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Importance of the fat content within the cheese-matrix for blood lipid profile, faecal fat excretion, and gut microbiome in growing pigs

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ABSTRACT

Cheese and butter have been shown to affect blood lipids differently. This parallel-arm, randomised, controlled study in 36 crossbred growing sows compared the effect of diets with either regular-fat cheese (REG), reduced-fat cheese + butter (RED) or butter (BUT) on blood lipids, faecal fat and energy excretion and gut microbiome in pigs. A 14-d run-in period was followed by 14-d interventions with macronutrient-matched diets. Fasting total cholesterol and HDL-cholesterol after 14 days were higher in REG compared with BUT, but only tended to be higher in RED. Compared with BUT, REG and RED had higher faecal fat excretion. Faecal energy excretion was only higher in REG, and this correlated with a lower microbiome *Firmicutes*-to-*Bacteroidetes* ratio. In conclusion, dairy fat consumed as cheese or butter caused different metabolic effects. Differences between reduced-fat cheese+butter and butter were less pronounced than differences between regular-fat cheese and butter, suggesting an impact of the dairy-matrix.

1. Introduction

Until recently, Heart Associations recommended limiting the intake of foods with high contents of saturated fat and cholesterol, such as high-fat dairy products. Accordingly, replacement of these with low-fat alternatives was recommended. Observational studies have though shown a modest inverse association between dairy product intake and cardiovascular risk (Elwood, Pickering, Givens, & Gallacher, 2010; Soedamah-Muthu et al., 2011). Likewise, observational data suggest that cheese consumption is unlikely to be detrimental to heart health (German et al., 2009; Gibson, Makrides, Smithers, Voevodin, & Sinclair, 2009; Houston, Driver, Bush, & Kritchevsky, 2008; Tholstrup, 2006). Controlled human intervention studies have also shown that milk-fat from cheese increases cholesterol less than milk-fat from butter (de Goede, Geleijnse, Ding, & Soedamah-Muthu, 2015). In addition, cheese may also increase cholesterol slightly less than milk (Soerensen, Thorning, Astrup, Kristensen, & Lorenzen, 2014). This is, however, not fully explained by differences in calcium and fat content between these dairy products. The fact that cheese consumption may be innocuous to cardiovascular health, despite high levels of saturated fat, may be linked to the unique food matrix of cheese.

Cheese is a fermented and ripened dairy product, which has been suggested as a possible explanation for the distinct effect of cheese compared with non-fermented dairy products (Hjerpsted & Tholstrup, 2015; St-Onge, Farnworth, & Jones, 2000). Also, cheese is a nutrient-dense food, with amongst others a high content of calcium. Calcium has been shown to precipitate with fat in the duodenum in formation of insoluble calcium fatty acid soaps. In addition, calcium and phosphate may form amorphous calcium phosphate (ACP), which binds bile acids. Both mechanisms reduce the intestinal absorption of fat measured by an increased fat excretion in faeces (Ditscheid, Keller, & Jahreis, 2005; Jacobsen, Lorenzen, Toubro, Krog-Mikkelsen, & Astrup, 2005; Lorenzen et al., 2007). The high calcium content of cheese, therefore, directly affects the blood lipids through a reduced fat absorption. It may, however, also indirectly affect blood lipid concentrations in case of ACP formation and increased faecal bile acid excretion. This would

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upregulate of de novo synthesis of bile acids from hepatic cholesterol and increase blood low density lipoprotein cholesterol (LDL-C) clearance (Ditscheid et al., 2005).

It may be crucial that calcium, phosphate, and fat are closely situated for these components to interact. In cheese calcium, phosphate, and fat is encapsulated in the protein structure. Also, these compounds will be simultaneously present in the duodenum after cheese consumption, which may facilitate their precipitation. In contrast in milk, fat is present in small globules enclosed by a membrane, but calcium and protein are present in the aqueous phase (Moss & Freed, 2003). Butter contains virtually no calcium and protein and less phosphate than cheese. Hence, intake of butter would not be expected to cause formation of fatty acids soaps and ACP. Therefore, the degree of precipitation of fat, calcium and phosphate is likely to depend on the dairy-product matrix. Hence, the effect of milk-fat intake may depend on whether the fat is bound within a cheese-matrix or not. We hypothesised that intake of regular-fat cheese would lower LDL-C concentration compared with intake of reduced-fat cheese + butter and butter. Secondarily, we hypothesised that intake of regular-fat cheese would cause higher faecal fat excretion compared with reduced-fat cheese + butter and butter.

Dietary calcium and phosphate content has also been shown to affect the mucosal microbiome composition in pigs (Mann et al., 2014), which may additionally affect the host. For example, a higher degree of energy extraction from the food has been associated with a higher gut microbial *Firmicutes*-to-*Bacteroidetes* (F/B) ratio (Turnbaugh et al., 2006) and some bacterial metabolites have been inversely associated with serum high density lipoprotein cholesterol (HDL-C), LDL-C and total cholesterol (TC) concentrations in obese women (Druart et al., 2014). Owing to differences in the dairy-matrix of cheeses and butter these foods may differently affect the gut microbiome composition which in turn may affect blood lipid profile and faecal energy excretion in the host.

The aim of the present study was to examine the effect of the matrix of regular-fat and reduced-fat cheese in diets with equal milk-fat on blood lipids, faecal excretion of fat and energy and gut microbiome in growing pigs.

2. Materials and methods

2.1. Animals

The animals included in the study were 36 LYDD crossbred growing sows, with an initial weight of 60–70 kg and age of 3–4 months. The pigs were obtained from the Danish Institute of Agricultural Science swine herd. Pigs were used because they have been suggested to be a good model for the human digestion and blood lipid response. Like humans, pigs are omnivorous and eat meals with postprandial breaks between and therefore have a bolus secretion of bile acids. In addition, the response to fat and cholesterol containing diets on blood lipid balance in pigs is comparable with that of humans (Dixon et al., 1999; Turk & Laughlin, 2004).

The experimental procedures were conducted according to protocols approved by the Danish Animal Experiments Inspectorate, Ministry of Food, Agriculture and Fisheries, Danish Food and Veterinary Administration, Copenhagen. In addition, the protocol applied with the Danish laws and regulations for the Humane Care and Use of Animals in Research Act 474 of May 15 2014, as in compliance with EU Directive 2010/63.

2.2. Experimental design

The study had a randomised parallel-arm design with three intervention diets. The primary end point was LDL-C concentration.

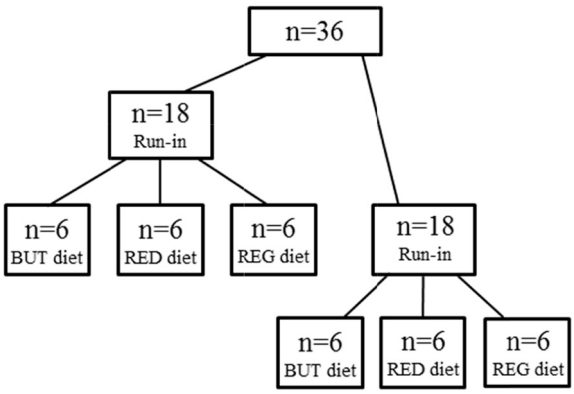

Fig. 1. Study design. Total number of pigs included n = 36, with n = 12 in each intervention arm.

The sample size calculation was based on an expected 0.3 mmol L^{-1} mean difference in serum LDL-C with a 0.25 SD between the two cheese diet groups. A total of 36 pigs with 12 pigs in each intervention arm were needed to detect the expected difference with an 80% power and a 0.05 significance level. Secondary end points were TC, HDL-C and TG concentrations as well as faecal excretion of fat and energy. Gut microbiota was included as an explorative end point.

Before the intervention there was a 14 days run-in period where pigs were kept together in large pens on bedding and adapted to a diet containing a higher fat energy percentage than that of the diet consumed before study commencement. Butter was chosen for the run-in diet to achieve a dietary fatty acid composition equal to that of the intervention diets. After run-in, pigs were randomised, according to initial TC concentration, to one of three intervention diets lasting for 14 days. During the intervention pigs were kept individually in pens without bedding. For logistical reasons the parallel-arm study was carried out in two equally numbered groups, where the first group started the run-in period two weeks before the second group (Fig. 1).

During the study fasting blood samples were drawn before the run-in period (used to analyse TC concentration for the randomisation), before the intervention period (pre-intervention), and after the intervention period (post-intervention). Body weight was measured on the same occasions. Three days before study completion the pigs were transferred to stainless steel metabolic cages, in which faeces and urine were collected over the last 48 h.

2.3. Diets

Three iso-caloric macronutrient-matched intervention diets were designed, which contained high amounts of (i) regular-fat cheese (REG), (ii) reduced-fat cheese + butter (RED), or (iii) butter (BUT). Based on body weight and growth curves (Andersen & Just, 1983) 90% of the habitual energy intake was provided to ensure complete consumption. The energy requirement was assessed before the run-in and again pre-intervention to adjust the energy provision to the 14 days increase in body weight. The three diets were designed to be iso-caloric with an equal fatty acid composition, and to match the macronutrient composition of the average Danish diet (Table 1). However, due to the lower fat tolerance limit of pigs, the fat content was slightly lower and the protein content slightly higher than that of the average Danish diet (Pedersen et al., 2010), Equal amounts of cheese (35.3 g per 100 g diet) were included in the two cheese diets. In a 60 kg pig this was equal to a daily consumption of ~700 g of cheese (providing