FATTY ACIDS OMEGA-3 IN MILK OF RABBITS DOES FED COMMON VETCH AND SARDINE OIL

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ABSTRACT

Four dietary treatments were assessed: AASO (alfalfa 66.95% and soybean oil 4.5%), VASO (common vetch 75.74% and soybean oil 5.33%), VASA (common vetch 75.83% and sardine oil 5.18%), and ALCO (commercial feed) on breeding does, of 3.2 kg during their first 2 lactations without homogenizing litters. The assessed variables were: content of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in milk fat. The feed was offered in pellets *ad libitum.* Milking was done manually. The milk concentration of DHA showed differences ($p<0.02$) among means of 0.36, 0.55, 1.26, and 0.15 mg mL^{-1}, corresponding to the order of the indicated feeding treatments. EPA content was different ($p<0.01$) by effect of treatments 1.72, 1.86, 3.64, and 0.94 mg mL^{-1}, according to the mentioned order. As it was expected, rabbits fed oils rich in omega-3 fatty acids are capable of delivering these acids into their milk.

Key words: *Vicia sativa* L., sardine oil, EPA, DHA, omega-3 fatty acids.

INTRODUCTION

The long chain fatty acids of milk fat of does can be modified by the dietary manipulation as shown by FRAGA *et al* (1989). (There are works at this respect also in rabbits). PASCUAL *et al*. (1999) indicated there was correlation between the fatty acids composition of milk does and dietary fat. The effect of feeding on fatty acids omega-3 content of rabbit milk has not been apparently studied. D´AMBOLA *et al*. (1991) found that suckling rabbits have better defenses against pulmonary diseases with diets supplemented with fish and safflower oils rich in omega-3 fatty acids. The effect on rabbit milk composition when using diets with sardine oil, rich in omega-3 fatty acid, apparently has not been reported. Therefore, the present study was done to assess the effect of diets with high content of common vetch, soybean or sardine oil, on the content of omega-3 fatty acids in rabbit milk.

MATERIAL AND METHODS

The experiment was carried out at the rabbit breeding unit of the Colegio de Postgraduados, in Montecillo, State of Mexico, with geographical coordinates 19°29' North latitude and 98°54' West longitude, and 2250 meters above sea level. Average monthly temperature was 18 °C during the experimental period (March-August 2003).

Diets

Four treatments were assessed AASO (alfalfa and soybean oil), VASO (common vetch and soybean oil), VASA (common vetch and sardine oil), and ALCO (commercial feed). The composition of the diets is shown in Table 1. Feeding was offered in pellets *ad libitum*.

Table 1. Ingredients and composition (calculated and analyzed) of the experimental diets (as fed basis).

Ingredients (%)	AASO	VASO	VASA	ALCO
Alfalfa	66.90	----	----	
Common vetch (*Vicia sativa* L.)	----	75.74	75.83	
Wheat	24.63	16.60	16.43	
Soybean oil	4.50	5.32	----	
Sardine oil	----	----	5.18	
L-Lysine-HCl	0.72	----	----	
DL-Metionine	0.16	0.09	0.09	
Calcium carbonate	----	0.61	0.84	
Calcium phosphate	2.15	0.80	0.80	
Coccidiostate	0.05	0.05	0.05	
Vitamin mixture	0.25	0.25	0.25	
Mineral mixture	0.10	0.10	0.10	
Etoxiquine	0.04	0.04	0.04	
Salt	0.50	0.40	0.40	
Chemical composition %				
Dry matter	90.29	91.04	91.25	91.38
Crude protein	18.75	18.10	18.44	18.16
Crude fiber	12.36	14.62	14.90	16.11
Ether extract	6.00	6.29	6.35	1.91
Ash	10.62	9.65	9.59	9.41
Neutral detergent fiber (NDF)	27.22	32.66	32.00	42.24
N-free NDF	26.36	31.62	31.12	40.98
Acid detergent fiber	14.99	19.51	21.63	23.64
Calcium	2.27	1.96	1.80	1.68
Total phosphorus	0.32	0.49	0.40	0.42
EPA (mg g feed^{-1})	1.41	1.47	3.20	1.01
DHA (mg g feed^{-1})	0.52	0.67	1.05	0.48

Animals

Two nulliparous New Zealand White female rabbits were taken randomly from a total of 20 animals per treatment, with an average weight at first conception of 3.2 kg, which were then placed in a rabbitry with uncontrolled conditions. Milk samples were manually taken on the 7^{th}, 14^{th}, 21^{st}, and 28^{th} day of lactation during the first 2 lactations and frozen them immediately for posterior analyze.

Chemical analyses

Milk (250 µL) were mixed with 250 µL of tris/EDTA/sucrose buffer, 4 mL of chloroform-ethanol (2:1), 4 mL of chloroform-ethanol-HCl (2:1:0.013), 1 mL of NH_4OH (2%). After centrifugation (3000 rpm/10min) the sediment (fat) was filtered and dissolved in one mL of hexane. Then, 100 µL were methylated, adding 100 µL of sodium methoxide and 800 µL of hexane. The mixture was centrifuged (3000 rpm/5min) and the supernatants were filtered (0.45 µm nylon acrodisc). Gas Chromatograph: GC-Perkin Elmer M-1020, column: PE-WAX (30mx25mmx20µm), carrier: helium 20 psi, injected aliquot: 2 µL. Temperature: oven 50 °C (2 min) to 210 ºC at 4 ºC/min (8min), injector 220 ºC and detector 250 ºC (FRITSCHE *et al*., 1993; ANONYMOUS, 1996). Standards: EPA (eicosapentaenoic acid; 20:5) and DHA (docosahexaenoic acid; 22:6) Supelco, 10 mg in 10 mL of hexane, retention times of 42.4 and 47.6 min for EPA and DHA respectively.

Dry matter, ether extract, ash, calcium, phosphorus, crude fiber and protein contents of diets were determined using the methods of the ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS (1990) and that of VAN SOEST *et al*. (1991) was used for the neutral detergent fiber, N-free NDF and acid detergent fiber.

Statistical analysis

The following mixed model was used:

$$Y_{ijkl} = \mu + T_i + C_{j(i)} P_k + D_l + T*D_{il} + E_{ijkl}.$$

Where:
Y_{ijkl}: Variable response (EPA y DHA)
μ :Population constant
T_i :Effect of the i^{th} treatment (i= 1, 2, 3, 4)
$C_{j(i)}$:Effect of the j^{th} rabbit nested in the i^{th} treatment
P_k :Effect of the k^{th} parity (k=1, 2)
D_l :Effect of the l^{th} day of lactation (l= 7,14, 21, 28)
$T*D_{il}$: Effect of the interaction of the i^{th} treatment X the l^{th} day of lactation
E_{ijkl} :Residual random error

Results, were analyzed as suggested by STEEL and TORRIE (1986), through the PROC MIXED procedure of the SAS (2003).

RESULTS AND DISCUSSION

Statistical analysis showed differences (P<0.05) by effect of the treatment for EPA and DHA, while the day of lactation affected (P<0.01) the concentration of DHA. The interaction of treatment X lactation day did not affect (P>0.05) either variable (Table 2).

Table 2. Effect of treatment, day of lactation and their interaction in the milk concentration of omega-3 fatty acids (mg mL^{-1}).

	Omega-3 fatty acids	
Source of variation	**EPA**	**DHA**
Treatment	*	*
Day of lactation	NS	**
Treatment X Day of lactation	NS	NS

* (P‹0.05), ** (P‹0.01), NS: Non significant, EPA = Eicosapentaenoic acid, DHA = Docosahexaenoic acid.

Effect of treatments

Significant differences were found (Table 3) in the concentration of EPA and DHA, being higher in VASA does than in the other treatments. This was due to the consumption of sardine oil in VASA does. The omega-3 values found for VASO and AASO confirm that soybean oil has this type of fatty acids as it was indicated by CASTRO-GONZÁLEZ (2002) and MIYAZAWA *et al*. (1994).

Table 3. Omega-3 acid content (mg mL^{-1}) in rabbit milk in function of dietary treatment (Least square means ± standard error).

Treatments	N	EPA	N	DHA
AASO	16	1.72±0.13^{B}	16	0.36±0.08^{B}
VASO	13	1.86±0.16^{B}	16	0.55±0.08^{B}
VASA	16	3.64±0.12^{A}	16	1.26±0.08^{A}
ALCO	16	0.94±0.15^{B}	16	0.15±0.08^{B}

N= Number of observations; $^{A, B}$: Means with different letters in the same column are significantly different (p‹0.05); EPA = Eicosapentaenoic acid; DHA = Docosahexaenoic acid.

Effect of the day of lactation

The day of lactation affected (p<0.01) the concentration of DHA in milk (1.16, 0.25, 0.27 y 0.60 mg mL^{-1}) on the 7th, 14th, 21st, and 28th day, respectively. These differences are explained by the higher concentration of total fat in the first days of lactation. CHRIST *et al.* (1996) found 20 vs 13% fat in does milk in the first and 20th day of lactation,

respectively. In this study, EPA content was not significantly affected (p>0.05) by the day of lactation, but showed the same tendency (Figure 1).

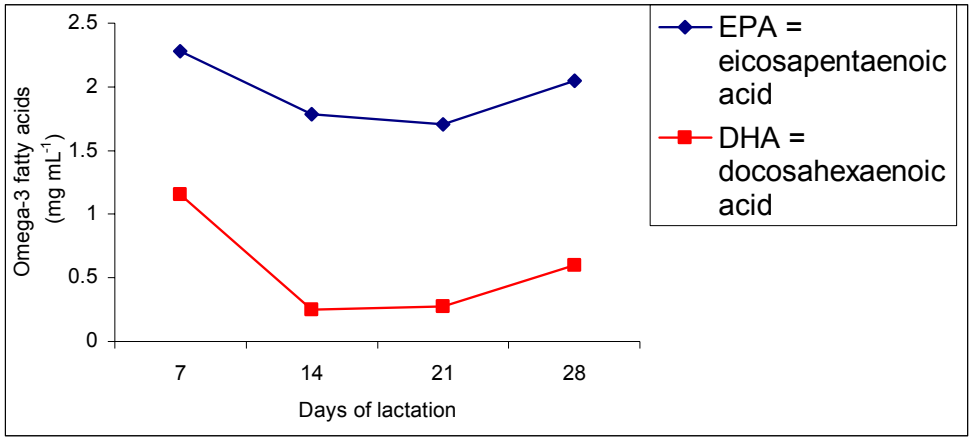

Figure 1. Effect of the day of lactation on the content of omega-3 fatty acids in rabbit milk.

Because of lack of information on EPA and DHA in does milk, the results obtained in this study cannot be compared.

CONCLUSIONS

As it was expected, rabbits fed oils rich in omega-3 fatty acids (EPA and DHA) are capable of delivering these acids into their milk.

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