behne D, Wolters W. Distribution of Selenium and Glutathione Peroxidase in the Rat. *J Nutr*. 1983:113:456-461.

48. Edwin EE, Diplock AT, Bunyan J, et al. The Distribution of Vitamin E in the Rat and the Effect of Alpha-Tocopherol and Dietary Selenium on Ubiquinone and Ubicheromenol in Tissues. *Biochem J*. 1961:79:91-105.

49. Lombardi-Boccia G, Lanzi S, Aguzzi A. Aspects of Meat Quality: Trace Elements and B Vitamins in Raw and Cooked meats. *J Food Comp Anal*. 2005;18: 39-46.

50. Musulin RR, Tully 3rd RH, Longenecker HE, et al. Vitamin C Synthesis and Excretion by the Rat. *J Biol Chem*. 1939:129:437-444.

51. Zotte AD, Szendr Z. The Role of Rabbit Meat as Functional Food. *Meat Sci*. 2011;88:319–331.

52. Conquer JA, Tierney MC, Zecevic J, et al. Fatty Acid Analysis of Blood Plasma of Patients with Alzheimer's Disease, Other Types of Dementia, and Cognitive Impairment. *Lipids*. 2000;35:1305-1312.

53. Heude B, Ducimetière P, Berr C. Cognitive Decline and Fatty Acid Composition of Erythrocyte Membranes — The EVA Study. *Am J Clin Nutr*. 2003;77:803-808.

54. Yurko-Mauro K, McCarthy D, Rom D, et al. Beneficial Effects of Docosahexaenoic Acid on Cognition in Age-Related Cognitive Decline. *Alz Dement*. 2010;6:456-464.

55. Jiang LH, Shi Y, Wang LS, et al. The Influence of Orally Administered Docosahexaenoic Acid on Cognitive Ability in Aged Mice. *J Nutr Biochem*. 2009;20:735-741.

56. Harris WS. The Omega-3 Index as a Risk Factor for Coronary Heart Disease. *Am J Clin Nutr*. 2008;87:1997S-2002S.

57. De Jager CA, Oulhaj A, Jacoby R, et al. Cognitive and Clinical Outcome of Homocysteine-Lowering B-Vitamin Treatment in Mild Cognitive Impairment: A Randomized Controlled Trial. *Int J Geriatr Psychiatr*. 2011. doi:10.1002/gps.2758.

58. Manukhina EB, Pshennikova MG, Goryacheva AV, et al. Role of Nitric Oxide in Prevention of Cognitive Disorders in Neurodegenerative Brain Injuries in Rats. *Bull Exp Biol Med*. 2008;146:391-395.

59. Rajapakse NW, De Miguel C, Das S, et al. Exogenous L-Arginine Ameliorates Angiotensin II-Induced Hypertension and Renal Damage in Rats. *Hypertension*. 2008;52:1084-1090.

60. Ast J, Jablecka A, Bogdanski P, et al. Evaluation of the Antihypertensive Effect of L-Arginine Supplementation in Patients with Mild Hypertension Assessed with Ambulatory Blood Pressure Monitoring. *Med Sci Monit*. 2010;16:CR266-CR271.

61. Association of American Feed Control Officials. Official Publication. 2011.