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Short communication: A nanoemulsified form of oil blends positively affects the fatty acid proportion in ruminal batch cultures

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ABSTRACT

Two consecutive rumen batch cultures were used to study the effect of nanoemulsified oils as a new type of supplement, on the in vitro fatty acid proportion and vaccenic acid formation. Three levels (3, 5, and 7%) of 2 different oil blends [soybean:fish oil (SF) or rapeseed-fish oil (RF)] were used. Both oil blends were used either in the raw form (SF or RF, respectively) or in the nanoemulsified form (NSF or NRF, respectively). The diets were the control (0%), which consisted of a dry total mixed ration without any supplements, the control plus 3, 5, or 7% of the SF or RF oil blend in appropriate form (raw or nanoemulsified). For each treatment, 6 incubation vessels were used. Each batch culture was incubated for 24 h and conducted twice in 2 consecutive days. All supplements were calculated as a percentage of the substrate dry matter (400 mg). Nanoemulsified supplements were recalculated to make sure the oil amount was equal to the raw oil supplementation levels. The results from both experiments indicated that the proportions of vaccenic acid and *cis*-9,*trans*-11 C18:2 increased when a raw oil blend was supplemented; on the other hand, no influence of nanoemulsified form of oil blend was observed on the proportion *cis*-9,*trans*-11 C18:2. Generally, supplementation with the nanoemulsified oil blends had less effect on biohydrogenation intermediates than the raw form of oil blends. However, the nanoemulsified form had a greater effect on the increase of n-3 and n-6 fatty acids. Nanoemulsified oil blends had a positive effect on decreasing the transformation rate of polyunsaturated fatty acids to saturated fatty acids in the biohydrogenation environment. Supplements of nanoemulsified oil blends tended to be more effective than supplements of raw oils in preserving a greater proportion of polyunsaturated fatty acids in the fermentation culture.
Key words: rumen, batch culture, nanoemulsion, oil blend, unsaturated fatty acid

Short Communication

For decades, various feed supplements have been introduced to moderate rumen fatty acid proportions and consequently to modify ruminant products. There is considerable interest in developing nutritional strategies to boost the UFA of milk and meat. The UFA offer potential benefits for human health, mainly attributed to CLA isomers and to n-3 and n-6 fatty acids. Increasing the supply of UFA in the human diet can help to prevent or delay atherosclerosis and coronary heart disease, while helping to avoid inflammatory conditions. Increasing the supply of UFA can also slow the growth of tumor cells (McCrorie et al., 2011; Koba and Yanagita, 2014). Some of the most commonly used forms of UFA supplementation are edible oils of plant or marine origin. Oils show an ability to modulate the rumen fatty acid proportion by affecting the activity of rumen microorganisms, especially the bacterial population (AbuGhazaleh and Ishlak, 2014; Boerman and Lock, 2014). Supplements do have some limits and restrictions in ruminant nutrition because of the possible negative effect on rumen fermentation (Martínez Marín et al., 2013; Ishlak et al., 2014). These limits make it desirable to find other dietary UFA sources that can affect and modulate the rumen fatty acid proportion without negatively affecting microorganisms or rumen fermentation indicators.

Recently, nanotechnology has found innumerable applications in different lines of work. Delivery of bioactive components using nanoscale technology has been documented in pharmaceutics as well as the cosmetic and food sciences (Fathi et al., 2012; Ghosh et al., 2014; Zhang et al., 2014). Nanoemulsion is one of the most important nanotechnology applications, with wide usage

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in several scientific and practical fields. A nanoemulsion is defined as a multiphase colloidal dispersion formed by a mixture of one liquid that is dispersed as nanoscale droplets in another immiscible liquid. Physical share-induced rupturing takes place, leading to a droplet diameters of <100 nm (Mason et al., 2006). The present preliminary experiments established new approaches for modulating the rumen fatty acid proportion by incorporating an oil-in-water nanoemulsion as a novel dietary component. The effect of incorporating an oil-in-water nanoemulsion into ruminant diets has never been investigated. We hypothesized that nanoscale droplets of oil blends rich in PUFA, added directly to the rumen fermentation culture, would inhibit microbial reactions; that is, lipolysis and biohydrogenation. This inhibition could prevent more UFA from being lost during those processes. Therefore, the main objective of the present study was to evaluate the effect of nanoemulsified oil blends rich in PUFA on rumen fatty acid proportions after 24 h of batch incubation.

Three hours after the morning feeding, the rumen inoculum was obtained from 3 ruminally cannulated Polish Holstein-Friesian dairy cows that had an average BW of 600 kg. The ruminal content was collected from the top, bottom, and middle of the rumen of each cow separately. The ruminal contents from all 3 cows were equally blended and then strained through 4 layers of cheesecloth into a Schott Duran bottle (Schott North America Inc., Elmsford, NY), and immediately transported to the laboratory in a water bath heated to 39°C. Two separate experimental trials on 2 consecutive days were carried out in the batch culture system according to the modified protocol of Szumacher-Strabel et al. (2004). Each in vitro run began with a single fresh collection of rumen fluid.

Briefly, rumen fluid was diluted with a buffer solution (292 mg of $K_2HPO_4 \cdot 3H_2O$, 240 mg of KH_2PO_4, 480 mg of $(NH_4)_2SO_4$, 480 mg of NaCl, 100 mg of $MgSO_4 \cdot 7H_2O$, 64 mg of $CaCl_2 \cdot 2H_2O$, 4 mg of Na_2CO_3, and 600 mg of cysteine hydrochloride per liter) at a ratio of 1:4. Then, a portion of this blend (40 mL) was put into 125-mL glass incubation vessels that already contained 400 mg of dried substrate (the TMR). The substrate used in our experiments was similar to the diet offered to the ruminally cannulated dairy cows (rumen fluid donors). All ingredients were dried first and then each dried ingredient was milled separately. A homogeneous mixture of the experimental substrate was made on a DM basis by mixing together the following amounts of the dried ingredients: maize silage (396 g/kg of DM), lucerne silage (71 g/kg of DM), grass silage (104 g/kg of DM), beet pulp (113 g/kg of DM), brewer's grain (85 g/kg of DM), extracted rapeseed meal (42 g/kg of DM), commercial concentrate containing 18% CP (185 g/kg of DM), and a mineral mixture (4 g/kg of DM). Subsequently, 400 g of the dried feed mixture was added to each bottle. The bottles were incubated for 6 h at 39°C before the start of the experiment.

Three different doses of 2 different oil blends, in raw and nanoemulsified forms, were evaluated as supplements in 2 consecutive, short-term in vitro batch fermentation trials. The doses of the raw oil blends (3, 5, and 7%) were calculated based on the substrate's DM. The raw oil supplements were then added on top of the 400 g of TMR. The oil contents of the prepared nanoemulsified form were equal to the oil doses used in the raw oil-blend treatments (3, 5, and 7%). The amount of the supplemented nanoemulsions, however, were recalculated based on the oils used in the nanoemulsion preparation (about 15% of the oil blends) to be about 20, 33, and 47% on a substrate DM basis. The nanoemulsified oil blends were then added directly to the bottle containing both the buffered rumen fluid and substrate.

Two different oil blends [a 1:1 soybean oil:fish oil blend (**SF**) and a 1:1 rapeseed oil:fish oil blend (**RF**)] in 2 forms [raw (SF and RF) and nanoemulsified (**NSF** and **NRF**)] were used during each experimental batch culture. We analyzed 3 levels (3, 5, and 7%) for each type of oil blend and included a control (0%), which consisted of TMR without any supplement. Each batch culture trial was repeated twice on 2 days with 2 different incubation fluids. Six incubation vessels (bottles) were used in each treatment. Six more vessels contained the control substrate (the dry TMR), and another 6 blank vessels contained culture fluid without the substrate. The bottles were filled with CO_2, closed with a rubber stopper, and sealed with aluminum. Then, the bottles were incubated for 24 h in anaerobic conditions at a pH was 6.5 and a temperature of 39°C. The bottles were agitated every 30 min while being incubated. The oil-in-water nanoemulsion was prepared using a Hielscher UP50H ultrasonic processor (80% amplitude for 20 min; Hielscher Ultrasonics, Teltow, Germany) as described by Ahmadi Lakalayeh et al. (2012). Rapeseed oil, soybean oil, and fish oil were used as the inner phase. The only surfactant used was Tween 80 (Sigma-Aldrich, Poznan, Poland). The oil-in-water emulsion formulation was composed of 15% oil, 5.6% Tween 80, and 79.4% deionized water as suggested by Kentish et al. (2008).

Fatty acid methyl esters in the TMR, oils, nanoemulsions, and fermentation fluid samples were extracted and analyzed after 24 h of incubation according to Cieslak et al. (2009) with some modification. Briefly, 2,500 μL of rumen fluid was suspended in 3 mL of 2 *M*