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Association between dietary polyunsaturated fatty acids and their concentration in blood plasma, red blood cell, and semen of dogs

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Abstract

Background: In dogs, dietary omega 3 polyunsaturated fatty acids (n-3 PUFA) affect the fatty acid (FA) profile of blood plasma, erythrocyte membrane (EM), and semen, but their correlation has not yet been investigated.

Aim: In this study, we evaluated the association between dietary PUFA and their profile in blood plasma, EM, and semen of dogs, with the possibility to predict the semen profile using the values of the three first.

Methods: Twelve male dogs received the same standard commercial diet for 4 weeks. The FA profile was analyzed by gas chromatography in paired diet, blood (plasma and EM determinations), and semen samples. Data were analyzed with SAS Proc Corr version 9.4. Pearson´s correlation coefficient (significant if $p < 0.05$) was used to assess the association of dietary FA profiles with those in blood plasma, EM, and semen.

Results: There was a positive correlation between dietary eicosapentaenoic acid (EPA) and blood plasma ($r = 0.97$), EM ($r = 0.94$) and semen ($r = 0.92$) EPA, and between dietary docosahexaenoic acid (DHA) and arachidonic acid (ARA) and semen DHA ($r = 0.93$) and ARA ($r = 0.92$), respectively. There was a negative correlation between dihomo-gamma-linolenic acid (DGLA) in the diet and EM DGLA ($r = -0.94$).

Conclusion: The dietary EPA is correlated with blood plasma, EM, and semen EPA concentrations, and dietary DHA and ARA are associated with semen DHA and ARA concentrations in dogs. These findings suggest that dietary EPA, DHA, and ARA concentrations could be helpful to predictive markers for such concentrations in the semen of dogs.

Keywords: Association, Diet, Dog, Polyunsaturated fatty acid, Semen..

Introduction

Omega 3 polyunsaturated fatty acids (n-3 PUFA), particularly eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3), and omega 6 (n-6) PUFA, mainly arachidonic acid (ARA; 20:4 n-6), are essential fatty acids (FA) for dogs and should be therefore incorporated into their diets (NRC, 2006a). In addition, these essential FA play specific biological roles associated with male fertility by providing the sperm plasma membrane necessary fluidity for fertilization (Wathes *et al.*, 2007).

In dogs, dietary n-3 PUFA supplementation increases EPA and DHA concentrations in blood plasma (LeBlanc *et al.*, 2005; Stoeckel *et al.*, 2013) and the erythrocyte membrane (EM) (Stoeckel *et al.*, 2011, 2013). In addition, we have previously shown that dietary supplementation with n-3 PUFA affected the dog semen FA profile by increasing EPA and ARA (Risso *et al.*, 2016) and total n-3 PUFA (Risso *et al.*, 2016, 2017) concentrations.

The effect of dietary supplementation with n-3 PUFA on blood plasma, EM, and semen FA profiles has been widely studied. However, as far as we know, the correlation between the dietary PUFA profile and blood plasma, EM, and semen PUFA profiles has not been investigated as yet. Thus, based on the hypothesis that dietary PUFA profiles there are associated with the profiles of blood plasma, EM, and semen, we evaluated the association between dietary PUFA and their profile in blood plasma, EM, and semen of dogs, with the possibility to predict the semen profile using the values of the three first.

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Materials and Methods

Animals and diet

The study included 12 male mongrel dogs. Inclusion criteria to homogenize the population were: age (range 4–6 years), body weight (BW, range 20–25 kg), and body condition (BC, scoring 3 on a 5-score scale) (Mori *et al.*, 2015). In addition, reproductive condition and health status were evaluated with comprehensive information collected from medical records (medical history and clinical exams) and routine laboratory tests. The dogs received a standard commercial diet and had free access to water for 4 weeks. Daily rations (kcal/day) were calculated based on the maintenance energy requirements (MER) of dogs [MER = 132 kcal × kg metabolic BW ($BW^{0.75}$)] (NRC, 2006b) and adjusted to maintain the animal BC. Dogs were taken to the School of Veterinary Sciences, National University of La Plata, Buenos Aires, Argentina, twice a week during the 4 weeks to register their food intake and BW by investigators. After 4 weeks, standard commercial diet, blood, and semen samples were taken on the same day for each dog for correlation evaluation.

Dogs belonged to private owners who signed a written informed consent and agreed to feed their dogs only with the diet and rations assigned in the study protocol.

Blood sample collection

Dogs were fasted for at least 12 hours before blood collection. Venous blood samples (5 ml) were aseptically drawn through venipuncture of the cephalic vein with a 21G needle. Whole blood was transferred to EDTA tubes and centrifuged at 1,400 × g for 5 minutes. Blood plasma (supernatant) was recovered and stored at −18°C until FA profile analysis. Erythrocytes (bottom of the sample, or other best term) were held at 4°C until analysis of the FA profile in the EM (within 4 hours after blood withdrawal).

Semen sample collection

Semen samples were collected by manual stimulation on the same day of blood sample collection, and the spermatic fraction of ejaculate was used for the FA profile. Semen was snap-frozen and stored at −80°C until FA profile analysis.

Standard commercial food analysis

The standard commercial food was pooled and analyzed for dry matter (DM, 80°C for 48 hours), neutral detergent fiber (NDF, Ankom200 Fiber Analyzer, ANKOM Technology, Fairport, NY), crude protein (CP, Kjeldahl N × 6.25), lipid (Ether extract, XT101 ANKOM Technology Method 2), and ash according to the Association of Official Analytical Chemists (AOAC, 1990). The analysis found 93.1% DM, 1.8% NDF, 28.6% CP, 17.0% ether extract, 7.5% ash, 45.1% nitrogen-free extract, and 3.8 kcal/g metabolizable energy.

FA profile analysis of standard commercial food, blood plasma, EM, and semen samples

The FA profile of samples was determined by gas chromatography with a 30°Cmm capillary column (Omega Wax 250; Supelco, Bellefonte, PA). The temperature was programmed for a linear increase of 3°C per minute from 175°C to 230°C. The chromatographed peaks were identified by comparing their retention times with standards (Table 1). Samples for lipid extraction were analyzed following the method described by Folch *et al.* (1957). The lipids obtained were saponified with potassium hydroxide dissolved in ethanol for 45 minutes at 80°C and then acidified with a 0.5 ml hydrochloric acid concentrate. The acids were esterified with boron trifluoride at 64°C for 1.5 hours.

Statistical analysis

Data were analyzed with SAS Proc Corr version 9.4. Pearson´s correlation coefficient was used to assess the association between dietary FA profiles and the

Table 1. FA profile of the standard commercial dog food (diet), blood plasma, EM, and semen in the study population (n = 12 dogs).

FA profile	Diet		Blood plasma		EM		Semen	
	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM
Σ SAT	26.89	1.46	49.50	5.15	47.26	0.52	47.56	3.47
Σ MUFA	32.67	2.22	22.15	4.98	14.40	0.25	11.41	1.07
Σ PUFA	40.44	2.37	28.35	2.78	38.34	0.43	41.03	4.59
Σ n-3 PUFA	10.58	3.57	1.78	0.56	1.38	0.38	12.72	4.94
Σ n-6 PUFA	29.86	1.47	26.57	5.01	36.96	0.48	28.31	4.25
EPA (20:5 n-3)	4.31	0.76	0.60	0.72	0.60	0.50	9.97	7.57
DHA (22:6 n-3)	3.01	0.88	0.99	0.80	0.65	0.50	2.75	2.31
ARA (20:4 n-6)	0.21	0.48	9.93	6.28	23.94	0.95	3.77	0.99
DGLA (20:3 n-6)	0.43	0.77	0.90	0.61	1.38	0.11	0.42	0.40

(SAT): saturated; (MUFA): monounsaturated; (PUFA): polyunsaturated; (n-3): omega 3; (n-6): omega 6; (EPA): eicosapentaenoic acid; (DHA): docosahexaenoic acid; (ARA): arachidonic acid; (DGLA): dihomo-gamma-linolenic acid; (LSM): least squares mean; (SEM): standard error of the mean.

Table 2. Correlation between the FA profile of the standard commercial dog food (diet) and the corresponding concentrations in blood plasma, EM, and semen in the study population (n = 12 dogs).

The dietary FA profile	Blood plasma		EM		Semen	
	r value	*p*-value	*r* value	*p*-value	*r* value	*p*-value
EPA	0.97	<0.01	0.94	0.01	0.92	0.02
DHA	0.02	>0.05	0.81	>0.05	0.93	0.02
ARA	0.40	>0.05	−0.37	>0.05	0.92	0.02
DGLA	−0.66	>0.05	−0.94	0.01	0.11	>0.05

(EM): erythrocyte membrane; (EPA): eicosapentaenoic acid; (DHA): docosahexaenoic acid; (ARA): arachidonic acid; (DGLA): dihomo-gamma-linolenic acid; (r): Pearson's correlation coefficient.

individual concentrations in blood plasma, EM, and semen. The alpha level of significance was set at $p < 0.05$. Results are reported as least squares means (LSM) with standard errors of the mean (SEM).

Ethical approval

This study was approved by the Institutional Animal Care and Use Committee (protocol 34-1-13) of the School of Veterinary Sciences, National University of La Plata, Buenos Aires, Argentina.

Results and Discussion

In agreement with our hypothesis, dietary PUFA profiles were associated with their profiles in blood plasma, EM, and semen. To our knowledge, this is the first report correlating dietary PUFA with semen PUFA profile in dogs. To respect this, we had a positive correlation ($r \geq 0.92$; $p = 0.02$) between studied dietary PUFA EPA, DHA, and ARA, affecting their respective concentrations in semen (Table 2).

In a previous report, we found an increase in EPA, ARA, and total n-3 PUFA percent concentrations in the semen of dogs fed with a diet supplemented with fish oil (FO) for 120 days (Risso *et al.*, 2016). Those changes were associated with the high level of n-3 contained in FO supplementation.

Unlike that study, in the present work, dogs did not receive any treatment with additional n-3 dietary supplements. We observed a positive correlation between dietary and semen EPA, DHA, and ARA concentrations.

Considering that, in dogs, PUFA are not detected or are found in low proportions in seminal plasma (Díaz *et al.*, 2014), in this work, we used spermatic fraction to measure FA profile as a reliable indicator of the sperm PUFA profile.

In accordance with the literature (LeBlanc *et al.*, 2005; Stoeckel *et al.*, 2011), we also found a positive correlation between dietary EPA and EPA concentration in blood plasma ($r = 0.97$; $p < 0.01$) and EM ($r = 0.94$; $p = 0.01$) (Table 2). In addition, we observed a negative correlation ($r = -0.94$; $p < 0.05$) between dietary and EM dihomo-gamma-linolenic acid (DGLA, 20:3 n-6) (Table 2).

Commercial dog food usually contains different concentrations of essential α-linolenic acid (ALA, 18:3 n-3) and linoleic acid (LA, 18:2 n-6), which are, respectively, precursors of EPA and DHA (ALA) and ARA (LA). It should be noted that ALA conversion into EPA competes with LA conversion into ARA because the same enzymes are used during their biosynthesis (Calder, 2013). Besides, since the ALA conversion rate into EPA and DHA is quite limited in dogs (Lenox and Bauer, 2013), these FA should be offered in diet to fulfill their specific functions. In this sense, FO (n-3 PUFA) is an efficient source of EPA and DHA for dietary supplementation (Lenox and Bauer, 2013). In the present study, dogs were fed a standard commercial diet containing FO as a source of EPA and DHA.

Previous research by Mueller *et al.* (2005) has demonstrated that dogs fed with preformed EPA and DHA showed higher levels of blood plasma EPA and DHA associated with lower levels of ARA. In our study, the correlation of the dietary FA profile (4.31% EPA and 3.01% DHA) was positive only with blood plasma EPA concentration ($r = 0.97$).

It has been proposed that the analysis of blood plasma FA in clinical settings is sufficient to know whether dietary PUFAs increase tissue n-3 PUFA content in dogs, but EM FA should be evaluated when more accurate FA profiles are needed, particularly n-6 PUFA (Stoeckel *et al.*, 2013). In this sense, n-3 and n-6 PUFA levels in the red blood cells of mice have been reported to correlate with n-3 and n-6 PUFA levels in the heart, muscles, spleen, lungs, and adipose tissue (Fenton *et al.*, 2016). These authors observed a correlation between red blood cell EPA, DHA, EPA+DHA, and ARA and the mentioned tissues (Fenton *et al.*, 2016). Corroborating such findings, we found a positive correlation ($r \geq 0.94$) between dietary EPA and blood plasma and EM EPA. Contrarily, we found a negative correlation between dietary DGLA and EM DGLA ($r = -0.94$). Further research is needed to elucidate the latter effect.

Conclusion

We conclude that dietary EPA is correlated with blood plasma, EM, and semen EPA concentrations, and dietary DHA and ARA are correlated with semen DHA and ARA concentrations in dogs. These results suggest that dietary EPA, DHA, and ARA could be helpful

to predictive markers for these values in dog semen. Future studies in a larger sample of dogs would support the current findings.

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Conflict of interest

The authors declare that there is no conflict of interest.

Authors' contribution

A Risso: design of the study, data analysis, and manuscript writing; FP: animal experiments, data analysis, and manuscript writing; YC: manuscript writing and editing, SP: manuscript writing and editing; AR: funds for sample and data analysis. All authors have read and approved the final manuscript.

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