The effect of salmon oil freshness on the palatability of dog foods

Het effect van de versheid van zalmolie op de smakelijkheid van hondenvoeders

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ABSTRACT

The effect of the feeding history of dogs and of the addition of different fat sources (chicken lard , rapid harvested salmon oil and non-rapid harvested salmon oil) to their diet on the palatability of dog foods was investigated. Three diets were tested in twelve healthy adult Beagle dogs using a modified two-pan preference test: a basal diet with chicken lard (CL) and two diets with 1% salmon oil, one being with rapid-harvest salmon oil (RS) and the other with non-rapid-harvest salmon oil (NRS). Substitution of 1% chicken lard by 1% salmon oil decreased the (n-6):(n-3) fatty acid ratio from 9 to 5. The oxidative status in both oils and foods was rather low, but the difference between the two salmon oils was prominent. Absolute food intake did not differ significantly among the diets, most likely due to numeric differences in absolute food intake between test periods. Relative intake showed a significant difference between CL (38.5 % of total food intake) and NRS (28.1 %), whereas no difference was noted between RS (33.4 %) and NRS and between CL and RS. The preceding diet had no effect on diet preference.

SAMENVATTING

Het effect van de diëtaire voorgeschiedenis van honden en het effect van de toevoeging van verschillende vetbronnen (kippenvet, snel gewonnen zalmolie en minder snel gewonnen zalmolie) aan hun voeder op de smakelijkheid van hondenvoeders werden onderzocht.

Drie voeders werden getest op smakelijkheid bij twaalf gezonde volwassen Beagles, gebruikmakend van een gewijzigde "two-pan" smakelijkheidstest: een basisvoeder met kippenvet (CL) en twee voeders met 1% zalmolie, hetzij met snel gewonnen zalmolie (RS) of met minder snel gewonnen zalmolie (NRS). De vervanging van 1% kippenvet door 1% zalmolie verminderde de (n-6):(n-3) vetzuurratio van 9 naar 5. De oxidatieve status van zowel de olies als de voeders was nogal laag, maar het verschil tussen de twee zalmolies was opvallend. De absolute opname van de voeders was niet significant verschillend, hoogstwaarschijnlijk wegens numerieke verschillen in absolute voederopname tussen de testperioden. De relatieve voederopname daarentegen toonde een significant verschil tussen CL (38,5% van de totale voederopname) en NRS (28,1%). Tussen RS (33,4%) en NRS, alsook tussen CL en RS werd geen verschil waargenomen. Het voordien verstrekte voeder bleek geen duidelijk effect te hebben op de opname van het geteste voeder.

INTRODUCTION

The potential benefits of dietary supplementation with long-chain n-3 fatty acids (FA), which are found in large concentrations in fish oil, have aroused great interest. Some of the benefits attributable to n-3 FA are due to their effect on the immune system through their regulation of the eicosanoid production.

Polyunsaturated long-chain fatty acids (PUFAs) are the major components of the cell membrane-associated phospholipids. Changes in the FA composition of the diet are known to alter the proportion of FA in the membrane phospholipids. PUFAs are important regulators of numerous cellular functions, including those related to inflammation and immunity. Arachidonic acid (C20:4n-6; AA), eicosapentaenoic acid (C20:5n-3; EPA) and dihomo-gamma-linolenic acid (C20:3n-6; DGLA) are the major precursors for eicosanoid production. In general, the EPA-and DGLA-derived eicosanoids are much less potent inducers of inflammation than the AA-derived eicosanoids (Ackerman, 1995a; Shapiro *et al.*, 1993; Wander *et al.*, 1997). Consequently, n-6 FA will result in a pro-inflammatory status with production of prostaglandins of the

2-series and leukotrienes of the 4-series. Supplementation with n-3 FA increases the relative amount of EPA and promotes the generation of prostaglandins of the 3-series and leukotrienes of the 5-series, while decreasing the generation of eicosanoids from AA. Prostaglandins of the 3-series and leukotrienes of the 5-series are considered to be less inflammatory (Shapiro *et al.*, 1993; Wander *et al.*, 1997).

Hence, n-3 FA are used in the management of several disorders in dogs, including canine atopy (Ackerman, 1995b; Mueller *et al.*, 2004), chronic renal failure (Brown *et al.*, 1996), inflammatory bowel disease (Zoran, 2003), cancer (Ogilvie *et al.*, 2000), arthrosis/arthritis (Bierer and Bui, 2002) and heart disease (Freeman *et al.*, 1998).

The present trial deals with the palatability of fish oil, the most important source of n-3 FA (EPA and docosahexaenoic acid (C22:6n-3; DHA)) (Ackerman, 1995a). Palatability is a measure of the dog's subjective food preference and is defined by the sensory reaction to specific chemical and physical properties of the food: odor, taste and texture (Araujo and Milgram, 2004). It is known that dogs prefer ingredients of animal origin. Hence, animal fat is generally considered as a palatable ingredient for dogs. Nevertheless, chemical reactions in the food, mainly in fat, may deteriorate the palatability of the food. Unsaturated lipids are highly susceptible to the deteriorative reactions of oxidative free-radical chemistry, particularly during storage. As a result of the free-radical driven formation and breakdown of hydroperoxides, organic substances including short chain aldehydes, ketones and alcohols are released. These volatiles are sensed as off-flavors and lead to quality losses and finally to rejection due to the perceived rancidity of the fat or food (German, 1999).

The present trial investigated the effect of salmon oil freshness on dog food palatability when compared to a dog food with chicken lard as a fat source, and it also went into the effect of preceding diets on the measured palatability of a test diet.

Table 1. Composition of the diets (% on dry matter basis).

Nutrient CL diet RS diet NRS diet 91.1 Dry matter 90.8 90.8 22.3 22.9 Crude protein 22.6 Ether extract 11.5 11.7 11.6 Crude fiber 3.2 3.4 3.3 Crude ash 7.3 7.3 7.5 Nitrogen-free extract 46.2 46.4 45.5 Starch 19.2 19.5 19.8 2.4 2.5 2.4 Sugars 14.5 Metabolizable energy (MJ/kg)* 14.5 14.4

CL diet: dog food with chicken lard; RS diet: dog food with rapid-harvest salmon oil; NRS diet: dog food with non-rapid-harvest salmon oil.

Ingredients: cereal and vegetal byproducts, hydrolyzed animal protein and animal byproducts, vitamin- and mineral premix and fish oil (for RS and NRS diet). Antioxidant: CL diet:150 ppm butylhydroxytoluene; 11 ppm propyl gallate; RS diet: 150 ppm butylhydroxytoluene; 12 ppm propyl gallate; NRS diet: 150 ppm butylhydroxytoluene; 12 ppm propyl gallate.

MATERIAL AND METHODS

Animals and housing

Twelve healthy adult beagle dogs, five females and seven males, with a mean body weight of 11.04 kg and between 3 and 9.5 years of age, were included in the palatability trial. The dogs were housed individually in indoor kennels during feeding time. After feeding, the dogs were put two by two in a kennel with an outdoor part for the rest of the day.

The 12 dogs were selected from a group of 17 beagle dogs before the start of the test on the basis of a bowl preference test and a palatability differentiation test. In the bowl preference test, the dogs were tested for the absence of bowl preference. One diet was divided into two bowls. Dogs that had significantly higher feed intake from one bowl in comparison to the other were not selected for the palatability trial. In the palatability differentiation test, the dog's palatability was examined. Two diets, one of which was known to be more palatable (Hill's Science Plan Puppy Dry and Puppy Mini Dry) than the other (Hill's Prescription Diet Canine W/D Dry), were tested. Only the dogs choosing the more palatable diet were included in the palatability trial.

Diets and feeding

A commercial extruded dry dog food (Table 1) was used in this trial. Fish products were absent in the basal diet. During the production of the diet, 1% of animal fat (chicken lard) was exchanged for 1% of fish oil. All other ingredients remained the same and the ingredient ratios did not change. This way, three different diets were produced: the basal diet without fish oil as control diet (CL, chicken lard as fat supplement) and two diets with 1% salmon oil, one with rapid-harvest salmon oil (RS; very fresh Norwegian salmon oil according to European Pharmacopoeia Commission 01/2005:1910; Branded pro-

^{*}Calculated: 0.15 x %Crude protein + 0.36 x %Ether extract + 0.15 x % Nitrogen-free extract

Table 2. Latin square adaptation period.

Dog	Adaptation period 1	Adaptation period 2	Adaptation period 3
1-2	CL	RS	NRS
3-4	CL	NRS	RS
5-6	RS	NRS	CL
7-8	RS	CL	NRS
9-10	NRS	RS	CL
11-12	NRS	CL	RS

CL: dog food with chicken lard; RS: dog food with rapid-harvest salmon oil; NRS: dog food with non-rapid-harvest salmon oil.

duct tested; Xalar Virgin Salmon Oil, produced by Marine Harvest Ingredients) and one with non-rapid harvest salmon oil (NRS). The RS oil is harvested within two hours after catching the salmon and is rapidly stored under optimal conditions. The different fat sources were not top dressed or coated on the dry nibble, but rather mixed with the other ingredients during the production of the diets.

Experimental design

To test the CL, RS and NRS diets for their palatability, a variation on the traditional two-pan preference test was used (Sunday et al., 1983; Griffin et al., 1984; Rashotte et al., 1984). During the testing period, the three diets were fed simultaneously in three separated food bowls. On a daily basis, the position of the food bowls was changed randomly. The dogs were offered each food at an amount higher (650 kJ/kg^{0.75} for mean body weight, 11.04 kg) than their maintenance energy requirement (480 kJ/kg^{0.75}) (Debrackeleer et al., 2000). The food was available to the dogs for a maximum of 15 minutes. If one bowl was empty before that time, all three bowls were removed. Water was available ad libitum. Fifteen minutes after feeding, the food bowls were collected; the leftovers were weighed and recorded daily. The dogs were fed the diets for three consecutive days (testing period) and the data were collected from the first day on. Because a diet's palatability may be influenced by the preceding diet, the three test diets were given as an adaptation diet before the start of the testing periods. Hence, the adaptation diet was tested in a 3×3 Latin square (Table 2). For each dog, the three diets were tested for their palatability 3 times, but each time another adaptation diet preceded the test. The adaptation periods took 5 days (Figure 1). During these adaptation periods the dogs also had access to 3 food bowls, but only one was filled with food – in a random way and differently from the day before. During the adaptation periods the dogs were fed at maintenance energy requirements (415 kJ/kg^{0.75} for dogs > 7 years and 480 kJ/kg^{0.75} for dogs between 2 and 7 years) (Debraekeleer et al., 2000).

To acclimatize the dogs to the time-limited feeding, the dogs were fed accordingly three days before the start of the trial (acclimatization period). During these three days they also had access to three different food bowls, each filled with their usual food for a period of 15 minutes.

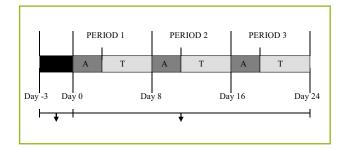


Figure 1. Time schedule.

Statistical analysis

The data (absolute and relative food intake) were analyzed by a repeated measures analysis in which the adaptation diets (CL, RS and NRS) were the repeated measure (within subject variable) and the test diets were the between subject factors. A mean value of each three test days was used for statistical analysis. Statistical significance was accepted at P < 0.05. The Tukey test was used as a post hoc test.

RESULTS

The analyzed FA composition of the salmon oils and the foods is shown in Tables 3 and 4. The FA analysis was performed at the Laboratory of Animal Nutrition and Product Quality (Melle, Belgium) as described by Raes *et al.* (2001). The two salmon oils had a similar but not identical fatty acid pattern, meaning high values in the long-chain n-3 FA, EPA and DHA. When included in the dog foods, these salmon oils reduced the (n-6):(n-3) ratio from 9 to 5 in comparison with the dog food containing chicken lard. Nevertheless, the PUFA content of the three dog foods was similar, being around 2 g PUFA / 100 g matter.

To evaluate the oxidative status of the salmon oils and the foods, oil and food samples were taken simultaneously at the start of the trial. Both the peroxide value (POV) (Table 5) and the amount of free fatty acids (FFA) (Table 3 and 4) were measured. A prominent difference in oxidative status was seen between the two salmon oils, whereas the differences between the three test diets were only moderate.

Table 3. Fatty acid profile of the salmon oils.

	RS	oil	NRS oil		
	mgFA/100 g matter	mg FFA/100 g FA	mgFA/100 g matter	mg FFA/100 g FA	
SFA	18933	34.4	14111	24.4	
MUFA	39905	26.8	37577	31.9	
C18:2n-6	5267		7490		
C18:3n-6	213		237		
C20:2n-6	359		588		
C20:3n-6	156		219		
C20:4n-6	628		577		
C22:4n-6	250		400		
n-6 PUFA	6873	6.61	9511	11.3	
C18:3n-3	1805		3256		
C18:4n-3	1761		757		
C20:5n-3	6293		4972		
C22:5n-3	3052		2625		
C22:6n-3	9637		6146		
n-3 PUFA	22549	9.01	17756	13.4	
n-6:n-3	0.3		0.5		

RS oil: rapid-harvest salmon oil; NRS oil: non-rapid-harvest salmon oil; FA: fatty acids; FFA: free fatty acids; SFA: saturated long-chain fatty acids; MUFA: monounsaturated long-chain fatty acids; PUFA: polyunsaturated long-chain fatty acids.

Table 4. Fatty acid profile of the experimental dog foods.

	CL diet		R	S diet	NRS diet	
	mgFA/100 g matter	mg FFA/100g FA	mgFA/100 g matter	mg FFA/100 g FA	mgFA/100 g matter	mg FFA/100 g FA
SFA	2908	40.4	2524	32.5	2744	35.7
MUFA	4103	33.7	3599	39.2	3808	35.7
C18:2n-6	1712		1466		1549	
50053 C18:3n-6	ND		3.60		3.13	
C20:2n-6	37.2		42.4		39.6	
C20:3n-6	8.42		8.44		8.73	
C20:4n-6	38.7		37.2		43.6	
C22:4n-6	11.6		7.50		9.06	
n-6 PUFA	1808	22.0	1565	22.3	1653	30.2
C18:3n-3	164		157		142	
C18:4n-3	5.58		8.18		7.92	
C20:5n-3	3.09		48.2		58.5	
C22:5n-3	11.7		34.2		36.0	
C22:6n-3	6.33		63.5		88.8	
n-3 PUFA	191	2.25	311	2.36	333	2.90
n-6:n-3	9.5		5.0		5.0	

CL diet: dog food with chicken lard; RS diet: dog food with rapid-harvest salmon oil; NRS diet: dog food with non-rapid-harvest salmon oil; FA: fatty acids; FFA: free fatty acids; SFA: saturated long-chain fatty acids; MUFA: monounsaturated long-chain fatty acids; PUFA: polyunsaturated long-chain fatty acids.

Table 5. Oxidative status of the experimental dog foods and salmon oils.

	Peroxide value (POV) Meq O ₂ /kg fat		
CL diet	3.65		
RS diet	3.68		
NRS diet	4.06		
RS oil	1.97		
NRS oil	4.1		

CL diet: dog food with chicken lard; RS diet: dog food with rapid-harvest salmon oil; NRS diet: dog food with non-rapid-harvest salmon oil; RS oil: rapid-harvest salmon oil; NRS oil: non-rapid-harvest salmon oil.

During the trial no adverse effects were noted and none of the dogs refused to eat any of the diets. The absolute food intake did not significantly differ among the three diets, due to numeric differences in absolute food intake between testing periods. In terms of relative intake, a significant difference between the CL diet and the NRS diet was shown. No significant difference was noted between the RS diet and the NRS diet, nor between the CL and the RS diet (Table 6). Although the effect did not reach the level of significance, it was notable that when the dogs were adapted to the NRS diet, then the differences were less pronounced because of a greater acceptance of NRS. Nevertheless, clear effects of the adaptation diet on diet preference were not demonstrated.

DISCUSSION

The traditional two-pan preference test, which is the most common method of assessing palatability in dogs,

involves providing two food bowls, each of which contains a separate food, to a dog and calculating the amount of each food consumed. The food that the dog ingests in higher quantity is considered to be the preferred food (Griffin *et al.*, 1984; Araujo *et al.*, 2004). This approach allows palatability to be determined rapidly, but does not control for satiety effects or food interactions. The two-pan test may not be useful for long-term palatability trials because the nutritional or caloric characteristics of the foods may interfere with the results. As dogs do not self-limit their food intake, large quantities of foods consumed may be detrimental to the health of these dogs. Furthermore, the palatability of one food can affect the preference for another food.

A novel cognitive palatability assessment protocol (CPAP) for dogs was determined, based on a discrimination-learning procedure in which the dogs are provided simultaneously with three objects and are allowed to respond to only one. One of the objects is associated with no reward, whereas each of the other two objects is associated with a particular food. Dogs are able to associate a particular object with a preferred food. This way, the CPAP can be used to test palatability and to establish food preferences in dogs. This approach provides an objective measure of food preference using a limited number of animals, while controlling for other factors influencing feeding, such as satiety (Araujo and Milgram, 2004; Araujo et al., 2004). In the present study the novel CPAP was not used because of the intensive training protocol. The training and learning component may result in an increase in the amount of time needed to obtain results (Araujo et al., 2004).

Instead, the traditional two-pan test was modified to control satiety effects and food interactions. At first, the dogs only had 15 minutes feeding time. This way their food intake was limited and satiety effects were mini-

Table 6. Results: Absolute and relative intake of the diets during the testing period (chicken lard (CLt), rapid-harvest salmon oil (RSt) and non-rapid-harvest salmon oil (NRSt)) in relation to the adaptation diets (chicken lard (CLa), rapid-harvest salmon oil (RSa) and non-rapid-harvest salmon oil (NRSa)).

			Testing per	Testing period		
			CLt	RSt	NRSt	n
Absolute food intake, g/d*	uo	CLa	130±18	132±15	117±18	4
	tati d	RSa	152±17	125±10	83±14	4
	Adaptation period	NRSa	121±15	106±17	122±22	4
		MEAN	134±10	121±8	107±11	12
Relative food intake, % of total intake*	_ =	CLa	35.9±5.5	33.3±2.9	30.7±4.7	4
	rtio_	RSa	42.5±3.9	36.9 ± 4.1	20.6±3.6	4
	Adaptation period	NRSa	37.0±5.6	30.0±4.5	33.0±6.0	4
		MEAN	38.5°±2.9	$33.4^{ab}\pm2.2$	28.1 ^b ±2.9	12

^{*}Mean value \pm SE; Mean values with unlike superscripts are significantly different (p<0.05).

Absolute food intake: P=0.710 for adaptation diet effect; P=0.276 for test diet effect and P=0.127 for the interaction between adaptation diet and test diet.

Relative food intake: P=1.000 for adaptation diet effect (dependent); P=0.049 for test diet effect and P=0.170 for the interaction between adaptation diet and test diet.

mized. In a later phase, the three test diets were also tested as adaptation diet, to measure the food interactions. The palatability of the test diets was tested three times, but each time another adaptation diet preceded the test. However, in this study the adaptation diets did not significantly influence the test results, though it was notable that when the dogs were adapted to the NRS diet, then the differences in palatability were less pronounced. The same was noted for the CL diet, but only for the absolute food intake and not for the relative food intake. Future tests might need to go more into this phenomenon, as it is of practical importance.

The oxidative status of the salmon oils and foods was evaluated by the measurement of POV, which reflects the presence of lipid peroxides, which are intermediate products of the lipid oxidation. It has been demonstrated that EPA and DHA are more susceptible to lipid oxidation than other FA under identical conditions, because they have a relatively high number of double bounds and readily form hydroperoxides. Since fish oils contain higher quantities of n-3 FA, they are more vulnerable to oxidative degradation than vegetable oils and other animal fats (Turner et al., 2006). In the present trial, the NRS diet had a higher POV compared to the CL diet. Since the NRS diet was also shown to be significantly less palatable than the CL, the oxidative status of dog foods might at least partly determine their palatability. Nevertheless, the POVs of the three diets were rather low. The use of chicken lard instead of other animal fat sources might explain the absence of prominent differences in oxidative status between the three diets, because chicken lard is rather unsaturated when compared to other animal fat sources (De Meyer et al., 1998). The similar PUFA content of the three dog foods might also be caused by the use of chicken lard, but the relative low supplementation of salmon oil might be a second possible cause. After all, it has to be mentioned that POV does not necessarily predict the extent of lipid oxidation. The present study, also showed a prominent difference in oxidative status between the two salmon oils. The rapid harvesting of salmon oil may limit the presence of lipid oxidation and thus maintain the sensory quality of the food when the salmon oil is added to it.

Fritsche and Johnston (1988) showed that purified diets containing fish oil without added antioxidant had increased POV followed by a marked reduction in POV within 7 days of testing.

CONCLUSION

The results of the present study demonstrated that the addition of NRS oil made the basal diet less palatable than the CL diet. This might be due to the higher peroxide value. It was also notable, but did not reach the level of significance, that when the dogs were adapted to the NRS diet, the differences were less pronounced. The RS diet was not significantly less palatable than the CL diet, and not significantly better than the NRS diet. The adaptation periods did not influence the results.

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Uit het verleden

DE VOORLOPER VAN DE IDENTIFICATIECHIP: MERKEN VAN 'EDELE' JACHTHONDEN (1502)



Tijdens het ancien régime was de jacht het exclusieve voorrecht van de adel en enkele andere groepen (tot in de 16^{de} eeuw de Gentse beenhouwers). De hedendaagse jacht en wat er mee samenhangt dragen er hier en daar nog de sporen van. Voor boeren en andere niet-geprivilegieerden was het streng verboden jachthonden te houden. Wel mochten ze hun huizen, huisdieren en velden verdedigen met bastaarden die aangeduid werden als 'rekels' (mâtins). Ook de herdershonden en de veehonden behoorden er toe. Deze 'onedele' werden enkel onderverdeeld naar hun functie. Zo onderscheidde men ook nog trekhonden en slagershonden.

Om te voorkomen dat rekels toch zouden jagen, moest men deze honden een stok om de hals hangen. Hieraan werd uiterst streng de hand gehouden. Een uitvoerig regeringsbesluit over de jacht (1613) van de aartshertogen Albrecht en Isabella omvat naast bepalingen over jachthonden ook allerlei reglementen over het houden van alle mogelijke andere honden. Deze mochten immers onder geen beding de edele jacht concurrentie aandoen. Artikel 48 stelt dat dienaren van de adel of anderen wel 'edele' honden mochten kweken en verzorgen voor hun *seigneurs ou maîtres* maar deze moesten steeds goed herkenbaar het (brand)merk van deze meesters dragen.

Dit gebruik was al veel ouder. De afbeelding toont een bladzijde uit een document met dergelijke merken in het Rijksarchief Brugge (Fonds Boesinghe nr. 424A). Het draagt als hoofding: *Dit sijn marcken van den hon-*

den van sommige prochien ende heerlichheden bij rapporten overgegeven inde Camere van reckenijng(en) ten jaere 1502 ende andere. Men herkent onder andere het merk van Gent en van de Gentse beenhouwers.

De tekst van het besluit (Placard sur la chasse) van 1613 is te vinden in Brants, V., *Recueil des ordonnances des Pays-Bas. Règne d'Albert et Isabelle (1597 – 1621).* Vol. 2, Goemaere, Brussel, 1912. Noteren we nog even dat art. 114 stipuleert dat universiteitsstudenten tijdens hun studie niet mochten jagen of stropen.