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Linseed oil and *DGAT1* K232A polymorphism: Effects on methane emission, energy and nitrogen metabolism, lactation performance, ruminal fermentation, and rumen microbial composition of Holstein-Friesian cows

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ABSTRACT

Complex interactions between rumen microbiota, cow genetics, and diet composition may exist. Therefore, the effect of linseed oil, *DGAT1* K232A polymorphism (*DGAT1*), and the interaction between linseed oil and *DGAT1* on CH_4 and H_2 emission, energy and N metabolism, lactation performance, ruminal fermentation, and rumen bacterial and archaeal composition was investigated. Twenty-four lactating Holstein-Friesian cows (i.e., 12 with *DGAT1* KK genotype and 12 with *DGAT1* AA genotype) were fed 2 diets in a crossover design: a control diet and a linseed oil diet (LSO) with a difference of 22 g/kg of dry matter (DM) in fat content between the 2 diets. Both diets consisted of 40% corn silage, 30% grass silage, and 30% concentrates (DM basis). Apparent digestibility, lactation performance, N and energy balance, and CH_4 emission were measured in climate respiration chambers, and rumen fluid samples were collected using the oral stomach tube technique. No linseed oil by *DGAT1* interactions were observed for digestibility, milk production and composition, energy and N balance, CH_4 and H_2 emissions, and rumen volatile fatty acid concentrations. The *DGAT1* KK genotype was associated with a lower proportion of polyunsaturated fatty acids in milk fat, and with a higher milk fat and protein content, and proportion of saturated fatty acids in milk fat compared with the *DGAT1* AA genotype, whereas the fat- and protein-corrected milk yield was unaffected by *DGAT1*. Also, *DGAT1* did not affect nutrient digestibility, CH_4 or H_2 emission, ruminal fermentation or ruminal archaeal and bacterial concentrations. Rumen bacterial and archaeal composition was also unaffected in terms of the whole community, whereas at the genus level the relative abundances of some bacterial genera were found to be affected by *DGAT1*. The *DGAT1* KK genotype was associated with a lower metabolizability (i.e., ratio of metabolizable to gross energy intake), and with a tendency for a lower milk N efficiency compared with the *DGAT1* AA genotype. The LSO diet tended to decrease CH_4 production (g/d) by 8%, and significantly decreased CH_4 yield (g/kg of DM intake) by 6% and CH_4 intensity (g/kg of fat- and protein-corrected milk) by 11%, but did not affect H_2 emission. The LSO diet also decreased ruminal acetate molar proportion, the acetate to propionate ratio, and the archaea to bacteria ratio, whereas ruminal propionate molar proportion and milk N efficiency increased. Ruminal bacterial and archaeal composition tended to be affected by diet in terms of the whole community, with several bacterial genera found to be significantly affected by diet. These results indicate that *DGAT1* does not affect enteric CH_4 emission and production pathways, but that it does affect traits other than lactation characteristics, including metabolizability, N efficiency, and the relative abundance of *Bifidobacterium*. Additionally, linseed oil reduces CH_4 emission independent of *DGAT1* and affects the rumen microbiota and its fermentative activity.

Key words: dairy cow, enteric methane production, linseed oil, *DGAT1* K232A polymorphism

INTRODUCTION

Several dietary strategies have been proposed to mitigate enteric CH_4 production, including the use of feed additives and improving forage quality (Beauchemin et al., 2009; Martin et al., 2010). Numerous studies have shown the potential of dietary lipid supplementation to reduce CH_4 emission, many of which have been

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reviewed by Grainger and Beauchemin (2011) and Hristov et al. (2013). To date, linseed is considered to be one of the most effective dietary lipid sources to reduce enteric CH_4 production from dairy cows (Beauchemin et al., 2009; Martin et al., 2010). Relatively few studies have considered the wider consequences of dietary linseed oil on the functioning of the rumen microbial ecosystem. Veneman et al. (2015) reported no effect of linseed oil supplementation on CH_4 emission or the rumen microbiota as a whole. Martin et al. (2016) reported significant decreases in CH_4 emissions upon extruded linseed supplementation for both corn-silage-based and hay-based diets, whereas the abundance of rumen methanogens was not affected by linseed supply in the corn-silage-based or hay-based diets.

Little is known whether host genetics can also influence the responses to dietary linseed oil. The acyl CoA:diacylglycerol acyltransferase 1 gene, located on chromosome 14, mediates the final step in triglyceride synthesis (Schennink et al., 2008). Many studies have investigated associations between the K232A polymorphism of this gene (i.e., a lysine to alanine substitution on the 232nd amino acid; *DGAT1*) and milk production traits of dairy cows. Although *DGAT1* has no effect on fat- and protein-corrected milk (**FPCM**) yield, the *DGAT1* K allele is associated with a higher fat content, protein content, and fat yield, but lower milk production and protein and lactose yield (e.g., Banos et al., 2008; Näslund et al., 2008; Bovenhuis et al., 2015). Additionally, *DGAT1* has a marked effect on milk fatty acid (**MFA**) composition. The *DGAT1* K allele is associated with a larger fraction of C16:0, and smaller fractions of C18 UFA in milk fat (e.g., Schennink et al., 2007; Duchemin et al., 2013). Several of the MFA that have been associated with CH_4 emission (van Gastelen and Dijkstra, 2016) are also affected by *DGAT1*, in particular C18 UFA in both the *cis* and *trans* isomers.

The *DGAT1* gene is expressed in the small intestine, liver, adipose tissue, and the mammary gland (DeVita and Pinto, 2013; Muise et al., 2014). Thus, effects of *DGAT1* on traits other than milk production might be expected. S. van Engelen (Wageningen University & Research; unpublished data) performed a genome-wide association study (**GWAS**) to determine regions of the bovine genome that are associated with predicted CH_4 yield (g/kg of DMI) using the CH_4 prediction equations based on MFA profile published by Dijkstra et al. (2011) and Van Engelen et al. (2015). The association with *DGAT1* was significant in the GWAS for predicted CH_4 yield, suggesting that the *DGAT1* K allele is associated with higher predicted CH_4 yield. The association between *DGAT1* and CH_4 yield has not been studied before and could be of statistical and biological significance. To the best of our knowledge, no study has investigated if the genetic variation of dairy cows, namely *DGAT1*, affects the rumen bacterial and archaeal composition, one of the potential biological explanations for the relation between *DGAT1* and CH_4 yield. In addition, little information is available on the association of *DGAT1* with nutrient digestion or energy and N balance of dairy cattle.

Therefore, the objectives of the present study were to investigate the effects of dietary linseed oil, *DGAT1*, and the interaction between dietary linseed oil and *DGAT1* on CH_4 and H_2 emission, energy and N metabolism, lactation performance, ruminal fermentation, and rumen bacterial and archaeal composition of dairy cows.

MATERIALS AND METHODS

Experimental Design

The experiment was conducted from January to April 2015, in accordance with Dutch law and approved by the Animal Care and Use Committee of Wageningen University & Research (Wageningen, the Netherlands). The experiment followed a crossover design with 2 dietary treatments and 24 lactating Holstein-Friesian cows (i.e., 12 cows with *DGAT1* KK genotype and 12 cows with *DGAT1* AA genotype; each group had 6 primiparous and 6 multiparous cows). The 12 cows with *DGAT1* KK genotype were sired by 10 bulls, and the 12 cows with *DGAT1* AA genotype were sired by 9 bulls. Additionally, 1 bull sired 2 cows with the *DGAT1* KK genotype and 1 cow with *DGAT1* AA genotype. At the start of the experiment, the cows with the *DGAT1* KK genotype and *DGAT1* AA genotype were, on average, 215 ± 65 and 216 ± 68 DIM (means ± SD) and produced 23.9 ± 5.66 and 26.9 ± 5.87 kg of milk/d, respectively. The cows were blocked in pairs according their *DGAT1* genotype, parity, DIM, and milk production. Within each block, cows were randomly allocated to a dietary treatment sequence in a crossover design with 2 periods: a control diet (**CON**) and linseed oil diet (**LSO**). Treatment periods lasted 17 d and were composed of a 12-d adaptation period followed by a 5-d measurement period. There was a 14-d wash-out period between the treatment periods of the same block of cows.

Diets, Feeding, and Housing

Both the CON and LSO diet consisted of 40% corn silage, 30% grass silage, and 30% concentrates on a DM basis. The ingredient and chemical composition of both diets are presented in Table 1. Linseed oil (Linagro NV, Lichtervelde, Belgium) was added to the concentrate of