EFFECT OF DIETARY N-3 FATTY ACIDS ON THE COMPOSITION OF DOE'S MILK AND TISSUES OF SUCKLING RABBITS

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ABSTRACT

The effect of dietary n-3 fatty acids on the fatty acid profile of doe's milk and pup's body was studied. Two experimental groups, each of 10 New Zealand White does, were fed diets enriched with α-linolenic acid (LNA) or long chain (≥20C) polyunsaturated n-3 fatty acids (LCPn-3), respectively. A standard commercial feed was given to the control group. The fatty acid profile of milk was well correlated with that of the diet showing higher concentration of n-3 in both experimental diets. The fatty acid composition of plasma, liver and fat of pups was affected by the profile of the milk ingested. The tissues showed higher concentrations of LCP fatty acids by feeding does the n-3 enriched diets and the increase was higher when LCP were supplemented. The increased levels of EPA and DHA were associated with a decline in AA.

Key words: rabbit doe, n-3 fatty acids, suckling rabbits.

INTRODUCTION

Linoleic (C18:2n-6-LA) and linolenic (C18:3n-3-LNA) fatty acids are essential for animals being the precursors of long chain (≥20C) polyunsaturated fatty acids (LCP), that play a structural and functional role in cell membranes: particularly those of retina, brain, neural and reproductive tissues (JENSEN *et al*., 1996). An adequate provision of LCPn-3 or their precursor is critical to ensure optimal growth and development of foetus and young born. The possibility of dietary improvement of the output of these fatty acids in mother's milk, and of increasing their level in tissues has been shown in different animal species and in human (FRITSCHE *et al.,* 1993; YEH *et al.,* 1998; HORNSTA, 2000; YEOM *et al.,* 2001; ROOKE *et al.,* 2001). LESKANICH and NOBLE (1999), in a review on the comparative roles of polyunsaturated fatty acids (PUFA) in pig neonatal development, underlined their relevance and gave recommendations on dietary intake of gestating sows. In rabbit, FRAGA *et al.* (1989) observed a higher proportion of PUFA in the milk of does fed a diet supplemented with lard and LEBAS *et al.* (1996) obtained similar results feeding does with a diet containing sunflower oil. The aim of this study was to analyse the effect of supplying to rabbit does diets rich in n-3 fatty acids (precursor or derivatives) on lipid profile of milk and of some tissues of suckling rabbits.

MATERIAL AND METHODS

Animals and diets

Forty New Zealand White (NZW) rabbit does selected by the National Association of Italy Rabbit Breeders were transferred, at 120 days of age, to the experimental rabbitry of Animal Production Department (University of Perugia). The environmental temperature ranged from +15 to +20 °C and relative humidity from 65 to 70%. At 150 days of age groups of 10 animals each were performed and supplying *ad libitum* the following diets (Table1):

- Standard commercial diet (Control group);
- Basal diet +5% extruded flaxseed (Omegalest®, Valorex – LNA group);
- Basal diet +2% fish oil (Nordos fat® - LCP group).

The fish oil was a coated blend of concentrated and refined oils adequately protected.

Sampling and analysis

At 16 d of lactation 2 pups per doe were killed and blood and liver samples were taken; whole carcasses were then lyophilised. At the same lactation day, milk samples were obtained from the mothers with a manual apparatus (moulded glass tube and vacuum pumps), similar to that described by SCHLEY (1975), after an injection of 2 IU of oxytocine.

Chemical analysis of diets was done according to AOAC procedures (1995). The chemical composition of the milk was determined on lyophilised samples quantifying total solids, crude protein, fat (methanol-chloroform) and ash according to AOAC procedures (1995). The lactose content was estimated by difference (organic matter minus crude protein and fat). Feeds, milk, plasma, liver and carcass lipids were extracted in a homogeniser with 20 ml of chloroform-methanol 2:1 (FOLCH *et al*., 1957 modified, explain modifications!!), and then filtered through Whatman No. 1 filter paper. Fatty acids were determined as methyl esters (FAME) with a Mega 2 Carlo Erba Gas Chromatograph, model HRGC (Milano, Italy), using a D-B wax capillary column (25 mm ∅, 30 m long). Statistical analysis was done with a linear model (SAS/GLM, 1990) considering the effect of dietary treatment.

RESULTS AND DISCUSSION

Both the diets supplemented with n-3 fatty acids showed higher percentage of ether extract (Table 1) and differed also in the fatty acid profile, showing lower level of saturated (SFA) and monounsaturated (MUFA) fatty acids and higher of PUFA. Among PUFA the total n-6 amount was lower and the n-3 higher, consequently the n-3/n-6 ratio increased. Differences between n-3 diets (LNA and LCP) were also found and concerned mainly the amount of LCPn-3 which was higher in LCP diet (3.60% *vs.* 0.34%). The chemical composition of milk (Table 2) was not affected by dietary treatment in spite of the greater level of ether extract in the two n-3 diets.

Table 1. Chemical composition of diets (%) and fatty acid profile (% of total fatty acids) of flaxseed, fish oil and diets

Ingredients		Flaxseed	Fish oil
C18:2n-6		17.35	2.30
C18:3n-3		56.35	7.12
C20:4n-6		0.01	1.18
C20:5n-3		0.02	5.20
C22:5n-3		0.04	2.24
C22:6n-3		0.01	9.23
	Control	LNA	LCP
Crude protein	16.91	16.32	16.11
Ether extract	3.93	4.72	6.63
Crude fiber	15.40	15.58	15.44
Ash	8.21	8.78	8.59
SFA	19.76	18.54	18.42
MUFA	18.46	17.14	16.55
PUFA	61.78	64.32	65.03
n-6	39.27	33.42	32.25
n-3	22.51	30.90	32.78
LCPn-3	0.32	0.34	3.60
n-3/n-6	0.57	0.92	1.02

The lacking of relation was presumably due to the time of sample harvesting (close to the lactation top), in such period the high milk yield reduces the concentration of substances and probably mask the differences. In fact the fatty acid profile was strongly affected by feed. The concentration of SFA was similar in all the groups because the proportions of individual fatty acids showed an irregular trend, with balanced variations (data not shown). The percentage of MUFA was inexplicably higher only in milk from mother ingesting LNA diet. Further PUFA showed a different repartition in n-3 supplemented does with a percentage of n-6 and n-3 respectively lower and higher than control group. The milk from does receiving preformed LCP showed a very high proportion of LCPn-3 (3.39 *vs.* 0.75 and 0.87%). The n-3/n-6 ratio was higher in the milk of both n-3 groups because, in LNA group the lower presence of LCPn-3 was compensated by the higher amount of LNA. The fatty acid profile of plasma in suckling rabbits reflected that of the milk ingested, particularly for n-3. In both n-3 groups, respecting to the control, the concentration of n-3 was higher and that of n-6 lower; mainly due to the variations in LNA and AA. The n-3/n-6 ratio was particularly high in the LCP group (0.38 *vs.* 0.23 and 0.13 for LNA and control groups), where the proportion of LCPn-3 was much higher than the other groups (4.95 *vs.* 1.03 and 0.82%). Such an increase of n-3 was associated with a decrease of AA (3.18 and 3.59 *vs.* 4.09%, respectively for LCP, LNA and Control). Even the fatty acid profile of the liver reflected that of the milk and showed variations analogous to those found in plasma.

Concerning the fatty acid composition of the whole carcass, the n-3 supplemented groups showed lower percentages of SFA, higher of MUFA and a reduction of LA (20.35 and 20.41 *vs.* 22.11%) and AA (2.20 and 2.09 *vs.* 2.55%); LNA group showed an

increase of LNA (6.08 *vs.* 4.05 and 4.80%). Some differences were also found between LNA and LCPn-3 group; in the last, the levels of EPA and DHA and even the n-3/n-6 ratio was higher (0.33 and 0.35 *vs.* 0.21).

Table 2. Chemical composition of milk (%) and fatty acid profile (% of total fatty acids) of milk, plasma, liver and carcass

Milk	Control	LNA	LCP	SE
Moisture	72.15	71.84	71.99	2.56
Crude protein	12.41	12.58	12.50	0.87
Ether extract	9.99	10.25	10.09	1.54
Lactose	3.04	2.79	3.03	0.25
Ash	2.40	2.54	2.39	0.14
SFA	76.82	76.02	76.57	1.02
MUFA	10.73a	11.76b	10.07a	1.25
C18:2n-6 LA	8.99b	7.42a	7.45a	0.53
C20:4n-6 AA	0.63	0.59	0.57	0.24
Total n-6	9.62a	7.94b	8.02b	0.97
C18:3n-3 LNA	1.79a	3.43b	1.82b	0.89
C20:5n-3 EPA	0.05a	0.06°	1.16b	0.19
C22:6n-3 DHA	0.03a	0.02a	0.91b	0.31
LCPn-3	0.87a	0.75a	3.39b	0.74
Total n-3	2.83a	4.28b	5.34b	1.98
PUFA	12.45	12.22	13.86	1.06
n-3/n-6	0.29a	0.54b	0.67b	0.58
Plasma				
SFA	52.76	52.63	52.20	3.76
MUFA	20.45	20.22	19.83	2.59
C18:2n-6 LA	19.58c	18.45b	17.02a	3.38
C20:4n-6 AA	4.09	3.59	3.18	0.91
PUFA n-6	23.67c	22.03b	20.20a	3.52
C18:3n-3 LNA	2.05a	3.86b	2.63a	0.52
C20:5n-3 EPA	0.11a	0.12°	1.24b	0.05
C22:6n-3 DHA	0.32°	0.34a	1.81b	0.51
LCPn-3	0.82a	1.03a	4.95b	0.97
Total n-3	3.12a	5.14b	7.77c	1.04
PUFA	26.79	27.15	27.97	4.09
n-3/n-6	0.13a	0.23b	0.38c	1.54
Liver				
SFA	53.18	53.72	52.75	4.93
MUFA	17.41	17.05	16.14	2.95
C18:2n-6 LA	20.22b	19.01a	19.35a	1.30
C20:4n-6 AA	3.83b	3.36b	2.45a	0.34

Total n-6	24.81b	23.14a	22.31a	1.55
C18:3n-3 LNA	1.83a	3.74b	3.37b	1.82
C20:5n-3 EPA	0.20a	0.21a A	0.97b	0.29
C22:6n-3 DHA	1.37°	1.07°	2.67b	0.81
Total n-3	4.60a	6.11b	8.81c	1.15
PUFA	29.41	29.25	31.11	2.95
n-3/n-6	0.18a	0.26b	0.39c	1.91
Whole carcass				
SFA	56.03b	49.67a	51.16a	2.04
MUFA	13.54b	19.57b	17.88b	2.62
C18:2n-6 LA	22.11b	20.35a	20.41a	0.65
C20:4n-6 AA	2.55b	2.20a	2.09a	0.16
Total n-6	25.19b	23.15a	22.94a	1.86
C18:3n-3 LNA	4.05a	6.08b	4.80a	2.20
C20:5n-3 EPA	0.11a	0.19a	0.67b	0.29
C22:6n-3 DHA	0.15A	0.41B	1.30C	0.81
Total n-3	5.24a	7.61b	8.02c	1.82
PUFA	30.43	30.76	30.96	2.58
n-3/n-6	0.21a	0.33b	0.35b	0.14

no: 20/treatment. [a b c] Means in the same row with different superscripts differ significantly (P<.05, respectively).

Presumably, the differences showed in the pups suckling 'n-3 enriched' milked, could be better explained considering that the real intake of LCPn-3 (g fatty acid) depends on the percentage of the fatty acid and on the milk fat which, during the first days of lactation should be higher (PASCUAL *et al.,* 1999). A strategy based on diets enriched with LNA gave satisfactory results, even if a relevant enrichment of pup's tissues in DHA was obtained only by the feed containing preformed LCP. The milk from does given this diet had very high level either of EPA or DHA as well as the plasma and the liver of pups nursed by such mothers. In spite of the low amount in diets and respective milks, in two groups not supplied with LCPn-3 the liver the DHA level was rather high. This fact confirmed the crucial role of DHA and the capacity of does to synthesise it from the precursor. The increased level of EPA and DHA were associated with a decline in AA, as observed in previous works in other species. GOUSTARD-LANGELIER *et al.* (1999), found a reduction of AA in liver and plasma phospholipids by adding 4.5% of fish oil in a formula for piglets, while using the same source at 1.5% this decline was controlled. ALESSANDRI *et al.* (1998), by administering a fish-oil low in EPA, supplied the DHA required by the piglets without increasing EPA. CARLSON (1982), by feeding human infants formulas supplemented with fish oil high in EPA, found a prejudicial reduction of AA which was accompanied by growth reduction. Nonetheless, when fish oil high in DHA but low in EPA was supplemented no such adverse effects were observed. ROOKE *et al.* (2001), feeding with fish oil to pregnant sows defined the optimum amount to obtain the greatest accumulation of DHA in brain, with minimum reduction in AA. The EPA competition with AA for the production of different prostaglandins and leukotrienes

is the presumable cause of metabolic abnormalities (LANDS, 1991). In our trial the reduction of AA was registered in plasma, liver and body fat but we did not observe abnormalities during the brief life period of rabbits. Probably, the amount of supplemented source of LCP was moderate (2%) and the ingestion of breast milk was a more natural mean, because the mother's organism presumably carried out a modulator action to prevent an unbalance in EPA/AA ratio in milk.

CONCLUSIONS

This study assesses that important enrichments in n-3 in young rabbits could be obtained by different dietary strategies. The enrichment of tissues in DHA was very relevant by administering preformed LCP. However, growing rabbits confirmed their ability to elongate-desaturate dietary LNA and to fix LCPn-3 in cell membrane. Such a strategy, considering the negative correlation between meat LCP and oxidative stability (DAL BOSCO *et al.,* 2004) could be more equilibrate to match the enrichment either in LCPn-3 or and stability. Further research must be performed to ascertain the possible benefits for young rabbits in terms of body development and growth, immune response and resistance to diseases.

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