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Research Article

Encapsulation Method to Protect Unsaturated Fatty Acids from Rumen Biohydrogenation *In Vitro*

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Abstract

Enrichment of ruminants' products with polyunsaturated fatty acids is one of the possibilities to introduce these fatty acids into the human diet. However, the rumen biohydrogenation of unsaturated fatty acids limits their quantity and thus bioavailability in the rumen as well as in animal products. The alginate/carrageenan calcium beads of linseed oil were evaluated *in vitro* to verify the ability of these products to protect polyunsaturated fatty acids from biohydrogenation by ruminal microbes. Encapsulation efficiency was evaluated by measuring the quantity of oil protected by alginate/carrageenan calcium beads as well as changes on linseed oil fatty acids content before and after encapsulation. Experiment was evaluated *in vitro* using batch culture system. The treatments were: control (Control) without supplements, experimental I (Linseed oil) (control + 4% of linseed oil), experimental II (Beads 1) control + 4% of linseed beads containing 15% oil, experimental III (Beads 2) control + 4% of linseed beads containing 20% oil. Linseed oil and both linseed oil beads were supplemented to substrates at 4% of dietary dry matter. The substrate was composed of a mixture of meadow hay and barley meal in the ratio of 60:40 and incubated for 48h. All samples were analyzed for fatty acids content. The results were 87% and 86% for alginate/carragenan beads loaded with 15vol% linseed oil and alginate/carragenan beads loaded with 20vol% oil, respectively. The encapsulation process didn't have a significant effect on PUFA fraction ($P<0.01$).Results indicated that there is no significant difference ($P<0.01$) between linseed oil fatty acids content before and after encapsulation process. After incubation in batch culture system, linseed beads decreased ($P<0.01$) total rumen saturated and monounsaturated fatty acids content. Omega 3 and omega 6 fatty acids contents increased statistically ($P<0.01$) by beads 1 and beads 2 and numerically by linseed oil treatment. In conclusion, new encapsulation method has the potential to protect linseed oil from rumen biohydrogenation *in vitro*, however, further *in vivo* experiments are required.

Key words: Polyunsaturated fatty acids, Rumen, Biohydrogenation, Encapsulation

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1. Introduction

Milk fat consumption has been widely discouraged by nutritionists due to high level of saturated fatty acids (SFA) and low content of unsaturated fatty acids (UFA) [1]. However, recent research has demonstrated that unsaturated, including n-3 FA in milk fat can be naturally increased by dietary lipid supplementation. Major natural dietary sources of lipids are relatively rich in linolenic (*c*-9, *c*-12, *c*-15-C18:3; n-3) and linoleic (*c*-9,*c*-12C18:2; n-6) acids. The proportion of unsaturated n-3 fatty acids in milk fat, however, is generally low (<1% of FA; [2]) because in the rumen, unsaturated dietary lipids undergo extensive transformations by ruminal microorganisms in 2 major processes; lipolysis and biohydrogenation (BH) [3]. Consequently, in ruminants, marked differences exist between the FA profile in the diet (mainly unsaturated FA) and the FA profile in duodenal digesta (mainly saturated FA; [4].

Many attempts have been undertaken to avoid biohydrogenation and to improve milk fatty acid content. The inclusion of linseed in the diet has been used to increase the α-linolenic acid (ALA) content in milk from cows [5], ewes [6] and goats[7]. The final contents of ALA in milk were however modest and seldom surpassed 2% of total FA. This result is attributable to the high rate of rumen biohydrogenation of ALA, reducing its absorption in the small intestine and consequently limiting its supply available to the mammary gland for incorporation into milk fat [8].

To overcome the ruminal transformations, protection technologies have been developed, which aim to prevent ruminal FA metabolism and/or ensure the accumulation of biohydrogenation intermediates [9]. Protection of linseed oil with formaldehyde treatment can increase the proportion of C18:3n-3 in milk fat up to 6.4% of total FA [10]. However, use of formaldehyde-based products to alter milk FA has not been commercially feasible because of regulatory prohibition of feeding formaldehyde to ruminants [11]. A whey protein emulsion gel complex (WPEG) that does not use formaldehyde has been developed [11]. Although, supplementing WPEG in general was shown to be a useful method for a persistent change of the FA pattern in milk, the high water content of WPEG may cause deterioration of WPEG quality during storage, which inhibits a large-scale practical application of WPEG ([12] and [13].

Currently, the only one commercially used method to protect unsaturated fatty acids from microbial action in the rumen is their supplementation as calcium salts. Although saturated and mono-unsaturated fatty acids react well with calcium thus forming an insoluble product that resist rumen degradation (biohydrogenation), polyunsaturated fatty acids (PUFA) do not readily react to form calcium salts and therefore the salts provides little to none protection against the breakdown of PUFA by rumen biohydrogenation [14] and [15]. Therefore, there is a need for finding a more effective method by which PUFA present in fat supplements can be protected to bypass the rumen and become available for absorption in

ruminant animals' lower digestive tract. The overall objective for this study is to increase PUFA outflow from the rumen and by that means the delivery to ruminant tissues using new encapsulation technology, in order to produce milk enriched in PUFA.

2. Material and Methods

The main objective of this study was to evaluate the effectiveness of new encapsulation method to protect vegetable oils rich in polyunsaturated fatty acids mostly omega 3 and omega 6, from rumen biohydrogenation and thereby allowing a high proportion of PUFA to be available for digestion and absorption in the small intestine and to be eventually incorporated into animals body tissues and milk.

The study involved two main steps as follow:

I. Preparation and evaluation of linseed oil beads using new encapsulation method.
II. Testing the effect of linseed oil beads compared to unprotected linseed oil on rumen fatty acids content after 24 and 48h of incubation using batch culture system.

The first step of this work, linseed oil encapsulation process, was conducted in Advanced Material and Nanotechnology Lab., Scientific Excellence Centre, National Research Centre, Egypt. The second step, *in vitro* evaluation, was conducted in Department of Animal Nutrition and Feed Management, Poznan University of Life Sciences, Poznan, Poland.

Materials

To prepare linseed oil beads, new encapsulation method using natural and safe polymers was used. Linseed oil was used as a model for plant oils rich in polyunsaturated fatty acids, mainly omega 3 and omega 6. Linseed (Sakha 2 type) was purchased from Fibre Research Institute, Agriculture Research Centre, Ministry of Agriculture, Giza, Egypt. The extraction of linseed oil was made by Al Raeed Jet Master manufactories for food industries, Giza, Egypt. Sodium alginate from brown alga and k-Carrageenan (MO: 154,000; sulfate ester 25%) were obtained from Fluka. $Tween_{80}$ used as emulsifier for preparing linseed oil emulsion gel was obtained from Sigma Aldrich Company. Calcium chloride (anhydrous) was used as a cross linking agent and was purchased from Sigma Aldrich. All other chemicals were of Analar grade.

Methods

Preparation of linseed oil beads

Sodium alginate/k-Carrageenan gel was prepared by dissolving sodium alginate/k-carragenan (1:1 w:w) in distilled water to get a concentration of 2.5% (w/v) alginate/carragenan gel. The gel solution was mixed thoroughly using an overhead mechanical stirrer until complete dissolving had occurred. The linseed oil emulsion gel was prepared by mixing 15 and 20 vol% linseed oil with alginate/ carragenan gel (v/v) using $Tween_{80}$ as an emulsifier (0.5ml/100ml gel) to get tow linseed oil beads concentrations. A solution of 2.5 % calcium chloride was prepared to use as cross-linking agent. Uniform linseed oil beads were formed through injection of the linseed emulsion gel solution using the Encapsulator instrument (model IE-50R purchased from Encap. Biosys., Switzerland) (Figure 1). The process was performed under the conditions as follow: nozzle 1m, frequency 1700 Hz, flow rate 4 ml/min^{-1} and air pressure of 1 bar. The formed beads were received in 2.5% $CaCl_2$ (w/v) and left in the hardening solution for up to 30 min.

The linseed oil beads were filtered and dried using oven dryer (45^{0}C)/2h.

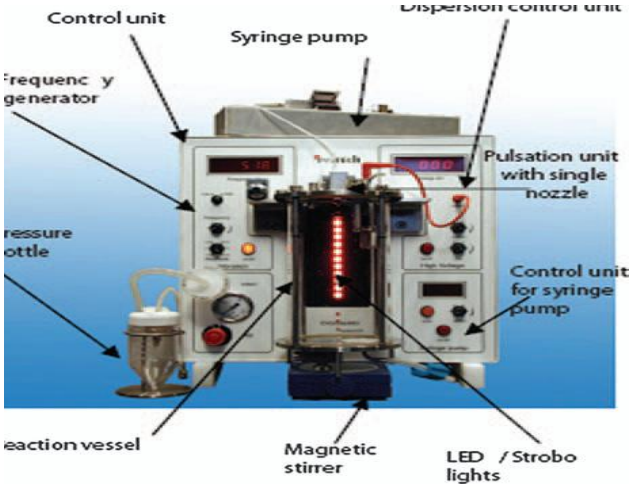

Figure 1. Encapsulator for gel beads preparation

Encapsulation efficiency measure ments

The encapsulation efficiency expressed in their quality and quantity was measured. As an indicator of the amount, ether extract from linseed oil beads at tow levels (15% and 20% oil content) was analyzed according to [16] to determine the quantity of oil protected by alginate/carrageenan calcium beads. To measure the encapsulation efficiency as an indicator of quality, the linseed oil fatty acids content was analyzed according to [17]. The process was performed in three steps:

Hydrolysis and free fatty acids extraction of samples

Hydrolysis was carried out in screw-capteflon stoppered tubes (Pyrex, 15 ml) after adding 3 ml of 2M NaOH to 150 mg of feed or digesta. Afterwards, samples were incubated in a block heater at 90°C for 40 min. After cooling to room temperature 1.7 ml of 4 M HCl were added to lower pH below 2.0. Dichloromethane was then used to extract free fatty acids. The extraction was performed according to [18]. After hydrolysis, 1 ml of freshly prepared internal standard (IS, Triundecanin, TAG C11:0 in dichloromethane, Sigma) and 3.5 ml of dichloromethane were added and the tubes were shaken vigorously using a Vortex-Genie 2. The same solvents for internal standard (IS) and extraction, without any side-effects, were used. A closed system of hydrolysis was used, assuming that no loss of fatty acids occurred. Furthermore IS was easily accessible and soluble. This procedure allowed the extraction to be strictly monitored. The lower organic phase was passed over a short column of Na_2SO_4. The extraction procedure was repeated twice. The solvent was then evaporated at 30°C for 10 min under a flux of nitrogen. The remaining residue was used for derivatization.

Derivatization procedure

The extracted fatty acids were esterified using 0.5 M NaOH in methanol and subsequently converted to fatty acid methyl esters (FAME) using borontrifluoride (Fluka) as described by [19]. A 0.34 M NaCl solution and hexane were added and shaken vigorously. The organic phase containing the fatty acid methyl etsres (FAME) was used for gas chromatographic analyses.

Gas chromatographic procedure

The fatty acids in linseed oil, linseed oil beads and digesta were quantified by gas chromatography (GC; Hewlett-Packard model 6890 equipped with a flame ionization detector). A Chrompac CP-Sil 88 column (100 m, 0.25 mm, 0.2 µm film thickness, Varian) was used. Hydrogen was used as a carrier gas at a constant flow of 30.0 ml/min and a split ratio of 1:10. The oven temperature was programmed as follows: initially 175°C for

25 min, then increasing at 5°C/min to 235°C. The fatty acid peaks were identified by comparison with the retention times of known standards (FAME Mix, Supelco, Belfonte, USA) according to [20]. Fatty acid standards (Supelco, Belfonte, USA) were used to create calibration curves for the quantification of fatty acids.

Batch culture system

Incubation and sampling procedures

Rumen fluid was collected from 3 ruminallyfistulated Polish Holstein-Friesian cows (weight 680±20 kg). The cows were fitted with ruminal cannula by surgical procedure approved by the guidelines of Polish Local Ethical Board for animal treatment. The animals diet was composed of grass silage (7 kg DM), corn silage (5 kg DM), meadow hay (1.8 kg DM) and concentrate (1 kg DM). The animals were fed at the rate of 2.5 kg dry matter/100 g of body weight/day. The collected rumen fluid (before morning feeding) was mixed and squeezed through a 4-layers cheesecloth into a Schott Duran® bottle (1 L) with an O_2-free headspace and immediately transported to laboratory at 39°C where was used as a source of inoculum. A mixture of meadow hay and barley meal in the ratio of 60:40 was used as a substrate. The treatments were: control (Control) without supplements, experimental I (Linseed oil) (control + 4% of linseed oil), experimental II (Beads 1) control + 4% of linseed beads containing 15% oil, experimental III (Beads 2) control + 4% of linseed beads containing 20% oil. Linseed oil and both linseed oil beads were supplemented to substrates at 4% of dietary dry matter. Each treatment was tested in four replicates accompanied by blank vessels (no substrate). A set of incubation vessels comprised of a four vessels for each tested level and a four control vessels without any supplements as well as 4 vessels as blanks (without substrate). 400 mg of milled substrate was added to the incubation vessels of 120 ml capacity. Each vessel was filled with 40 ml of the incubation medium (292 mg K_2HPO_4, 240 mg KH_2PO_4, 480 mg $(NH_4)_2SO_4$, 480 mg NaCl, 100 mg $MgSO_4x7H_2O$, 64 mg $CaCl_2x2H_2O$, 4 mg Na_2CO_3 and 600 mg cysteine hydrochloride per 1 liter of double distilled water (ddH_2O)) and dispensed anaerobically in the 1:4 (v/v) ratio. Then the samples were incubated at 39°C for 48h. Samples of rumen fluid after fermentation were collected after 24 and 48h of incubation and then analysed for fatty acids content as described by [17].

3. Statistical analysis

The data were analyzed using general linear method of statistical analysis system [21]. Duncan's multiple range test [22] was carried out for separation among means.

Data of linseed oil, linseed oil beads and rumen fluid fatty acids content after 24 and 48 h of incubation were analyzed according to one way analysis of variance, where the model was:

$$Y_{ij} = \mu + T_i + E_i$$

Where,

Y = is the effect of the observation.

μ =is the overall mean.

T =is the effect of the treatment.

E = the experimental error.

4. Results and discussion

Preparation of linseed oil beads

To protect vegetable oils rich in polyunsaturated fatty acids from biohydrogenation in the rumen, different biopolymers at different levels and distinct combinations were studied to encapsulate the linseed oil. Linseed oil

was used as a model for vegetable oils rich in polyunsaturated fatty acids. The biopolymers were sodium alginate and k-carrageenan and they were used under different conditions and separate concentrations. From the obtained data, the best result was achieved when sodium alginate and k-carrageenan were mixed together. The main advantage of this mixture was lack of oil leakage during prepared beads storage, moreover, the gel beads were uniform in shape and their morphology was almost spherical to eclipse shape (Figure 2). Alginate-k-carrageenan beads have the advantage of a smoother morphology than that of polysaccharide network. This is probably due to the ability of sodium alginate to instantaneously gelation in the presence of divalent cations such as calcium. In the same time, because of its gelation, viscosity enhancing, and proven safety properties, k-carrageenan can be used as a sustained-release composition.

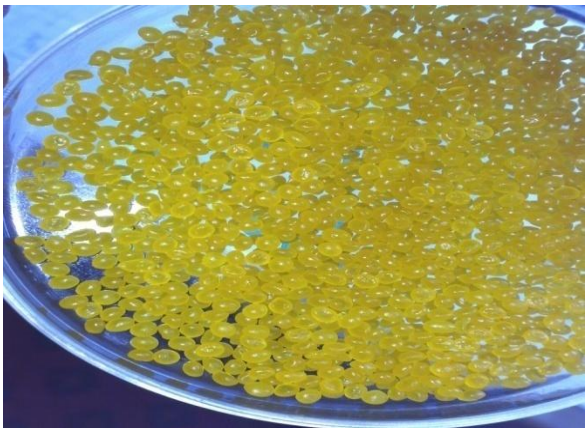

Figure 2. Linseed oil beads

Encapsulation efficiency

The results of encapsulation efficiency as an indicator of beads' quantity and quality are illustrated in Table 1. The encapsulation efficiency was expressed as the final quantity of ether extract in the oil protected by alginate/carrageenan after drying. The results were 87% and 86% for alginate/carragenan beads loaded with 15 vol% linseed oil and alginate/carragenan beads loaded with 20vol% oil, respectively. The quantity of protected linseed oil by alginate/carragenan calcium beads was measured to determine how much of linseed oil beads can be used for dietary supplementation. The present results of encapsulation efficiency are in agreement with those of [23] who prepared the Ca-alginate beads containing high oil content (30vol%) for pharmaceutical utilization. He found that the maximum encapsulation efficiency before drying was over 90%, with alginate concentrations higher than 15 g/L and oil loadings up to 40 vol%. Lowering the alginate concentration below 15 g/L, or increasing the oil loading higher than 40vol%, decreased the encapsulation efficiency. The explanation for both cases is that the encapsulation efficiency depends on the degree of cross-linking at the surface of the extruded emulsion droplet. If there was a lower concentration or volume fraction of alginate, once the emulsion droplet dropped into the gelling bath, there was insufficient cross-linking between the alginate and calcium ions at the droplet surface that resulted in formation of loose Ca-alginate hydrogel wall barriers. Moreover, [24] reported an encapsulation efficiency of about 90% at oil loading of 20vol%.

Results presented in Table 1 indicate that there is no significant difference ($P<0.01$) between linseed oil fatty acids content before and after encapsulation process. The results indicated also that the encapsulation process didn't have a significant effect on PUFA fraction ($P<0.01$) and other linseed oil fatty acids. One of the most important polyunsaturated fatty acids, linolenic acid (C18:3*c*9, *c*12, *c*15), was not affected by encapsulation process. Linolenic acid

Table 1.Encapsulation efficiency as a quantity (E.E.) and quality (fatty acids fraction) for linseed oil and linseed oil beads before and after encapsulation process.

Item	*Treatment			±SE
	Linseed oil	Beads 1	Beads 2	
**E.E.	100	87	86	
***Fatty acids				
C16:0	5.94	5.89	5.76	0.09
C18:0	4.65	4.70	4.47	0.12
C18:1*c*9	15.18	15.41	15.34	0.12
C18:2*c*9,*c*12	14.69	14.14	14.59	0.39
C18:2*c*9,*c*15	0.34	0.99	0.55	0.28
C18:3*c*9,*c*12,*c*15	56.49	55.10	56.26	0.75
Sum of fatty acids	97.29	96.23	96.97	0.54
Other fatty acids	2.71	3.77	3.04	0.40
Total fatty acids	100	100	100	

* linseed oil, Beads 1 and beads 2 are expressed non-encapsulated linseed oil, linseed oil beads 1 (15% oil) and linseed oil beads 2 (20%), respectively

** Ether extract as a measure to evaluate encapsulation efficiency as a quantity (%)

*** The fatty acids values are expressed as mg/100 mg linseed oil

values were 56.49, 55.10 and 56.26 mg/100mg oil for linseed oil, beads 1 and beads 2 treatments, respectively. The obtained results are in an agreement with those reported by [25] who used ultrasonic atomizer to encapsulate fish oil using chitosan, maltodexteren and whey protein isolated materials within three processing steps: emulsification, ultrasonic atomization, and freeze drying. They demonstrated that tuna oil before encapsulation had a slightly higher content of total saturated fatty acids (SFA) and PUFA but lower of monounsaturated fatty acid (MUFA). They also postulated that the encapsulation process using an atomizer could preserve the important fatty acids as can be seen, also ultrasonic atomizer was the promising alternative method for tuna oil encapsulation.

Batch culture system

Fatty acids content in rumen fluid

The main objective of this trial was to evaluate the effectiveness of alginate/carrageenan calcium beads on PUFA protection from rumen biohydrogenation. The fatty acids content of rumen fluid after 24 and 48h fermentation are represented in Table 2 and 3, respectively. Results of Table 2 indicated that beads 1 and beads 2 treatments significantly decreased C16:0, C18:0, C18:1*t*10, C18:1 *t*11, MCFA, SFA and MUFA *t* fatty acids in addition to n6/n3 ratio. Beads 1 and beads 2 treatments increased significantly C18:1*c*9, C18:2 n6 *c*9 *c*12, C18:3 n3 *c*9 *c*12 *c*15, MUFA *c*, PUFA, n3 and n6 fatty acids.

Table 2. Rumen liquor fatty acid fraction affected by linseed oil and linseed oil beads supplementation after 24h of incubation using batch culture system

Item	Treatments				±S.E
	Control	Linseed	Beads 1	Beads 2	
C8:0	0.1	0.1	0.1	0.1	0.007
C10:0	0.2	0.1	0.2	0.3	0.039
C12:0	2.2^{a}	0.8^{b}	1.1^{b}	1.4^{ab}	0.19
C16:0	28.5^{a}	21.4^{b}	21^{b}	20.3^{b}	0.95
C18:0	24.8^{a}	18.8^{bc}	20.7^{b}	16.6^{c}	0.88
C18:1*t*10	1.4^{a}	1.5^{a}	0.9^{b}	0.6^{b}	0.11
C18:1*t*11	4.6^{b}	5.9^{a}	3.4^{c}	2.8^{c}	0.33
C18:1*c* 9	7.9^{c}	13.1^{ab}	10.6^{b}	13.4^{a}	0.68
C18:2n6*c*9*c*12	4.4^{c}	6.8^{ab}	6.1^{b}	7.2^{a}	0.30
C18:3n3*c*9*c*12*c*15	0.5^{c}	11.4^{b}	15.0^{ab}	20.8^{a}	2.20
C18:2*t*10*c*12	0.1	0.3	0.1	0.3	0.038
C18:2*c*9*t*11	0.7^{a}	0.4^{b}	0.6^{ab}	0.6^{ab}	0.041
SCFA	0.9	0.8	0.7	0.8	0.69
MCFA	46.6^{a}	31.4^{b}	34.8^{b}	30.8^{b}	1.84
SFA	68.8^{a}	49.1^{b}	53.5^{b}	46.6^{b}	2.48
MUFA	24.18^{b}	29.69^{a}	23.40^{b}	23.32^{b}	0.35
MUFA *c*	15.76^{c}	19.49^{a}	16.90^{bc}	18.36^{ab}	0.31
MUFA *t*	8.42^{b}	10.21^{a}	6.50^{c}	4.96^{d}	0.25
PUFA	7.83^{c}	21.24^{b}	23.55^{ab}	30.71^{a}	0.77
n3	1.2^{c}	12.17^{b}	15.81^{ab}	21.40^{a}	0.70
n6	6.12^{c}	8.88^{a}	7.56^{b}	8.40^{a}	0.23
n6/n3	5.09^{a}	0.73^{b}	1.16^{b}	0.39^{b}	0.039

*Treatments were control, linseed oil, Beads 1 (15% oil content) and Beads 2 (20% oil content) (4% on DM basis).

Significant differences (P<0.01) a, b and c between treatments means are indicated by dissimilar superscripts.

n3 and n6 are expressed omega 3 and omega 6, respectively.

Results presented in Table 3 clearly showed that beads 1 and beads 2 significantly decreased C8:0, C12:0, C18:0, C18:1*t*11, C18:2 *t*10*c*12, MCFA, SFA and MUFA *t* fatty acids. Beads1 and beads2 significantly increased content of C18:1*c*9, C18:3 n3 *c*9*c*12*c*15, MUFA *c*, PUFA and n3 fatty acids.

Summarizing obtained results we can stated that short chain fatty acids were not affected by experimental treatments after either 24 or 48h of incubation whereas medium chain fatty acids were decreased significantly (P<0.01). Both protected and unprotected form of linseed oil decreased significantly (P<0.01) saturated fatty acids content after 24 and

48h of incubation compared with the control. Monounsaturated fatty acids were significantly (P<0.01) increased by linseed oil treatment in comparison to protected forms and control group after 24h of incubation. However, it were decreased significantly (P<0.01) by all treatments compared with control after 48h of incubation. Monounsaturated fatty acids in *cis* form were increased by all experimental treatments when compared to control group either after 24 or 48h of incubation. Monounsaturated fatty acids content in *trans* form decreased significantly (P<0.01) when linseed oil was supplemented as beads.

Table 3. Rumen liquor fatty acid fraction affected by linseed oil and linseed oil beads supplementation after 48h of incubation using batch culture system

Item	Treatments				±S.E
	Control	Linseed	Beads 1	Beads 2	
C8:0	0.11^{a}	0.11^{a}	0.07^{b}	0.07^{b}	0.008
C10:0	0.22	0.2	0.24	0.12	0.035
C12:0	1.9^{a}	1.3^{ab}	0.8^{b}	1.0^{b}	0.16
C16:0	28.6^{a}	23.1^{b}	23.0^{b}	22.8^{b}	0.02
C18:0	21.9^{a}	16.7^{b}	16.7^{b}	16.1^{b}	0.83
C18:1*t*10	1.8	1.4	1.3	1.3	0.10
C18:1*t*11	5.9^{a}	6.6^{a}	3.9^{b}	3.9^{b}	0.36
C18:1*c* 9	7.9^{b}	12.9^{a}	12.6^{a}	12.0^{a}	0.67
C18:2n6*c*9*c*12	5.6	6.1	6.3	7.6	0.32
C18:3n3*c*9*c*12*c*15	1.0^{b}	8.6^{ab}	17.3^{a}	17.6^{a}	2.43
C18:2*t*10*c*12	0.19^{b}	0.34^{a}	0.15^{c}	0.15^{c}	0.02
C18:2*c*9*t*11	0.8	0.5	0.6	0.4	0.06
SCFA	1.8	1.6	1.3	1.4	0.10
MCFA	43.8^{a}	33.5^{b}	32.9^{b}	32.3^{b}	1.74
SFA	64.5^{a}	50.3^{b}	49.0^{b}	47.8^{b}	2.45
MUFA	25.13^{b}	30.79^{a}	25.16^{b}	24.56^{b}	0.19
MUFA *c*	14.94^{c}	19.32^{a}	17.84^{ab}	17.29^{b}	0.59
MUFA *t*	10.19^{a}	11.46^{a}	7.31^{b}	7.31^{b}	0.51
PUFA	10.34^{b}	18.97^{ab}	26.28^{a}	27.28^{a}	1.10
n3	2.52^{b}	9.66^{ab}	17.94^{a}	18.47^{a}	0.59
n6	7.80	7.93	9.34	9.14	0.53
n6/n3	3.50	0.94	1.21	0.53	0.64

*Treatments were control, Beads 1 (15% oil content), Beads 2 (20% oil content) and linseed oil on 4% (DM basis).

Significant differences (P<0.01) a, b and c between treatments means are indicated by dissimilar superscripts.

n3 and n6 are expressed omega 3 and omega 6, respectively

Content of polyunsaturated fatty acids, omega 3 and omega 6 fatty acids increased significantly (P<0.01) when linseed oil was supplemented as beads 1 and beads 2 when compared with control. Omega 6/omega 3 ratio was decreased (P > 0.01) by all treatments compared with control after 24h of incubation but it was insignificant after 48h of incubation. Moreover, the results of current study indicated that the bio hydrogenation of polyunsaturated fatty acids and its transformation to saturated fatty acids was decreased.

According to many authors, activity of the microorganisms involved in the processes of lipolysis and biohydrogenationis the cause of the decreased amount of polyunsaturated fatty acid leaving the rumen [26, 3, 8].

Hence, methods to reduce the susceptibility of unsaturated fatty acids to such activity are desirable in term to increase their outflow from the rumen [11]. The results of current study are in consistent with those reported by[27]. They studied the effect of oils rich in linoleic acid on rumen fermentation parameters of sheep, goats and dairy cows using batch culture system. Their results indicated that using oils in the free form was more likely for biohydrogenation. They indicated that saturated fatty acids were decreased numerically but not significantly whereas monounsaturated fatty acids content increased in dairy cows rumen fluid and numerically but not significantly in sheep and goat rumen fluids after fermentation. Also, polyunsaturated fatty acids content was decreased insignificantly by oil treatments compared with control. Many methods were analysed in order to reduce biohydrogenation of unsaturated fatty acids in the rumen. [28] Studied the *in vitro* biohydrogenation of n−3 polyunsaturated fatty acids protected against ruminal microbial metabolism. In their study linseed oil was used as a rich source of linolenic acid (18:3n−3), and was compared with whole seeds of linseed, formaldehyde-treated linseed seeds, formaldehyde-treated linseed seeds that was pre-treated with sodium hydroxide, formic acid or ammonium tetra format, xylose treated linseed seeds or linseed oil absorbed into a fiber source. The authors used also crude fish oil as the source of eicosapentaenoic (20:5n−3) and docosahexaneoic acids (22:6n−3) that was compared with fish oil encapsulated with saturated fat, marine algae or fish oil absorbed into a fiber source. All results were compared to the control group without any oil supplement. The oil sources were incubated using a batch culture technique, and the fermentation terminated after 6, 12, 24 and 48h. They concluded that biohydrogenation of 18:3n−3 in linseed oil and linseed seeds was rapid and extensive whilst absorption of the linseed oil into fibre, or treatment of linseed with xylose or formaldehyde alone, offered little additional protection. However, pre-treatment of the seeds with sodium hydroxide, formic acid or ammonium tetraformate to disrupt the seed coat, followed by formaldehyde treatment, reduced the rate and extent of biohydrogenation, with sodium hydroxide pre-treatment offering the best protection. However, this method has limited possibilities of applications in many parts of the world due to potential adverse health effects and legal prohibition of using formaldehyde in ruminants' nutrition[11]. Moreover, the extent of biohydrogenation of fish oil fatty acids is controversial. Unsaturated fatty acids having a carbon chain longer than 18 carbons undergo isomerization processes rather than biohydrogenation.

Biohydrogenationof 20- and 22-carbon fatty acids was reported to be limited *in vitro* [29, 30] and *in vivo* [29].

As the effect of presented study we can conclude that using alginate/carrageenan calcium beads to encapsulate linseed oil is the effective way of protection of polyunsaturated fatty acids, including omega 3 and omega 6 fatty acids, from rumen biohydrogenation and transformation to saturated form. Making beads with 20% oil content have a better effect than beads with 15% oil content. Further *in vitro* and *in vivo* studies should be conduct to confirm these results.

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