PROCEEDINGS OF THE

II International Conference on Environmental Stressor in Biology and Medicine

Siena (Italy), October 5-7, 2011

Evaluation of different dietary lipid supplements on oxidatively generated biomarkers in periparturient dairy goats.

Bellagamba F.[1], Busetto M. L.[1], Caprino F.[1], Invernizzi G.[1], Moretti V. M.[1], Savoini G.[1]

[1] Dipartimento di Scienze e Tecnologie Veterinarie per la Sicurezza Alimentare, Università degli Studi di Milano, Via Trentacoste 2, 20134 Milano, Italy

Summary

This study aimed at analyzing the impact of different dietary lipid supplements on oxidative stress status in 26 Alpine dairy goats fed with diets just differing in lipid sources: protected fish oil (FO), calcium stearate (ST), and control group (C), without any supplement. Blood samples were collected weekly starting from day 130 of gestation (about 20 days before delivery) until 21 days of lactation. Analytical determination of malondialdehyde (MDA) and 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodGuo) have been carried out in serum samples. Gas chromatographic determination of plasma fatty acids (FAs) was also performed. MDA and 8-oxodGuo resulted both higher in FO group when compared to ST and C group, but not at significant level. Dietary lipid supplements produced remarkable change in plasma FAs and after 21 days of supplementation, 20:5 n-3 (eicosapentaenoic, EPA) and 22:6 n-3 (docosahexaenoic, DHA) in FO group resulted significantly different ($P \leq 0.05$) from ST and C groups. The significant increase of EPA in FO diet was assessed right after 7 days of supplementation. Positive and significant ($P \leq 0.05$) correlations between some FAs and MDA, and 8-oxodGuo were obtained.

Introduction

In farm animals, altered redox balance is involved in several metabolic disorders with relevant consequences for animal health and productivity [1-2]. Fish oil supplementation, rich in EPA and DHA, produces significant change in mature goat milk fatty acids composition [3]. However, there are concerns that involve n-3 long chain polyunsaturated fatty acids (LC-PUFAs) supplementation in oxidative damage enhancement, with potential untoward effects during critical metabolic status, like gestation [4]. This study aimed at analyzing the impact of different dietary lipid

	C		C 7dd		C 21dd		FO		FO 7dd		FO 21dd		ST		ST 7dd		ST 21dd	
14:0	0.31	±0.02	0.25	±0.04	0.38	±0.05	0.33	±0.01	0.38	±0.03	0.33	±0.05	0.32	±0.02	0.31	±0.03	0.26	±0.05
15:0	0.25	±0.06	0.33	±0.03	0.46	±0.06	0.44	±0.05	0.39	±0.04	0.39	±0.06	0.38	±0.02	0.25	±0.04	0.27	±0.06
16:0	17.01	±0.80	17.08	±0.55	17.31	±0.25	17.00	±0.63	17.21	±0.38	17.60	±0.51	17.38	±0.32	16.99	±0.45	16.58	±0.52
16:1n-7	0.74	±0.05^{a}	0.68	±0.03^{a}	0.70	±0.08^{a}	0.73	±0.05^{a}	1.07	±0.15^{ab}	1.11	±0.13^{b}	0.73	±0.08^{a}	0.71	±0.04^{a}	0.68	±0.05^{a}
17:0	0.73	±0.07	0.68	±0.05	0.80	±0.05	0.86	±0.08	0.93	±0.04	0.76	±0.12	0.77	±0.04	0.73	±0.04	0.66	±0.06
17:1	0.56	±0.07	0.41	±0.06	0.42	±0.06	0.49	±0.07	0.51	±0.06	0.32	±0.05	0.45	±0.11	0.55	±0.05	0.46	±0.03
18:0	16.71	±1.43	16.63	±0.94	19.32	±0.62^{b}	17.38	±1.30	16.14	±1.46	13.64	±1.05^{a}	17.90	±0.90	18.64	±1.11	17.36	±1.21
18:1n-9	23.15	±4.29	20.17	±2.95	15.97	±1.78	20.47	±3.68	19.58	±2.65	14.42	±2.45	19.78	±2.46	20.93	±2.52	19.24	±3.15
18:1n-7	1.62	±0.14^{a}	1.54	±0.22^{a}	1.79	±0.12^{a}	2.61	±0.51^{a}	3.02	±0.80^{a}	5.26	±0.83^{b}	1.62	±0.17^{a}	1.85	±0.18^{a}	1.93	±0.08^{a}
18:2n-6	28.87	±1.44	31.17	±1.29	32.40	±1.99^{b}	27.82	±1.07	26.12	±0.74^{a}	30.95	±1.74	28.26	±1.01	28.35	±1.17	32.03	±1.45
18:3n-6	0.34	±0.10	0.53	±0.08	0.49	±0.10	0.61	±0.07	0.49	±0.12	0.30	±0.07	0.62	±0.05	0.54	±0.06	0.62	±0.14
18:3n-3	1.21	±0.12	1.21	±0.07	1.46	±0.20	1.44	±0.10	1.34	±0.10	1.41	±0.07	1.27	±0.14	1.02	±0.10	1.30	±0.06
20:3n-6	0.00	±0.00	0.08	±0.05	0.31	±0.02	0.10	±0.04	0.13	±0.06	0.47	±0.23	0.02	±0.02	0.00	±0.00	0.10	±0.05
20:4n-6	5.06	±0.58	5.90	±0.68	5.21	±0.22	5.34	±0.52	5.62	±0.45	4.41	±0.78	6.56	±0.60	5.53	±0.52	5.54	±0.50
20:5n-3	0.37	±0.07^{a}	0.47	±0.06^{a}	0.42	±0.04^{a}	0.88	±0.27^{a}	2.57	±0.78^{b}	3.96	±0.87^{c}	0.53	±0.06^{a}	0.37	±0.05^{a}	0.44	±0.10^{a}
22:0	0.16	±0.05	0.19	±0.04	0.22	±0.02	0.17	±0.03	0.21	±0.03	0.18	±0.04	0.17	±0.07	0.18	±0.04	0.14	±0.05
22:4n-6	0.25	±0.08	0.23	±0.05	0.29	±0.03	0.27	±0.07	0.20	±0.06	0.00	±0.00	0.26	±0.10	0.37	±0.07	0.16	±0.08
22:5n-3	1.58	±0.21	1.58	±0.19	1.22	±0.09^{a}	1.86	±0.21	2.13	±0.17^{b}	2.05	±0.15	1.89	±0.29	1.45	±0.18	1.23	±0.13^{a}
C24:0	0.20	±0.08	0.33	±0.14	0.24	±0.09	0.20	±0.06	0.30	±0.07	0.24	±0.06	0.10	±0.06	0.27	±0.06	0.22	±0.08
22:6n-3	0.89	±0.20^{a}	0.53	±0.17^{a}	0.58	±0.21^{a}	1.01	±0.22^{a}	1.66	±0.37^{ab}	2.19	±0.39^{b}	0.99	±0.18^{a}	0.98	±0.16^{a}	0.76	±0.14^{a}
SFA	35.36	±2.31	35.50	±1.30	38.73	±0.45	36.37	±1.94	35.55	±1.59	33.14	±1.29	37.02	±1.21	37.36	±1.55	35.50	±1.68
MUFA	26.06	±4.35	22.80	±2.99	18.88	±1.84	24.30	±3.56	24.18	±2.26	21.11	±2.65	22.58	±2.65	24.03	±2.61	22.32	±3.15
PUFA	38.58	±2.16	41.70	±1.95	42.39	±1.96	39.33	±1.92	40.27	±1.20	45.75	±1.67	40.39	±2.03	38.61	±1.67	42.18	±1.73
N-3	4.06	±0.37^{a}	3.79	±0.29^{a}	3.68	±0.17^{a}	5.20	±0.51^{a}	7.71	±1.16^{b}	9.62	±1.34^{b}	4.68	±0.53^{a}	3.82	±0.32^{a}	3.74	±0.26^{a}
N-6	34.52	±2.01	37.91	±1.74	38.71	±1.99	34.13	±1.55	32.56	±1.26	36.13	±1.97	35.72	±1.55	34.79	±1.40	38.45	±1.66
n-3 LC PUFA	2.84	±0.42^{a}	2.58	±0.28^{a}	2.22	±0.23^{a}	3.75	±0.58^{a}	6.37	±1.14^{b}	8.20	±1.33^{b}	3.41	±0.46^{a}	2.80	±0.36^{a}	2.43	±0.29^{a}
n-6 LC PUFA	5.31	±0.63	6.22	±0.66	5.82	±0.24	5.71	±0.59	5.94	±0.51	4.88	±0.72	6.84	±0.59	5.90	±0.57	5.80	±0.53

Table1: total plasma fatty acids (% of total fatty acids) of goats fed with different dietary supplementations. The value are expressed as means ± SEM.

supplements on oxidative stress status in 26 Alpine dairy goats during the peripartum period.

Materials and methods

At day 130 of gestation goats housed in single boxes were fed with experimental diets just differing in lipid sources: a dietary protected fish oil (FO) group, rich in n-3 LC-PUFAs (EPA, 10.4 %; DHA, 7.8 %); a dietary calcium stearate (ST) group, rich in 16:0 (26 %) and 18:0 (69.4 %) saturated fatty acids, and a dietary control group (CO), without any supplement. Vitamins supplementation were identical in all diets and α-tocopherol supplementation was 25 mg Kg^{-1}. Blood samples were collected weekly starting from day 130 of gestation (before supplementation) until 21 days of lactation. The MDA was quantify in serum by high performance liquid chromatography [5]. The serum level of 8-oxodGuo was determined using a competitive ELISA method (Japan Institute for Control Aging Fukuroi, Japan). Analysis of total plasma fatty acids composition was performed [6]. Data were analysed by means of ANOVA and results presented as mean ± standard error (SEM). The effects were considered to be significant at $P \leq 0.05$. Pearson's correlation coefficients were calculated between parameters measured in this study.

Results and discussion

Dietary lipid supplements produced remarkable change in plasma fatty acids (table 1) with a significant ($P \leq 0.05$) increase of EPA (3.96 ± 0.87 %) and DHA (2.19± 0.39%) after 21 days of supplementation in FO group compared to ST and C diets. The significant increase of EPA (2.57 ± 0.78%) in FO diet was assessed since 7 days of supplementation (figure 1). No effect of diet was noted for serum

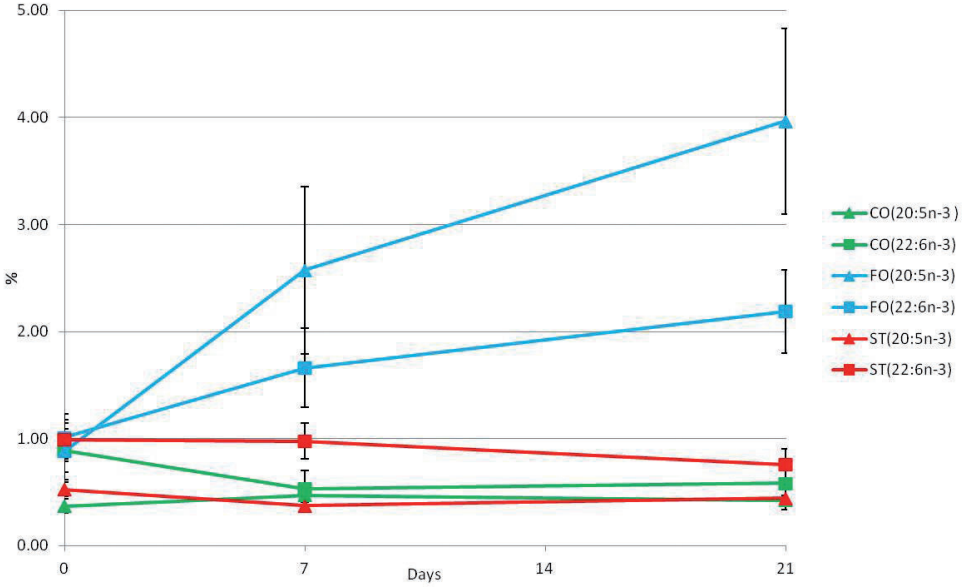

Figure 1: plasma content of 20:5 n-3 (EPA) and 22:6 n-3 (DHA) during dietary supplementations (FO: fish oil; ST: calcium stearate; CO: control).

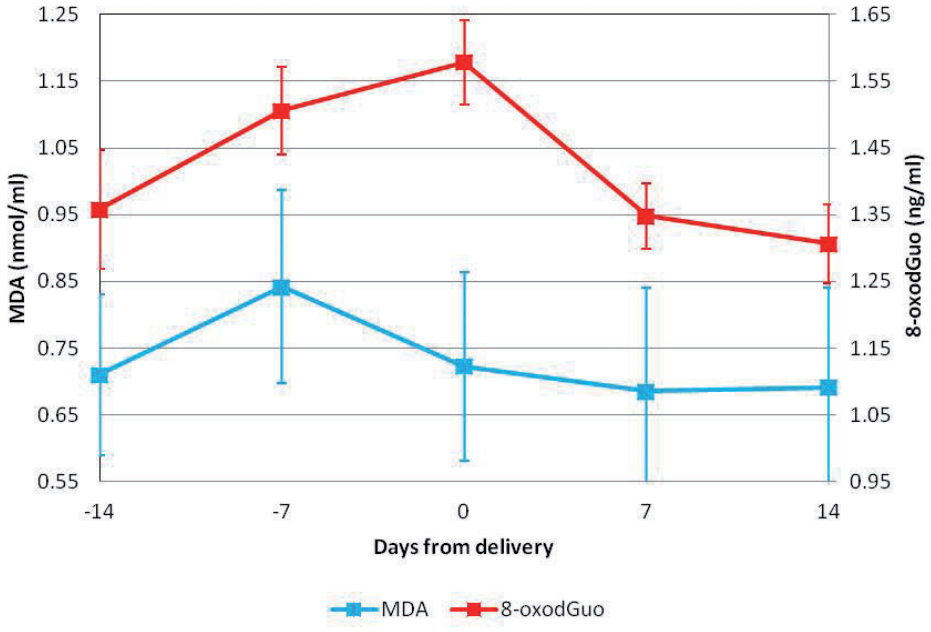

Figure 2: Serum 8-oxo-7,8-dihydro-2'-deoxyguanosine(8-oxodGuo) and malondialdehyde (MDA) levels measured during the peripartum period.

n=79	Pearson's correlation coefficients *r* and significance level[a]							
	18:0	18:1 n-9	18:3 n-3	20:5 n-3	22:6 n-3	MUFA	n-3	n-3 LC-PUFA[b]
8-oxodG	0.11 (0.34)	-0.28 (**0.01**)	-0.30 (0.79)	0.23 (**0.04**)	0.30 (**0.01**)	-0.25 (**0.03**)	0.26 (**0.02**)	0.27 (**0.02**)
MDA	-0.23 (**0.04**)	0.24 (**0.03**)	0.35 (**0.002**)	0.04 (0.72)	-0.13 (0.27)	0.23 (**0.04**)	0.05 (0.65)	-0.002 (0.98)

[a] significant correlation ($P<0.05$) in bold

[b] n-3 LC-PUFA = 20:5n-3 + 22:5n-3 + 22:6n-3

Table 2: Correlations among plasma fatty acids, serum 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) and malondialdehyde (MDA).

levels of MDA and 8-oxodGuo, but they both resulted higher (822.02±50.45 μmol ml^{-1} and 1.60±0.24 ng ml^{-1}, respectively) in FO diet when compared to ST and C. Similarly, no significant effect of time of sampling (distance from delivery) x feeding supplementation interaction was obtained. However, the 8-oxodGuo trend showed an increase from 2 weeks before until partum (figure 2). The plasma oleic and linoleic acids resulted positively correlated ($P \leq 0.05$) with serum MDA, whereas EPA and DHA were correlated with 8-oxodGuo. There were no significant correlations between MDA and 8-oxodGuo (table 2).

Conclusions

Modifications of dietary fatty acids composition produce valuable and significant variations of plasma FAs. The oxidative stress biomarkers measured did not produce significant results, however their changing during the peripartum period and correlations to some plasma FAs levels might revealed different profiles of the oxidative status

References

[1] Lykkesfeldt J., O. Svendsen (2007). Oxidants and antioxidants in diseases: oxidative stress in farm animals. *The Veterinary Journal*, 173: 502-511.

[2] Celi P. Biomarkers of oxidative stress in ruminant medicine. *Immunopharmacology and immunology*(2011). 33(2): 233-240.

[3] Savoini G., A. Agazzi, G. Invernizzi, D. Cattaneo, A. Baldi (2010). Polyunsaturated fatty acids and choline in dairy goats nutrtion: production and health benefits. *Small Ruminant Research*, 88: 135-144.

[4] Shoji H., C. Franke, C. Campoy, M. Rivero, H. Demmelmair, B. Koletzko (2006). Effects of docosahexaenoic acid and eicosapentaenoic acid supplementation on oxidative stress levels during pregnancy. *Free Radical Research,* 40: 379-384.

[5] Mateos R., E. Lecumberri ,S. Ramos, L. Goya,L. Bravo (2005). Determination of malondialdehyde (MDA) by high-performance liquid chromatography in serum and liver as a biomarker for oxidative stress: Application to a rat model for hypercholesterolemia and evaluation of the effect of diets rich in phenolic antioxidants from fruits**.** *Journal of Chromatography B*, 827:76–82.

[6] Glaser C., H. Demmelmair, B. Koletzko (2010). High-throughput analysis of total plasma fatty acid composition with direct in situ transesterification. *Plos One* 5: e12045.

Via G. Verdi 15/1, 40065 Pianoro (Bologna), Italy
www.medimond.com • info@medimond.com

Printed in May 2012 by Editografica • Bologna (Italy)

ISBN 978-88-7587-643-2

monduzzi editore

INTERNATIONAL PROCEEDINGS DIVISION

is a registered trademark owned by Medimond s.r.l.