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Original article

Increasing levels of two different fish oils lower ruminal biohydrogenation of eicosapentaenoic and docosahexaenoic acid in vitro

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Abstract — Ruminal biohydrogenation of dietary *n-3* fatty acids limits any attempt to increase their contents in products of ruminants. The aim of the study was to determine whether total lipolysis, release rate of eicosapentaenoic (EPA) and docosahexaenoic acids (DHA) from triacylglycerols (TG), their biohydrogenation and their accumulation as unesterified fatty acids was affected by the fish oil type (FOa; FOb), inclusion level (12.5, 25, 50, 75, 100 and 125 mg per incubation flask) and incubation time (24 h; 48 h). The two fish oils which differed in EPA (FOa: 18.7%; FOb: 5.8%) and DHA (FOa: 11.7%; FOb: 7.6%) concentrations were incubated using a batch culture technique. Total lipolysis of fish oil decreased with increasing oil level at 24 h ($P < 0.001$). By contrast, at 48 h total lipolysis tended to be higher at 25 compared to 12.5 mg but remained constant (FOa: 74%; FOb: 81%) with inclusion levels above 25 mg. Although EPA and DHA proportions were markedly higher in FOa, release rates were similar for the two fish oils. Rates of EPA and DHA released from TG of both oils decreased with increasing levels ($P < 0.001$) and were higher at 48 h ($P < 0.001$). Biohydrogenation of EPA and DHA were lower with FOa and increasing inclusion levels ($P < 0.001$). Concurrently, the accumulation of unesterified EPA and DHA was more pronounced in FOa and increasing inclusion levels ($P = 0.06$). Compared to 24 h, 48 h of incubation enhanced the biohydrogenation of EPA and DHA ($P < 0.001$) and also increased their accumulation in the unesterified form ($P < 0.001$). In conclusion, the release and the biohydrogenation of EPA and DHA primarily depended on the amount of supplied oils and the accumulated unesterified fatty acids, respectively.

fish oil / EPA / DHA / lipolysis / biohydrogenation

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Résumé — Une supplémentation croissante de deux huiles de poisson diminue la biohydrogénation ruminale des acides eicosapentéanoïque et docosahexéanoïque in vitro. La biohydrogénation dans le rumen des acides gras *n-3* limite les possibilités d'augmenter leur teneur dans les produits des ruminants. Le but de cette étude était de déterminer si le type d'huile de poisson (FOa ; FOb), son niveau de supplémentation (12,5 ; 25 ; 50 ; 75 ; 100 ; et 125 mg par flacon d'incubation) et la durée d'incubation (24 h ; 48 h) avaient un effet sur la lipolyse totale, sur le taux de libération des acides eicosapentéanoïque (EPA) et docosahexéanoïque (DHA) à partir des triacylglycérols (TG), sur leur biohydrogénation et sur leur accumulation en tant qu'acides gras non estérifiés. Les deux types d'huile de poisson qui différaient par leur concentration en EPA (FOa : 18,7 % ; FOb : 5,8 %) et en DHA (FOa : 11,7 % ; FOb : 7,6 %) ont été incubés en utilisant une technique in vitro de type « batch ». La lipolyse totale de l'huile de poisson a diminué après 24 h avec l'augmentation du taux de supplémentation ($P < 0{,}001$). En revanche, après 48 h, la lipolyse a eu tendance à augmenter avec un niveau de supplémentation de 25 mg comparativement à un niveau de 12,5 mg, et est restée constante au-delà de 25 mg (FOa : 74 % ; FOb : 81 %). Les taux de libération ont été semblables pour les deux types d'huile, bien que les proportions d'EPA et de DHA aient été nettement plus élevées dans FOa. Les taux d'EPA et de DHA libérés par les TG des deux types d'huile ont diminué avec l'augmentation du niveau de supplémentation ($P < 0{,}001$) et furent plus élevés après 48 h ($P < 0{,}001$). La biohydrogénation de l'EPA et du DHA a été plus faible avec l'huile FOa et avec l'augmentation du niveau de supplémentation ($P < 0{,}001$). En même temps, l'accumulation des acides non estérifiés EPA et DHA fut plus élevée avec l'huile FOa et avec les taux croissants d'adjonction ($P = 0{,}06$). L'incubation de 48 h comparée à celle de 24 h a augmenté le taux de biohydrogénation de l'EPA et du DHA ($P < 0{,}001$) et leur accumulation sous forme non estérifiée ($P < 0{,}001$). En conclusion, le taux de libération et d'hydrogénation de l'EPA et du DHA dans le rumen dépend, principalement et respectivement, de la quantité d'huile ajoutée et de l'accumulation des acides gras non estérifiés.

huile de poisson / EPA / DHA / lipolyse / biohydrogénation

1. INTRODUCTION

Fish and fish oil are important sources of *n-3* fatty acids, mainly of eicosapentaenoic [EPA; C20:5(*n-3*)] and docosahexaenoic acids [DHA; C22:6(*n-3*)] [10], which are known to have beneficial effects on human health (reviewed by Givens et al. [10]; Williams [29]). EPA and DHA can either be provided directly by the diet or synthesised de novo in the tissue from their precursor, linolenic acid. However, the synthesis rate has been reported to be low in humans [29]. Furthermore, low EPA and DHA concentrations are found in meat, milk and milk products of ruminants which constitute an important part of Western diets [18]. Therefore, various studies have been conducted in view of demonstrating the potential of fish oil to increase the concentration of *n-3* fatty acids in milk and meat [16, 17].

In general, dietary unsaturated fatty acids are biohydrogenated in the rumen by micro-organisms thereby decreasing their availability for absorption in the small intestine. In vivo studies by Doreau and Chillard [5] and Scollan et al. [23] both revealed that rumen infusion or dietary supplementation of fish oil cause extensive biohydrogenation of EPA and DHA. Similarly, using the batch culture technique, Fievez et al. [8] reported an almost complete biohydrogenation of these fatty acids also provided as fish oil after 6 or 24 h. By contrast, using fish oil with a higher EPA and DHA concentration, Ashes et al. [1] showed negligible in vitro biohydrogenation of these fatty acids after 48 h. In a dose response study carried out in vitro, Gulati et al. [12] found that EPA and DHA biohydrogenation decreased when, at a constant fat concentration

of 5 mg·mL^{-1} rumen fluid, cotton seed oil was replaced by more than 1 mg of fish oil.

The objectives of the current study were to evaluate whether total lipolysis of fish oils, the individual release of EPA and DHA from triacylglycerols (TG), their biohydrogenation and their accumulation as unesterified fatty acids were influenced by (i) fish oils differing in the proportion of EPA and DHA, (ii) the increasing levels of these fish oils, and (iii) the length of the incubation time.

2. MATERIALS AND METHODS

2.1. Incubation method

In the present experiment a short term rumen simulation technique (batch culture; [25]) was used to study the effects of two fish oil types (FOa: Pronova Biocare, Sandefjord, Norway; FOb: Technological Laboratory of the Danish Ministry of Fisheries, Lyngby, Denmark) on total lipolysis as well as EPA and DHA release and biohydrogenation. The two oils differed in their fatty acid composition (Tab. I). The proportion of EPA and DHA in FOa amounted to 18.7 and 11.7%, respectively, whereas FOb contained 5.8% EPA and 7.6% DHA. Complete lipolysis of fats rich in linoleic acid [LA; C18:2(*n-6*)] and almost complete biohydrogenation of LA were demonstrated by Gulati et al. [12, 13] and therefore a commercial soy oil and its main fatty acid LA (Tab. I) were used as references. Each oil was incubated at six levels of oil supply (12.5, 25, 50, 75, 100 and 125 mg) for either 24 or 48 h in a rumen fluid-buffer-mixture. This mixture was prepared prior to the incubation experiment, from filtered rumen fluid obtained from four fistulated wethers receiving a grass hay diet ad libitum which was mixed at a ratio of 1:4 (vol/vol) with a phosphate buffer (containing per litre distilled water: 28.8 g $Na_2HPO_4 \times 12H_2O$; 6.1 g $NaH_2PO_4 \times H_2O$; 1.4 g NH_4Cl; adjusted to pH 7) and stored under anaerobic conditions (CO_2; 99.99% purity) at 39 °C.

Table I. Analysed fatty acid compositions (% of total fatty acids) of the oil supplements.

Fatty acids	Fish oil a	Fish oil b	Soy oil
C14:0	7.7	8.4	0.1
C16:0	17.0	13.5	11.6
C16:1	9.2	4.6	0.1
C18:0	3.4	1.2	3.7
C18:1 *trans*	1.7	ND[1]	ND
C18:1 *cis*	13.3	12.5	24.0
C18:2*n-6* (LA)	3.6	1.8	53.2
C18:3*n-3*	1.0	1.2	6.2
C18:4	2.7	3.1	ND
C20:1 *cis*	1.6	14.0	ND
C20:5*n-3* (EPA)	18.7	5.8	ND
C22:1 *cis*	2.2	23.0	0.8
C22:4*n-6*	1.2	1.2	ND
C22:5*n-3*	2.5	0.7	ND
C22:6*n*-6 (DHA)	11.7	7.6	ND
Others	2.5	1.6	0.2

[1] ND: not detected.

Because of the small quantities used for incubation, the oils were dissolved in hexane (1:100, wt/vol). The hexane-oil-mixture was placed into glass flasks according to predefined concentrations and the solvent was evaporated under N_2. Subsequently, 0.4 g ground hay (particle size between 0.2 and 2.0 mm) and 25 mL of the rumen fluid-buffer-mixture were added. One third of the flasks (seven per incubation series) were immediately prepared for lipid extraction and served as the control (0 h incubation). In order to maintain an anaerobic condition and to provide gas for the incubation process, the remaining flasks were flushed six times with CO_2 before incubation was started in a shaking water bath

at 39 °C. After 24 h, the incubation of one half of the flasks was stopped by preparing the contents immediately for lipid extraction. The other flasks were refilled with 25 mL fresh rumen fluid-buffer-mixture and 0.4 g hay and incubation was continued for a further 24 h. The re-inoculation was carried out because previous results of our group revealed no effects on rumen fermentation parameters in 24 h batch incubations when fish oil TG were supplemented, whereas changes in the fermentation pattern occurred when supplementing unesterified fatty acids (UFA). The final oil concentration per mL incubation medium differed between the 24 and 48 h incubation amounting to 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 mg oil·mL^{-1} and 0.25, 0.5, 1.0, 1.5, 2.0, and 2.5 mg oil·mL^{-1}, respectively. In the present experiment 12 incubation series lasting 24 or 48 h were carried out. Each incubation series contained three inclusion levels of one fish oil type and one inclusion level of the soy oil. The two fish oils were incubated three times at each inclusion level in duplicate (n = 6). Soy oil was incubated two times at each inclusion level (n = 2).

2.2. Analysis

Lipid extraction was performed as described by Van Nevel and Demeyer [26], with some small modification. Briefly, 10 mg heptadecanoic acid (Sigma, Bornem, Belgium) was added as the internal standard [4] to the content of each incubation flask and then extracted overnight with chloroform/methanol (2:1, vol/vol; C/M). The extracts were centrifuged 15 min at 3000 × *g*. This procedure was repeated twice and the extracts were combined. The extract was then washed three times with distilled water to avoid the formation of methyl ester during further analysis of the samples [25]. The final volume was brought to 100 mL with C/M. The TG and UFA were quantified as fatty acids by a combination of thin-layer chromatography (TLC) and gas chromatography (GC) according to Demeyer et al. [4]. Two mL of extract was dried by evaporation at 40 °C under N_2. The residue was dissolved in 200 µL chloroform and spotted on a glass plate (20 × 20 cm) coated with Silica Gel G (Fluka, Bornem, Belgium) and subsequently developed with hexane-diethylether-acetic acid (70:30:2, vol/vol/vol). Lipid classes were detected under ultraviolet light after spraying with a 0.1% (wt/vol) 2.7-dichlorofluorescein in 2-propanol. The TG and UFA bands were scraped from the plate into separate glass centrifuge tubes. The transestrification was performed with 0.5 mL benzene and 5 mL methanolic H_2SO_4 (115:1; vol/vol) and the methylesters were extracted as described by Demeyer et al. [4]. Individual fatty acids were determined by a gas chromatograph (HP6890, Hewlett Packard, Brussels, Belgium) with a flame ionisation detector, equipped with a CPSil88 fatty acid methyl ester (FAME) column (100 m length, 250 µm id, film 0.25 µm; Chrompack, Middelburg, The Netherlands) using hydrogen as the carrier gas in accordance with the method of Raes et al. [22]. The peaks were identified based on standard retention times and quantified using the peak area of the internal standard. The fatty acids of the fish oils and soy oil were extracted with C/M and FAME were prepared and analysed as described previously.

2.3. Calculation of total lipolysis, fatty acid release rate and biohydrogenation

The amount of fatty acids released from the TG was calculated as the difference between the amount of total fatty acids (total lipolysis), LA, EPA and DHA (release of individual fatty acids) in the TG fraction before and after incubation according to the following formula: rate (%) = 100 × [(difference of TG before and after incubation) / (TG before incubation)] (Eq. 1). The recovered amounts of unesterified EPA and DHA

in the flasks were used to calculate the biohydrogenation (%) of EPA and DHA, which were determined as 100 – [(fatty acid accumulation in the UFA fraction)/(amount of fatty acid released from TG)] × 100 (Eq. 2).

2.4. Statistical analysis

All statistical analyses were conducted using PROC MIXED (SAS, Version 8.00, SAS Institute Inc., Cary, NC, USA). Total lipolysis of FOa and FOb, rates of EPA and DHA released from TG and their biohydrogenation as well as the final amounts of unesterified EPA and DHA were analysed as repeated measurements (incubation time) including fish oil type and inclusion level as the main effects and incubation series as the random effect. Main effects and the respective two and three way interactions with probability levels of $P < 0.05$ were considered significant. In the graphs, two and three way interactions are only indexed when significance occurred. Statistical analysis of the soy oil results was omitted, because of the limited number of samples analysed.

3. RESULTS AND DISCUSSION

3.1. Total lipolysis of fish oils

Ruminal lipolysis of TG is generally described as an extensive and rapid process in which microbial lipases principally hydrolyse the acyl ester linkages, whereas plant enzymes play a secondary role [14]. The lipolysis of soy oil after a 24 h and 48 h incubation period was on average 86 and 90%, respectively, for all six inclusion levels (data not shown). This is in agreement with Gulati et al. [13] who reported the complete lipolysis of sunflower oil (10 $mg \cdot mL^{-1}$ incubation media) after a 24 h incubation period. Although the inclusion level did not seem to influence the extent of lipolysis after 24 and 48 h, Beam et al. [3] reported declining rates of lipolysis (expressed per hour) with increasing soy oil concentrations from 2 to 10% of the substrate corresponding to 0.6 and 3.4 mg oil per mL incubation media, respectively. Compared with soy oil, the lipolysis rate was distinctly lower for FOa and FOb. Regardless of the type of fish oil (Oil: $P = 0.47$), the inclusion level (In: $P < 0.001$) and incubation time (Ti: $P < 0.001$) had a significant influence on the rate of total fatty acids released from the TG (Fig. 1). The lipolysis rate tended to decrease with increasing oil concentrations at 24 h. By contrast, at 48 h lipolysis tended to increase from 12.5 to 25 mg and was constant when exceeding an inclusion level of 25 mg at an average rate of 74 (FOa) and 81% (FOb) (In × Ti: $P < 0.001$). Doreau and Ferlay [6] suggested that the rate of lipolysis depends on the microbial ecosystem and on variations of ruminal pH which affects lipase activity. Van Nevel and Demeyer [26] reported that at a rumen pH ≤ 6.0 lipolysis of soy oil was inhibited and a further decline was observed when the inclusion level was doubled. However, in the present study neither the pH nor the production of volatile fatty acids and methane were influenced by the inclusion level at 24 h, although the pH of the rumen fluid was low (5.7 to 5.9; results published by Fievez et al. [7]). Thus, an overall reduction of microbial activity can be excluded. Moreover, since the extent of lipolysis of soy oil averaging 88% (data not shown) was not affected at any inclusion level, it is unlikely that the availability of lipases was too low to hydrolyse the increasing amounts of fatty acids during the 24 h incubation period. Possibly, the sole presence of unesterified EPA and DHA as well as their increasing amounts due to higher fish oil supply reduced the lipase activity.

3.2. Release rates of EPA and DHA

The effects of the fish oil type, inclusion level and incubation time on the release

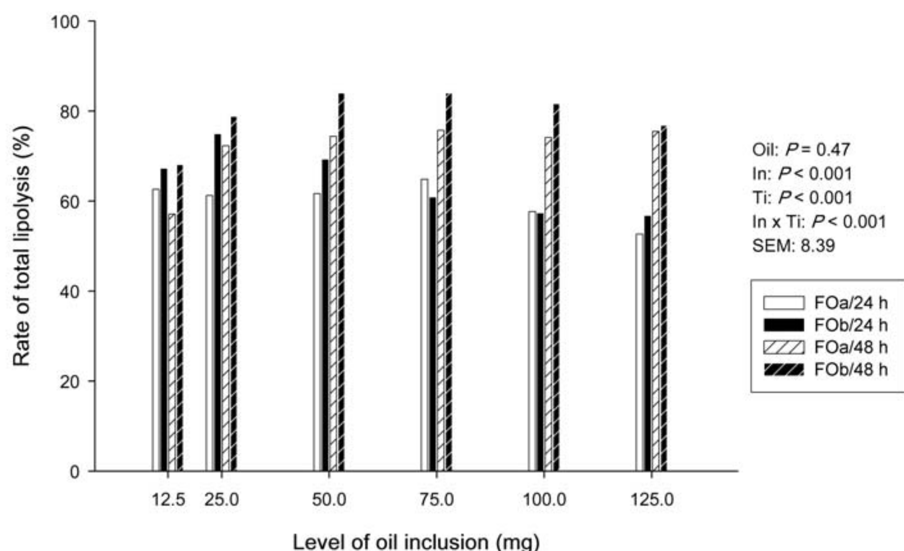

Figure 1. The effect of inclusion level (In) and incubation time (Ti) on total lipolysis of the fish oils (Oil). FOa, 24 h: fish oil a, 24 h incubation; FOb, 24 h: fish oil b, 24 h incubation; FOa, 48 h: fish oil a, 48 h incubation; FOb, 48 h: fish oil b, 48 h incubation; SEM: standard error of means.

rates of EPA and DHA are presented in Figures 2 and 3, respectively. Although EPA and DHA proportions were markedly higher in FOa compared to FOb, the release rate of these fatty acids from the TG did not differ (EPA: Oil: $P = 0.73$; DHA: Oil: $P = 0.52$). Beam et al. [3] has reported that the release rate of fatty acids may vary considerably depending on the source of the added lipids since they showed clear differences between the rates of LA released from tallow and soy oil. One can speculate that in the present study the characteristics of the two fish oils were too similar in order to influence the release rate. Regardless of the fish oil, the release rates of EPA (82, 80, 75, 70, 67, and 61%) and DHA (83, 78, 73, 66, 64, and 58%) decreased with increasing oil supply (In: $P < 0.001$ for each). Nevertheless, the total amount of unesterified EPA (0.30, 1.07, 2.81, 4.52, 5.89, and 7.19 mg) and DHA (0.30, 0.94, 2.21, 3.34, 4.33, and 4.89 mg) as well as the total amount of esterified EPA (0.74, 1.72, 3.62, 5.51, 7.96, and 10.09) and DHA (0.57, 1.31, 2.75, 4.42, 6.26, and 7.85 mg) (In: $P < 0.001$ for each) increased because the release rates decreased to a lesser extent than the supply increased. Similar to total lipolysis, EPA and DHA release was higher at 48 h compared to 24 h (Ti: $P < 0.001$ for each). However, on the contrary to total lipolysis the release reducing effect of the inclusion level at 48 h was obvious for EPA and DHA suggesting a specific influence on these fatty acids.

Moreover, the release of EPA and DHA were considerably lower than the rate of LA released from soy oil, which averaged 90% (data not shown) and which is in line with the findings of Gulati et al. [12]. The question arises why the release rate of LA differs distinctly from EPA and DHA. One could hypothesise that in the rumen, specific ester linkages of the TG are more prone to microbial lipases. If the percentage of EPA and DHA linked at those positions within the oil-TG is lower compared to LA, more EPA and DHA could escape hydrolysis in the

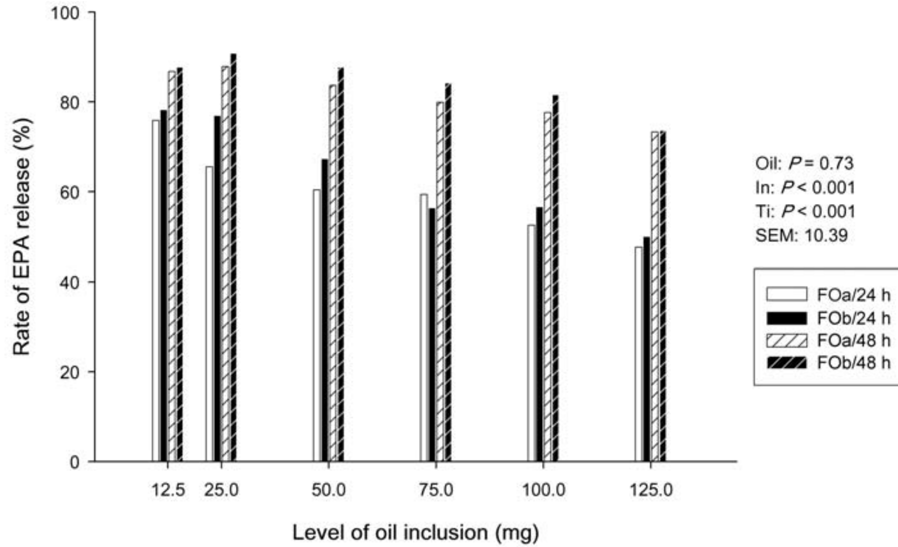

Figure 2. The effect of inclusion level (In) and incubation time (Ti) on the rate of EPA released from triacylglycerols of two different fish oils (Oil). FOa, 24 h: fish oil a, 24 h incubation; FOb, 24 h: fish oil b, 24 h incubation; FOa, 48 h: fish oil a, 48 h incubation; FOb, 48 h: fish oil b, 48 h incubation; SEM: standard error of means.

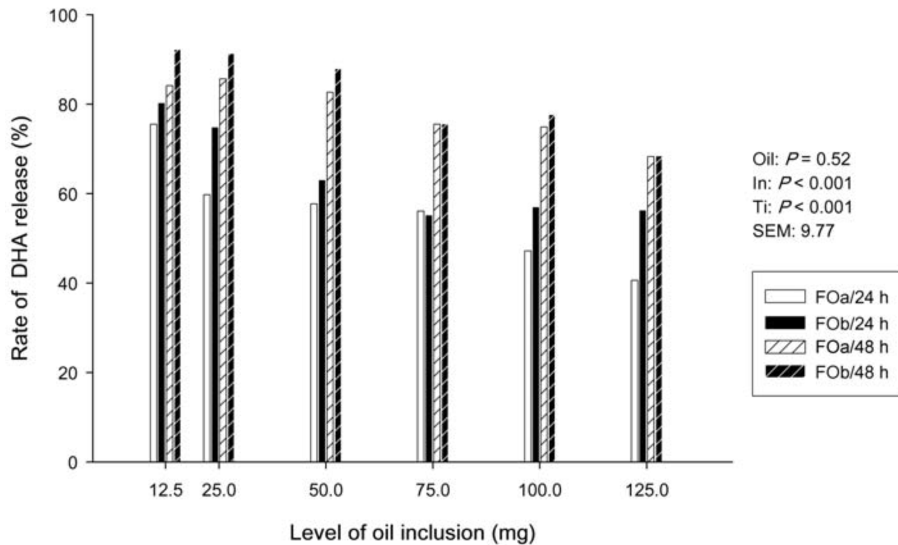

Figure 3. The effect of inclusion level (In) and incubation time (Ti) on the rate of DHA released from triacylglycerols of two different fish oils (Oil). FOa, 24 h: fish oil a, 24 h incubation; FOb, 24 h: fish oil b, 24 h incubation; FOa, 48 h: fish oil a, 48 h incubation; FOb, 48 h: fish oil b, 48 h incubation; SEM: standard error of means.

rumen and enter the small intestine as mono- and diacylglycerols. However, mono- and diacylglycerols containing EPA or DHA have not been found in the present study (data not shown). This observation is in agreement with data presented by Doreau and Ferlay [6] who could not detect any mono- and diacylglcerols in the rumen.

3.3. Biohydrogenation of EPA and DHA

A prerequisite for the biohydrogenation of unsaturated fatty acids in the rumen is their release in the unesterified form (Hawke and Silcock [15] cited by Moore and Christie [19]). In contrast to the release rate from the TG, EPA and DHA biohydrogenation was affected by the fish oil type (Oil: $P < 0.001$; Figs. 4 and 5), being lower in the oil containing more *n*-3 fatty acids (FOa). Concomitantly, the increase in the amount of unesterified EPA and DHA was more pronounced in FOa compared to FOb (Oil: $P = 0.06$; Figs. 6 and 7). Similar to the results reported by Gulati et al. [12], increasing the supply of EPA and DHA caused a decreased biohydrogenation of the two fatty acids (In: $P < 0.001$ for each) and the final amount of unesterified EPA and DHA increased (In: $P < 0.001$ for each). Thus, biohydrogenation of EPA and DHA was more sensitive to their proportion in the unesterified form than to their rates of release. With increasing concentration of FOa, the final amount of unesterified EPA was higher compared to FOb (Oil × In: $P < 0.001$). The elevated amount of unesterified EPA after incubation can be explained by the three times higher EPA concentration in FOa compared to FOb (FOa: 18.7%; FOb: 5.8%). By contrast, the differences in the DHA concentration between the two fish oils were less distinct (FOa: 11.7%; FOb: 7.6%) and might explain the lack of a significant Oil × In interaction. While biohydrogenation of EPA and DHA was higher

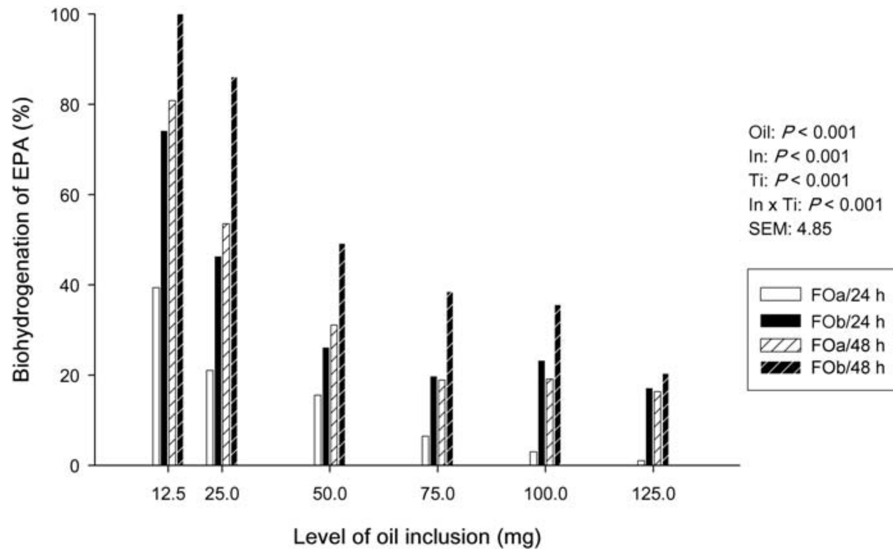

Figure 4. The effect of inclusion level (In) and incubation time (Ti) on biohydrogenation of EPA derived from two different fish oils (Oil). FOa, 24 h: fish oil a, 24 h incubation; FOb, 24 h: fish oil b, 24 h incubation; FOa, 48 h: fish oil a, 48 h incubation; FOb, 48 h: fish oil b, 48 h incubation; SEM: standard error of means.

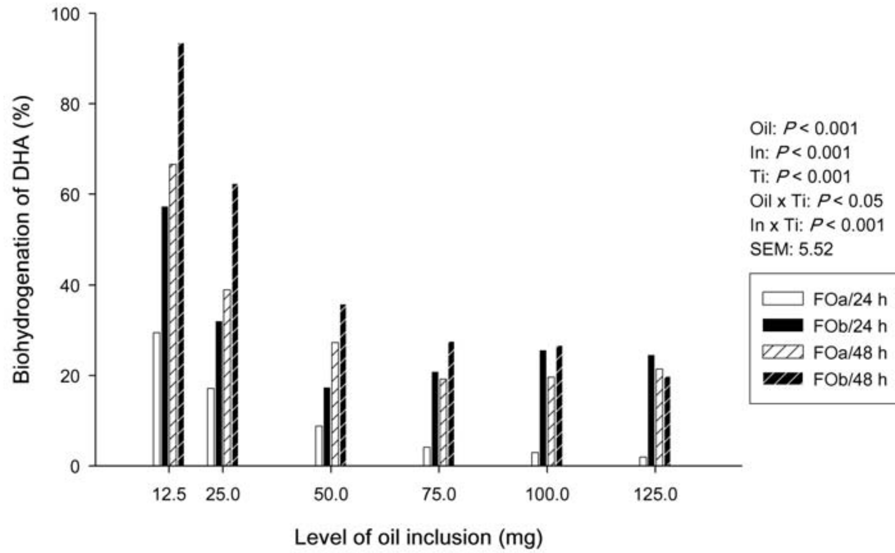

Figure 5. The effect of inclusion level (In) and incubation time (Ti) on biohydrogenation DHA derived from two different fish oils (Oil). FOa, 24 h: fish oil a, 24 h incubation; FOb, 24 h: fish oil b, 24 h incubation; FOa, 48 h: fish oil a, 48 h incubation; FOb, 48 h: fish oil b, 48 h incubation; SEM: standard error of means.

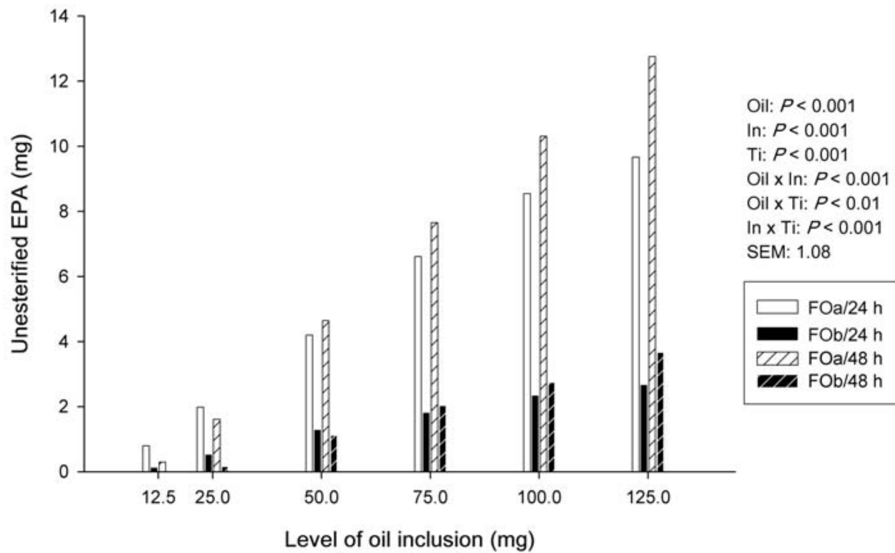

Figure 6. The effect of inclusion level (In) and incubation time (Ti) on the accumulation of unesterified EPA derived from two different fish oils (Oil). FOa, 24 h: fish oil a, 24 h incubation; FOb, 24 h: fish oil b, 24 h incubation; FOa, 48 h: fish oil a, 48 h incubation; FOb, 48 h: fish oil b, 48 h incubation; SEM: standard error of means.

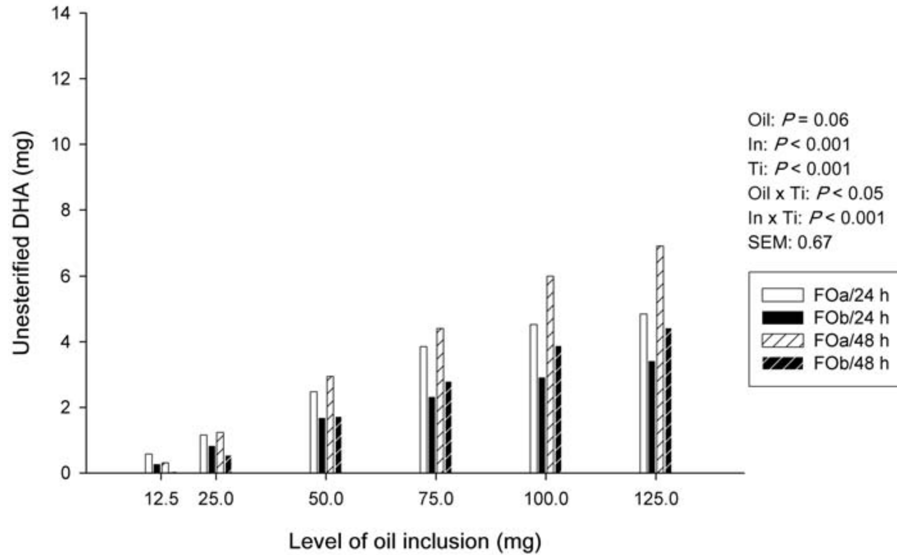

Figure 7. The effect of inclusion level (In) and incubation time (Ti) on the accumulation of unesterified DHA derived from two different fish oils (Oil). FOa, 24 h: fish oil a, 24 h incubation; FOb, 24 h: fish oil b, 24 h incubation; FOa, 48 h: fish oil a, 48 h incubation; FOb, 48 h: fish oil b, 48 h incubation; SEM: standard error of means.

at 48 h (Ti: $P < 0.001$ for each) the differences between 24 and 48 h of incubation were less pronounced with increasing oil supply (In × Ti: $P < 0.001$ for each). Nevertheless, the amount of unesterified EPA and DHA was markedly higher at 48 h (Ti: $P < 0.001$) and also depended on the fish oil inclusion level (In × Ti: $P < 0.001$). The reason for this observation was that the release rate during the second 24 h incubation period increased to a greater extent than the biohydrogenation. The amounts of unesterified EPA and DHA from FOa were significantly higher after 48 h compared to 24 h, whereas only slight changes occurred for FOb (Oil × Ti: $P < 0.05$ for each). However, on the contrary to EPA, where the pattern of biohydrogenation over time was similar for both oils, there were interactions between Oil and Ti ($P < 0.05$) for the biohydrogenation of DHA.

The release from TG without biohydrogenation of EPA and DHA could explain the toxicity of fish oil for ruminal micro-organisms reported by Palmquist and Kinsey [21]. Furthermore, Thompson and Spiller [24] have concluded from an in vitro study, that EPA impairs the proliferation of human colonic bacterial metabolism. Moreover, it has been suggested that polyunsaturated fatty acids and especially the more unsaturated ones adversely affect the activity of specific rumen bacteria [9] and rumen protozoa [20]. In the present study, the increasing amount of unesterified EPA and DHA observed with an increasing oil supply could be responsible for the inhibition of bacteria involved in biohydrogenation. Although the direct contribution of rumen protozoa to the process of biohydrogenation is of only minor importance [14], they could play an indirect role as the hydrogen producer. Using the

same fish oil types, Fievez et al. [7] reported an increasing hydrogen accumulation with an increasing oil supply. Therefore, in the present study, a lack of hydrogen needed for biohydrogenation of UFA seems to be unlikely.

Due to the limited numbers of soy oil samples (n = 2) analysed, no statistical comparisons of the biohydrogenation between LA and EPA and DHA were carried out. Nevertheless, the results indicated a numerically higher biohydrogenation for LA compared to the *n*-3 fatty acids. Despite the chain length and number of double bonds, biohydrogenation of LA was almost complete regardless of the inclusion level and incubation time (data not shown). Differences in the steric factors of the fatty acids could be the reason for the lower inclination to rumen biohydrogenation of EPA and DHA as compared to LA [1, 27]. The absence of specific enzymes might also influence the biohydrogenation rates of EPA and DHA [1] indicating again a microbial effect. Beam et al. [3] explained the differences in the biohydrogenation of LA compared to oleic acid with the activities of different microbial enzymes such as hydrogenases and isomerases. Bauchart et al. [2] suggested that the reduced biohydrogenation of unesterified LA, compared to linolenic acid, is due to its uptake by rumen bacteria. The same mechanism could be responsible for the lower biohydrogenation of EPA and DHA in the present study. However, it still has to be proven that rumen bacteria are able to incorporate fatty acids with more than three double bonds, because up to now these fatty acids are not detected in bacterial lipids (Goldfine [11] cited by Harfoot and Hazlewood [14]).

The contradictory results between studies on biohydrogenation of EPA and DHA might be due to the proportion of EPA and DHA in the fish oil used or to the inclusion level. Variations might also be attributed to the different sources and levels of fibre present in the ration [12] which would be supported by findings of Wang and Song [28] who described an increased biohydrogenation of LA when starch was added instead of cellobiose.

4. CONCLUSION

The ruminal release rates of EPA and DHA from TG were reduced by the increased fish oil supply. At the same time the amount of unesterified EPA and DHA increased which caused a decrease in the biohydrogenation of these fatty acids. These findings suggest that with increased fish oil supplementation, the EPA and DHA fluxes to the small intestine both in the form of TG and as unesterified fatty acids are improved. This effect can even be increased at least for EPA by the use of fish oils particularly rich in EPA.

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