RABBIT IS PARTICULARLY INTERESTING TO DEPOSIT DHA IN ITS MEAT, WITHOUT EFFECTS ON MEAT'S ORGANOLEPTIC QUALITY – A REVIEW

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ABSTRACT

Docosahexanoic acid (C22:6 ω3) generally known as DHA, is the predominant structural fatty acid of cell membrane phospholipids. The recent recommendation for humans is 250 mg DHA/day, about twice the present daily DHA intake in modern populations. Increasing the human DHA intake up to recommendations improves clearly the health status. Because fish as source of DHA cannot be increased, because level in other animal products is insufficient and because higher plants are not able to synthesize this fatty acid, DHA production with microalgae (MA) like *Schizochytrium* spp has been developed. DHA content of most MA cultures used in experiments was 18% but it can be increased up to 28% of dry matter. This product is well accepted by animals and is able to largely increase the DHA content of animal products. But excessive incorporation in animals diet may induce problems of palatability of the products. For rabbit the inclusion level must remain below 1.5% MA. Maximum limits vary widely between species *e.g* 0.8%¨MA for pigs and 3.7% MA for broiler chickens. With respect of the maximum incorporation level, fixation rate of the dietary DHA obtained from MA in edible parts of the rabbit is close to 40%. It is 31% for eggs production, 18% in chicken meat, 15% in pork meat, 10% for dairy milk and only 6% for beef meat or trout fillet. Simultaneous inclusion in diets of C18:3 ω3 sources with MA reduces the rate of fixation of DHA for all studied species, but in any case rabbit remains the most efficient species for fixation of DHA in its edible parts.

Key words : rabbit, DHA, lipids, human nutrition

INTRODUCTION

Docosahexanoic acid (C22:6 ω3) generally known as DHA, is the predominant structural fatty acid of cell membrane phospholipids, particularly those of the retina and the neuronal synapses in the brain (Bradbury, 2011). Physiologically, DHA is derived from linolenic acid (C18:3 ω3 - ALA) after elongation and desaturation. Unfortunately, even in presence of large amounts of ALA, the DHA synthesis is clearly too low to provide enough DHA to human organism. In addition, the increasing daily intake of ω6 fatty acids, as observed during the last century in westerns countries, reduces the endogenous DHA synthesis because of the competition for △6 desaturase enzyme (Givens and Gibbs, 2008).

This is why, a substantial contribution of preformed dietary DHA is necessary for a good health of the populations, but this is rarely observed. For example, in France average intake of DHA by human population is only 137 mg/day while the official minimum recommendation is 250 mg/day; the situation is similar in quite all modern societies (Givens and Gibbs, 2008; Anses, 2016; Bradbury, 2011). Increasing the human DHA intake up to recommendations, decreases the cardiovascular risk (Anses 2011), the insulin-resistance, the diabetes and obesity (Delarue et al., 2004), the metabolic syndrome (Delarue et al., 2006), reduces the Age-related Macular Degenerescence (Anses 2011) or the Alzheimer's diseases (Morris et al., 2003; Hooijmans et al., 2009). According to different results it improves also the cognition in the youth and decreases the depression (Nelson et Van Elswyk, 2015 ; Zhang et al, 2016).

SOURCES OF DHA

The plants generally used in human nutrition, like all others higher plants, are not able to synthesize DHA because of their universal lack of the necessary enzymes (Robert et al., 2005). Consequently higher plants cannot be included in the list of potential sources of DHA. Thus for human, the easiest way to obtain preformed DHA is to eat animal products. Among the animals, fishes have clearly a higher DHA content than terrestrial animals (Castro-Gonzales, 2002; Barnathan, 2007). But, the prospective increase of fish proportion in human food in order to better cover the requirement in DHA appears totally unrealistic, due 1/ to the world decrease of the fishery resource (Zambonino-infante, 2009) and 2/ to the increase of their contamination with heavy metals and persistent organic pollutants. Among the products issued of terrestrial animals (meat, milk,…), the normal DHA content is most generally relatively low between 5 and 20 mg/100 g (Givens and Gibbs, 2008). Chicken egg is the only classical animal product to have a significant content of DHA : 35 to 200 mg/100 egg (Simopoulos and Salem, 1992). However the DHA content of the classical rabbit meat is also relatively high compared to the other meats with an average content of 20 to 32 mg/100g meat and up to 45 mg/100 g in the liver (Bernardini et al., 1999; Combes 2004; Dal Bosco et al., 2007) but this proportion is insufficient to increase significantly the average human DHA intake.
An alternative to the consumption of fish is the increase of the DHA level in the feed of terrestrial animals in order to increase the DHA content of the animal products consumed by humans. For all tested animals, addition of a DHA source in their feed, effectively increases the DHA content of their products, but with an efficiency extremely variable between animals (Raes et al., 2004; Mourot, 2010; Betiku et al., 2016)
For the reasons previously explained concerning quantity and quality, the DHA source for animal feeding cannot be fish meal or fish oil. Other sources are therefore sought. Despite the fact that higher plants are basically not able to synthesize DHA, attempts to introduce the lacking enzymes by genetic engineering are in progress (Robert et al., 2005). But presently the encouraging Canadian or Australian works conducted with canola or *Camelina sativa* are still at the pilot step (Mansour et al., 2014; Walsh et al., 2016). On the contrary, some unicellular algae-like, known as microalgae and very rich in DHA (7 to 28%% of the dry matter content according to the production conditions) such as thraustochytrids (*e.g. Schizochytrium* spp), are now industrially produced and commercially available, even if some improvement are still desirable to reduce the cost of production (Barnathan, 2007; Winwood 2013; Smith, 2017; Martek Biosciences Corporation, 2020). The DHA content of the microalgae presently available on the market (2020) is comprised between 18 and 28%. However, data presented in this review were generally established with MA containing 18 % of DHA, the concentration in MA employed in most published experiments.

The purpose of this review is to compare the proportion of added DHA to animal feeds, which can be fixed in their edible parts by the different animal species, without alteration of the acceptability of products by human (taste, odours, texture, ….).

UPPER ACCEPTABLE LIMIT FOR INCORPORATION OF DHA (MICROALAGAE, …) IN ANIMAL FEEDS

The addition of DHA as microalgae in their feed is most generally well accepted by animals. However with the highest levels, some negative effects on the palatability of the products have been observed in different species. Before calculation of the rate of DHA fixation, it is necessary to determine the highest acceptable dietary level for microalgae incorporation or more generally the maximum DHA content in animal feeds which doesn't deteriorate the taste and smell of the products. Limits for the main species are given below and the recommended maximum level for microalgae supplementation (*Schizochytrium* spp – 18% DHA) are summarized in table 1.

➤ In a total of 7 experiments conducted with rabbits, no problem of meat quality was observed with 0.3% added DHA in the diet, while organoleptic alterations were described when the addition was increased at 0.8% DHA or more (Colin et al., 2017).
➤ Unpleasant tastes (aged flavor and strong fishy flavor) were found in dry-cured shoulders from pigs fed on diet with 0.3% DHA, while no problem was observed with only 0.1% additional DHA (Sarraga et al.,

2007; Meadus et al., 2009). The upper acceptable limit for pig diets is probably 0.2% added DHA whatever the origin (fish oil or microalgae) because with such an addition, no sensory problem was detected for dry cured ham in the work of Vossen et al. (2010)

➤ For young goats (kids), no sensory problem was observed when 0.9% DHA was added in the goat milk replacer, while with the double dose (1.8% DHA) kids produced meat with unusual odours, unpleasant flavours, and low overall appreciation scores (Moreno-India et al., 2012).

➤ Betiku et al. (2016) observed a deterioration of the texture of the trout fillet and an excessive unpleasant fishy odor when 1% of DHA was added to trout's diet as microalgae. But with one half this dose, texture and general acceptability were considered as unchanged in comparison with the control.

➤ In broiler chicken production, the overall acceptability of meat was negatively affected by the dietary supplementation with 1.25% of DHA (7.4% of microalgae in the diet), but with lower supplementation (0.62% DHA in the diet) meat sensory properties were not affected (Ribeiro et al., 2013)

Table 1: Maximum dietary level of microalgae to avoid sensory alterations (% diet) for different animal productions

Meat production of	% of diet
- Beef (Steer, Heifer)	< 0.5%
- Broiler chicken	3.7%
- Goat (young kids)	5.0%
- Pork	0.8%
- Rabbit	1.5%
- Trout	6.2%
Dairy milk	5.0%
Chicken eggs	4.8% *

* *microalgae with only 7.4% DHA in DM*

➤ When microalgae (MA) were added in daily beef ration, off-flavors (seaweedy flavor), were noticed in the steaks (meat slice or ground). Intensity of this defect grew with the increasing amount of MA in heifers concentrate, in the range of 0.5 to 3% MA (0.1 to 0.55% DHA) (Rodriguez et al, 2018, Phelps et al., 2016 a, b). This is why the maximum level of MA for beef production would most likely be less than 0.5% DHA of the ration.

➤ On the contrary, milk palatability was unaffected when dairy cows received 5% microalgae in their diet (0.9% DHA) in comparison with the control diet also distributed *ad libitum* (Franklin et al., 1999).

➤ Laying hens receiving a diet with up to 4.8% of experimental microalgae (0.36% additional DHA in this case) produced eggs without significant alteration of their acceptability (Herber and Van Elswyk, 1996). For their part, Delarue et al., (2017) observed that 42 % of the consumers detect a difference between the control eggs and those produced by laying hens receiving a feed containing 0.6% MA (0.17% DHA) but without statistical preference for one or the other type of eggs. But it must be underlined that if the same total ω3 fatty acid in the yolk was obtained with fish oil (3% of the diet, *i.e.* 0.28% DHA) than in the previously mentioned study of Herber and Van Elswyk (1998), eggs were of poor sensory quality with a fishy taste (Fraeye et al., 2012). In addition, use in laying hens diet, of oil extracted from microalgae strongly reduced acceptability of eggs. This is most probably in relation with the low level of carotenoids (natural antioxidants) after extraction and with the decrease of lipids stability during the extraction process (Cachaldora et al., 2008)

PERCENTAGE OF FIXATION OF DHA FOR DIFFERENT PRODUCTIONS

As seen in the previous part of this short review, the highest acceptable supplementation level of preformed DHA varies widely from one production to the other. As frequently as possible, the fixation rate was calculated by interpolation from various publications for a common average DHA supplementation of 0.15% DHA (~0.8% MA), a value below the maximum recommended level for rabbits, chicken broilers and egg production. Because of the scarcity of experimental results, higher supplementations were used in calculations for pig meat and trout, although some bad palatability problems were encountered at these levels. For ruminants (beef meat and dairy milk) supplementation was calculated as grams of DHA added daily in the concentrate.

Total intake of DHA was calculated according experimental results of daily intake x duration of the experimental periods taking place before slaughter, *i.e.* some weeks to some months. However, for eggs and milk production, only the daily feed intake was taken in consideration but after a sufficient period of adaptation. This period is of importance since in the dairy cow for example the hydrogenation of DHA in the rumen is negligible during the first days following the first distribution, but increases dramatically after some weeks, reducing in parallel a possible transfer into milk (Chilliard et al., 2001).

The quantity of preformed DHA fixed was estimated by difference between the total content of the DHA in the edible parts produced by control and by supplemented animals. The total quantity of DHA in the edible part was estimated according to 1/ the DHA content of the products (mg/100g) observed in experiments 2/ the proportion of edible parts in the carcass and 3/ the slaughter yield frequently obtained from general literature or experimental data.
The percentages of fixation of the DHA vary a lot between the different species (table 2). The rabbit appears as having the highest-percentage of fixation of the vegetable DHA. This percentage of deposit is particularly higher (39.4 *vs* 30.6%) and less variable (13.0 *vs* 68.2%) than that of the laying hens for the egg, considered as one of the best foods for human DHA supplying, apart from fishes (Simopoulos et al., 1992, Šefer et al., 2011). This characteristic is probably explained by the low transformation of the ingested fatty acids during their deposit in the rabbit lipids combined with a low endogenous fatty acids production in the rabbit (Ouhayoun et al., 1985, 1987) .

Table 2: Rate of fixation of the ingested DHA in their edible products by different species

Species	Product	DHA + ALA[1]	DHA in the diet	Number of experiments	DHA Fixation rate %	Fixation Var. coef.%	DHA in edible prod. mg/100g
Rabbit	meat	no	0.15%	6	39.4	13.0	200
Rabbit	meat	yes	0.15%	30	25.3	24.6	130
Chicken	eggs	no	0.15%	13	30.6	68.2	213
Chicken	eggs	yes	0.15%	20	21.3	56.2	195
Chicken	meat	no	0.15%	6	18.9	145.0	67
Trout	whole fish	no	1.38%	2	18.5	NA	699
Trout	fillet				3.3	NA	166
Pig	meat	no	0.60%	1	14.9	NA	56
Pig	meat	yes	0.34%	1	13.2	NA	45
Dairy cow	milk	no	28 g/d	18	10.3	51.0	12
Beef	meat	no	18 g/d	4	6.3	78.8	18

[1] No : Supplementation of feed with only DHA (Microalguae); Yes : supplementation with DHA and ALA (C18:3 ω3)

The fixation of the ingested DHA in the edible parts of the rabbit is higher when the feed contains DHA as unique source of omega 3 fatty acid than when it is associated to a source of ALA (flax or rapeseed), confirming the previous observations (Colin et al., 2012). The laying hens and pigs present the same phenomenon which can be explained either by an increase of the beta-oxidation due to the higher quantity of poly unsaturated fatty acid or by a decrease of the endogenous synthesis of DHA from ALA. But even with this decrease of DHA fixation due to ALA, the rabbit is the species presenting the highest percentage of ingested DHA fixed in the consumable parts.
Finally, the DHA content of edible products for each type of production is indicated in table 2, demonstrating meat of rabbit fed vegetable DHA is one of the richest product available for human nutrition. This aspect is developed in an other communication presented in this Congress (Colin et al., 2020)

ACKNOWLEDGMENT

Authors thank the French region of Brittany and the Department of Finistère for their financial help in this project.

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