COMPARISON OF AN ESTIMATED INDEX OF FATTY ACID METABOLISM AND LIVER Δ6-DESATURASE ACTIVITY IN RABBIT

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ABSTRACT

The aim of this study was to verify the accuracy of Δ6-desaturase (Δ6D) index estimated from fatty acid profile of liver and meat of rabbit in comparison with its real metabolic activity directly evaluated in the liver microsomes. Two experiment were carried out using different diets (Control *vs* Linolenic diets) and genotypes (selected *vs* local breeds) for the comparison of Δ6D index and enzyme activity assay. Concerning the feed effect, a lower estimated index was found in Linolenic samples, both in liver and muscle, and this trend was only confirmed by the desaturase activity towards the n-3 fatty acids. The total and n-6 fatty acid-enzyme activity did not show significant differences between groups, as well as any correspondences with the indexes. The "selected" rabbits showed lower indexes respect to the local ones. The activity of the enzymatic complex has instead highlighted an opposite trend for the n-3 and the n-6 series and total one. In particular, the enzyme activity toward n-3 followed the same tendency of the index, whereas the other two determinations showed an opposite trend. The results of this comparison demonstrate that the calculation of the proposed index could be used as surrogates of the measure of liver Δ6-desaturase activity in lipid metabolism of rabbit, but only if specific n-3 pathway is considered.

Key words: Rabbit, meat, lipid metabolism, desaturase, fatty acids

INTRODUCTION

In recent years, the fatty acid composition of meat has been intensively studied considering its strong implications in human health. Meat of monogastric animals could represent a source of long-chain n-3 polyunsaturated fatty acids (n-3LCP) conversely to the fish, whose consumption is generally low in European countries (Welch et al., 2002). Fish and livestock animals have developed different abilities to convert the precursor C18:3n-3 (α-Linolenic acid, LNA) into n-3LCP, greatly depending on the efficiency of elongase and desaturase enzymes (mainly represented by the Δ6-desaturase complex - Δ6D). Therefore, the assessment of activity, expression and substrate preference of this enzymatic complex is becoming of a certain importance in animal science in order to deepen the knowledge on lipid metabolism and to ameliorate the nutritional characteristics of the livestock products. Being the determination of the activity of these enzymes laborious and expensive, some Authors (Vessby et al., 2002) proposed certain indexes as surrogates of the measure of the "true" elongase/desaturase activity. The activity of Δ6D is downregulated by high dietary LCP fatty acids level, and hence, the degree of conversion of essential fatty acids to their longer and more unsaturated metabolites is lower (Nakamura et al., 2004; Rodríguez et al., 2019). Moreover, genetic selection can modify the expression of genes coding for enzymes involved in LCP synthesis as well as the relative enzymatic activity (Castellini et al., 2016).

To the best of our knowledge, no comparison between estimation and analytical determination have been carried out in rabbit; thus, the aim of the present study was to define the real applicability of an estimated index of fatty acid metabolism, through its comparison with the Δ6D enzyme activity assay.

MATERIALS AND METHODS

Animals and experimental design

Experiment 1

Forty male New Zealand White rabbits at the weaning age (30 days) were housed in the experimental farm of the University of Perugia (temperature 18-23 °C, humidity 50 ± 5%) and divided in two homogeneous groups. Each treatment consisted of 20 rabbits and each group receiving a different diet (Table 1) as follows:

· Control group was fed *ad libitum* with the control diet containing soya bean oil as the main source of fat;
· Linolenic group was fed the control diet, substituting 10% of the soy bean oil with extruded flaxseed;

Table 1. Formulation (%) of experimental diets.

Ingredients	Control	Linolenic
Barley meal	9.0	5.0
Fine wheat bran	30.0	26.4
Dehydrated alfalfa meal	40.0	50.00
Soybean meal 44% crude protein	4.0	3.50
Soybean oil	0.5	-
Sunflower meal 30% crude protein	9.0	5.00
Extruded linseed	3.0	10.0
Estimated digestible Energy**(MJ)	2.34	2.38

Experiment 2

Forty males (30 days) rabbits of two different genotypes were used and reared as in Experiment 1. In particular, both twenty commercial rabbits (New Zealand White rabbits) and rabbits of local strain (Grey-colored italian rabbits, conserved for more than 30 years in small-sized farms in Central Italy) were used and fed with the above described control diet.

Sampling and analysis

At 85 d, 10 rabbits per group of the two experiments, with weights close to the average of the group (±10%), were selected and killed by cutting their carotid arteries and jugular veins after electrical stunning. Handling and dissection of the refrigerated carcasses (24 h at 4 °C) were performed as proposed by Blasco and Ouhayoun (1996). Within a few minutes, samples of liver and *Longissimus lumborum* (LL) muscle were cut into appropriately sized pieces (30 mg). Livers samples were partially rinsed with RNAse-free water and frozen at -80 °C for subsequent evaluation of enzyme activity (Maranesi et al., 2015). Lipid extraction of liver and *Longissimus lumborum* muscle was performed according to Folch et al. (1957) and esterification was performed according to Christie (1982). The trans-metilation procedure was carried out using enicosenoic acid methyl esters (Sigma Chemical Co.) as the internal standard. The fatty acid composition was determined using a Varian gas-chromatograph (CP-3800) equipped with a flame ionisation detector and a capillary column of 100 m length × 0.25mm × 0.2 μm film (Supelco, Bellefonte, PA, USA). Helium was used as the carrier gas with a flow of 2 mL/min.

The Δ6-desaturase (Δ6D) activity was determined for the different PUFA series (n-6 and n-3) by measuring respectively, the amounts of 18:3n-6 produced from 18:2n-6 (Linoleic acid, LA) and 18:4n-3 produced from LNA. The reaction medium, in a total volume of 0.5 mL, contained the following: 4 mM ATP, 0.5 mM CoASH, 1.25 mM NADH, 2 mMMgCl2, 12 mM NaF, 1.5 mM glutathione, 0.5 M potassium phosphate, pH 7.2, 40 nmol 1-14C-labeled fatty acids (AS ≅ 3 nCi/nmol), and 5mg of microsomal proteins. Considering the amount of free LA and LNA in microsomes, in LA incubations totals of about 70 nmol and 64 nmol LA were present, whereas endogenous LNA was almost negligible

(about 4 nmol in F and 8 nmol in S). In LNA incubations, the activity was determined in the presence of about 30about30 nmol (F) and 24 nmol (S) LA.
To estimate the activity of Δ6-desaturaseΔ6D the following index was calculated from the fatty acid profile of liver and muscle (Dal Bosco et al., 2012):

[(C20:2n-6 + C20:4n-6 + C20:5n-3 + C22:5n-3 + C22:6n-3)/(LA + LNA + C20:2n-6 + C20:4n-6 + C20:5n-3 + C22:5n-3 + + C22:6n-3)] × 100.

Statistical analysis
A linear model (STATA, 2005, procedure GLM) was used to evaluate the effect of feeding and genotype on estimated and real Δ6-desaturase activity. The statistical significance of differences was assessed using t-tests.

RESULTS AND DISCUSSION

The estimated indexes and enzyme activity of Δ6-desaturase in rabbits fed different diets or belonging to two different genotypes are reported in Table 2.

Table 2 Estimated and enzyme activity of Δ6-desaturase (pmol in 30 min/mg protein) in rabbits fed different diets or belonging to different genotypes

	Diets		Pooled SE[1]
	Control	Linolenic	
Estimated Δ6-desaturase index (liver)	36.2[b]	28.9[a]	2.4
Estimated Δ6-desaturase index (LL muscle)	28.6[b]	25.3[a]	2.4
Δ6-desaturase for C18:3n-3	20.5[b]	17.2[a]	3.8
Δ6-desaturase for C18:2n-6	177.5	181.2	10.8
Total Δ6-desaturase activity	198.0	198.4	14.7
	Genotypes		Pooled SE[1]
	Commercial	Local	
Estimated Δ6-desaturase index (liver)	31.8[a]	33.1[b]	1.5
Estimated Δ6-desaturase index (LL muscle)	27.8[a]	30.6[b]	1.5
Δ6-desaturase for C18:3n-3	19.1[a]	26.9[b]	2.4
Δ6-desaturase for C18:2n-6	175.2[b]	41.5[a]	15.0
Total Δ6-desaturase activity	194.3[b]	68.4[a]	17.5

Means in the same row and tissue with different letters differ significantly ($P \leq 0.05$).
[1] SE: Standard Error

Concerning the feeding effect, lower estimated indexes were calculated in Linolenic group respect to Control (both in liver and muscle), and this trend was confirmed by the enzyme activity towards n-3. On the contrary, the total and n-6 oriented Δ6D activity did not show significant differences between groups. It is widely known that n-6 and n-3 fatty acid series compete for the same enzymes, but the affinity of the Δ6D is retained greater for n-3 than for n-6 fatty acids. Thus, the conversion of LA to n-6LCP metabolites decreases when the intake of n-3 fatty acids increases (Lands, 1992). Nevertheless, the above-mentioned preference of Δ6D for n-3 is neutralised by the fact that standard diet had higher content of n-6 fatty acids, especially LA, respect to Linolenic. The calculation of the indexes in different genotypes confirmed our previous findings (Dal Bosco et al., 2014) and in particular, that the fatty acid metabolism of animals selected for productive performance differed from those of local strains. Specifically, the NZW rabbits always showed lower indexes respect to the local ones.

The results of the present study confirm our previous study in poultry, where the estimated enzyme Δ6D activity was always higher in local breeds than in much selected commercial hybrids (Dal Bosco et al., 2012). Hence, the higher estimated Δ6D observed in local rabbits, can suggest a genetic effect on the

mechanisms of desaturase and elongase responsible for the synthesis of LCP fatty acids. Moreover, the real activity of the Δ6D enzymatic complex highlighted an opposite trend for the n-3 than the n-6 series and total activity. In particular, the enzyme activity for n-3 fatty acids followed the same trend of the estimated indexes, whereas in the other two determinations the trend was opposite. These results agree with Castellini et al. (2016), which observed a lower total Δ6D activity in slow growing rabbits respect to fast growing ones (45.2 *vs* 104.4 pmol/30 min/mg protein). In the latter research about 90 % of the activity was directed toward LA, whereas only 10 % toward LNA. In contrast, the Δ6D activity of slow-growing rabbits was 38.5% toward LNA and 61.5 % toward LA with higher affinity for LNA. These results showed that genetic selection for productive traits in rabbit modifies the expression and the activity of Δ6D. Thereafter, the Δ6-desaturase activity seems affected by precursor availability, but the major modifications in the LA/LNA ratio do not change substantially this tendency and that preference for LCP series in rabbit is mainly due to the intensity of genetic selection.

CONCLUSIONS

The results of this comparison represent a first step of research towards a hypothetical use of the proposed index in the study of the rabbit lipid metabolism as surrogate of the true desaturase/elongase activity. In this study, the Δ6D index only mimic the activity toward the n-3 series would seem usable; indeed; indeed, the trend of estimated indexes and the total and n-6 direct enzyme activities were not superimposable. Obviously, further and thorough studies are needed both to expand the available database and to deepen the knowledge on the lipid metabolism in rabbit, and finally, to validate the potential applicability of the indexes.

ACKNOWLEDGEMENTS

The research was funded by ‘Ricerca di base 2018’ University of Perugia

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