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The oil-rich alga *Schizochytrium sp*. as a dietary source of docosahexaenoic acid improves shape discrimination learning associated with visual processing in a canine model of senescence

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ABSTRACT

Whole cell *Schizochytrium sp*. is a rich source of omega-3 long-chain polyunsaturated fatty acids (n-3 LCPUFA) including docosahexaenoic acid (DHA), an important nutrient for brain health. Aged beagle dogs experienced on a visuospatial task of working memory, variable-delay delayed-non-matching-to-position were used to assess efficacy of DHA-rich microalgae based upon DHA wt% of total phospholipids and 8-iso-$PGF_{2\alpha}$ concentrations in plasma, and performance on cognitive assessments of visual object discrimination, learning, and memory consolidation after 25 weeks on fortified diet.

Improved DHA status ($p < 0.001$) and initial learning of the protocols for visual and variable contrast discrimination ($p < 0.05$), but not long-term recall of the concurrent discrimination task were observed in animals fed the algal-fortified diet. Overall, results were consistent with dried *Schizochytrium sp*. as a source of n-3 LCPUFA nutrition to support DHA status in large mammals, and healthy brain function in a canine model of senescence.

1. Introduction

Industrial monoculture of microbes, including marine algae, has been recognized for more than four decades as an important means of large-scale production of biomolecules such as, antibiotics, vitamins and other nutrients. Marine algae, is a rich source of omega-3 (n−3) long-chain polyunsaturated fatty acids (LCPUFAs), DHA (22:6n−3) and eicosapentaenoic acid (EPA, 20:5n−3). The composition of dried whole cell *Schizochytrium sp*. in particular includes approximately 20% DHA, on a per weight basis, making it a rich source of this nutritionally important n-3 LCPUFA. Extraction and refinement from whole cell algae result in oils in which DHA comprises greater than 50% of the total fatty acid for use as a concentrated source of dietary n-3.

DHA is the major n-3 LCPUFA in brain and comprises 30–40% of the aminophospholipid fatty acids in neuronal cell membranes [1,2]. As an integral component of neural cell membranes, DHA is involved in numerous processes including membrane order, receptor activation, and signal transduction [3]. There has also been considerable speculation that suboptimal DHA concentrations may contribute to cognitive dysfunction and dementia over the course of aging, in some individuals [4]. Epidemiological investigations associate low DHA status in plasma with age-related cognitive decline in aged healthy and in individuals with dementia [5–8]. Some investigations have indicated that long-term supplementation with algal DHA supports learning and memory in healthy elderly individuals with age-related mild cognitive decline [9]. Other investigations provide evidence of a positive relationship between supplementation with adequate amounts of n-3, increased status of DHA in plasma, and cognitive performance in aged subjects with subjective memory complaints [10].

The canine model of brain aging has emerged as an important tool for the study of age-related changes in cognitive functions in humans. Visual processing is among the earliest functions for which decrements in cognitive performance attain measureable limits during aging in both humans and dogs [11–14]. Gradual impairments in visuospatial learning and memory involving object recognition and discrimination are also common features of aging, in both humans and dogs [13,15]. To date, there are no published investigations of the relationship between the status of DHA during senescence and cognitive function in large non-human mammals. Dried whole cell biomass may provide an important alternate source of dietary n−3s for use in applications which do not require a highly refined source of DHA or EPA.

The objective of this study was therefore to assess whole cell algal biomass as a source of DHA based upon changes in the concentrations

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in plasma of n-3 LCPUFAs and 8-isoPGF$_{2\alpha}$. Evidence was also investigated for possible interactions between fortification with whole cell algae and performance on various cognitive tasks, using a well characterized canine model of aging [11,15–18].

2. Materials and methods

2.1. Animals and diets

Twenty-six colony beagles (CanCog Technologies, Toronto, Canada) ranging in age from 8.6 to 11.1 years (mean=9.8 years, SD=0.8, 10 females) were utilized in the study. The research protocol was approved by the CanCog Technologies Animal Care Committee, and in accordance with the guidelines of the Ontario Ministry of Agriculture. The animals were group housed at the CanCog site throughout the trial. Each animal used for the investigation was visually examined daily by a trained veterinary and animal research staff. All dogs had similar exposure to cognitive testing, as previously described [19]. Each animal underwent examination by a veterinarian to ensure that auditory, motor, and visual functioning was normal. Housing temperature was maintained at 21±6 °C and relative humidity levels ranged between 15% and 75%. Animals had free access to fresh water and approximately 1 h, to consume their food. Animals were acclimated for a period of 42 days to a commercially available, standard extruded dry diet which lacked the n-3 LCPUFAs (PUFAs) docosahexaenoic acid (DHA, 22:6n–3) or eicosapentaenoic acid (EPA, 20:5n–3). Metabolizable energy and nutrient composition, on an as fed basis, of the control diet were ~1.47×10^4 kJ/kg and 26%, 12%, 4% and 10% of protein, fat, fiber, and moisture, respectively. Total fat and protein content of dried *Schizochytrium sp*. (DSM Nutritional Lipids, Columbia, MD) were 45.3 and 12 wt%, respectively (Tables 1 and 2). The dehydrated single-cell algal (DHA) biomass comprised 0.4% of the experimental diet. Minor adjustments to the amounts of tallow and corn were made to the control diet, in order to maintain the same energy contents between the control and the test diets. The final content of DHA in the test diet, as fed, was 1 mg/g. Body condition scores were monitored on a weekly basis throughout the investigation. The amount of diet fed daily to each animal was 26 g/kg of body weight. Dietary amounts were adjusted weekly in increments of 50 g, as needed in order to maintain body condition score approximating 3 on the 5-point scale.

2.2. Group assignments

Twenty six dogs, which had been previously trained on the variable-delay delayed-non-matching-to-position (DNMP) task [15], were selected at baseline in order to identify 24 for use in experimental assessments. DNMP score was used to rank visuospatial performance for each of the animals at baseline (Fig. 1). To do so, the dog making the fewest incorrect responses was ranked as 1 and the dog making the most incorrect responses was ranked as 26. Two subjects were dropped as they showed the most frequency response failures. The remaining animals were assigned based upon descending rank to one of two groups (e.g. 1,2,2,1, etc.). The two cognitively equivalent groups (n=12/group) were then randomly assigned by lot drawing to the base diet (control) or to the DHA-fortified diet for the duration of the investigation.

The study was blinded to all personnel in the investigation with the exceptions of the person(s) involved with administering the investigational and control diets and the person responsible for performing allocation. Collection of data from the study by these individuals was limited to food consumption. The code assignments and other records and document that would reveal treatments to people collecting data remained archived in a secured room at the test facility until all data were collected and analyzed.

The treatment phase started on the day following group placement. The first 51 days provided the initial wash-in and no other procedures were introduced during this time. Over the next 124 days, the groups were tested on a battery of cognitive tests, including concurrent discrimination learning (days 51–90), contrast sensitivity learning (days 100–140), retest on DNMP (days 145-154), performance on variable contrast protocol (days 157–164) and retention of concurrent discrimination learning task (days 167–174).

Table 1
Nutritional profile analysis for diets used to evaluate cognitive behavior in 24 aged beagle dogs (all values are stated on an on as-fed basis).

Nutrient	Diet	
	Control	Fortified
Moisture (%)	5.9	7.4
Crude protein (%)	28.8	29.1
Fat (%)	11.8	11.8
Fiber (%)	1.9	2.2
Ash (%)	9.5	9.5
Dried whole cell algae (%)	0.0	0.4

Table 2
Nutritional profile, fatty acid, and vitamin composition of dried whole cell algae.

Basic components	Per 100 g
Moisture	2.5
Protein	12.1
Carbohydrates	32.0
Ash	8.2
Fiber	0.6
Crude fat	45.3
Fatty acid	
12:0 (Lauric)	0.2
14:0 (Myristic)	5.1
16:0 (Palmitic)	12.1
18:0 (Stearic)	0.02
18:3n6 (γ-linolenic)	0.1
20:3n6 (DHGLA)	0.2
20:4n6(Arachidonic)	0.2
20:5n3 (Eicosapentaenoic)	0.5
22:5n6 (DPAn6)	**6.3**
22:6n3 (DHA)	**18.0**
24:0 (Lignoceric)	**0.1**
Vitamins	
Biotin	0.3 mg
Choline	1440 mg
Folic Acid	0.1 mg
Niacin	14 mg
Vitamin A	33.6 μg
Beta Carotene	2.3 μg
Vitamin B_1	4.4 mg
Vitamin B_2	2.9 mg
Vitamin B_6	1.4 mg
Vitamin B_{12}	54.9 μg
Vitamin C	0
Vitamin E	0.45 μg
Pantothenic Acid	3.5 mg

2.3. Blood collection

Whole blood was collected on days −42, 1 and 175 and placed into EDTA tubes for separation into plasma and red blood cell. Plasma was prepared for the purpose of measuring 8-iso-PGF$_{2\alpha}$ by centrifugation at 1300*g* for 25 min to remove platelets. Samples were stored at −80 °C until analysis.