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Effects of fermented, ropy, non-dairy, oat-based products on serum lipids and the faecal excretion of cholesterol and short chain fatty acids in germfree and conventional rats

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Abstract

   Three fermented, ropy, non-dairy, oat-based products were evaluated for their effect on serum lipids, faecal cholesterol and faecal short chain fatty acids in germfree and conventional rats. Three different exopolysaccharide (EPS) producing lactic acid bacteria strains were used to ferment the non-dairy oat-base (Adavena® G40) (Ceba Foods AB, Lund, Sweden). Two commercial non-dairy products based on oats (Mill Milk™) (Ceba Foods AB, Lund, Sweden) and rice (Rice Dream®) (Imagine Foods, London, UK) were used as non-ropy and unfermented controls. All the standardized feeds were sterilized before being fed to the animals. Adult, germfree and conventional AGUS rats,

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were fed the above sterile diets *ad libitum* for 21 days. Blood samples and faecal samples were collected and the animals' weight gain was monitored throughout the study. No significant change in serum lipids or faecal excretion of cholesterol was observed between the groups on the different diets. A difference in faecal SCFA pattern was observed in conventional rats fed on the oat-based diets in comparison to the group fed on the rice-based diet. More evidence is needed to support the effect of fermented, ropy, oat-based products and their potential effect on serum lipids, faecal cholesterol/ coprostanol levels and amounts of short chain fatty acids. © 2002 Elsevier Science Inc. All rights reserved.

1. Introduction

Results of several studies have shown that elevated cholesterol levels in serum are associated with an increased risk to develop ischaemic heart disease (IHD) [1,2]. Efforts to reduce cholesterol in plasma low-density lipoprotein (LDL) by diet play an important role in the prevention of IHD [3,4]. It has been shown that foods containing soluble dietary fiber can reduce serum lipids in humans, mainly by reducing the absorption of cholesterol and bile acids from the intestine [5]. Oats have a high content of soluble fiber, most β-glucans [6]. To both widen and increase the use of oats as the major ingredients in processed food products, an oat-based, non-dairy milk product, Mill Milk^{TM} (Ceba Foods AB, Lund Sweden), has been developed based on a patented process [7] with the main carbohydrate species being maltose and β-limit dextrins. A recent study on healthy volunteers has shown that this product significantly reduces total serum cholesterol and LDL cholesterol after 4 weeks during which 0.75-1 liter/day, of which 0.4% is β-glucans, was consumed [8]. The process of producing an oat-based, non-dairy milk substitute has been further developed to also include a product containing glucose instead of maltose. Recent studies have shown that these non-dairy milk products are suitable substrates for the growth of different lactic acid bacteria (LAB) and also for the formation of bacterial exopolysaccharides (EPS) [9–11]. EPS produced by LAB during the fermentation play an important role for the final viscosity and the texture of the product. Depending on how these EPSs interact with the overall matrix in the food product they can also give a "ropy" characteristic to these fermented products.

A study has shown that also ropy milk, fermented by EPS-producing LAB, had a serum lipid lowering activity in rats [12]. The effect of EPS is assumed to depend on their digestive fate, i.e., if they are microbial degraded in the colon. As short-chain fatty acids (SCFA) are intermediate products of microbial metabolism of both exogenous and endogenous compounds in the gastrointestinal tract, the fermentation of EPS can result in the formation of SFCA. If they are not degraded, their chemical properties will bring about an increase in the stool bulk [13–15].

The intestinal flora has an important effect on physiological parameters, such as levels of lipid and SCFA [16–17]. A comparison of two different *in vivo* models, with and without the physiological impact of the intestinal microflora, is therefore of great concern in the course of investigating the physiological effects of new, fermented food products. The objective of

the present study was to investigate the effects of three fermented, ropy, non-dairy, oat-based products, produced with EPS-producing LAB, on serum lipids and the faecal excretion of cholesterol and SCFA in germfree (GF) and conventional (CONV) rats.

2. Materials and methods

2.1. Preparation of lactic acid bacteria and fermented oat-base

Three strains of LAB were used for the production of fermented, ropy, non-dairy, oat-based products. These were: *Lactobacillus brevis* G-77, *Pediococcus damnosus* 2.6 (Universidad de Pais Vasco, San Sebastian, Spain) and *Lactobacillus delbrueckii* subsp. *bulgaricus* NCFB 2772 (The National Culture Collection of Food Bacteria, Aberdeen, Scotland). The strains were stored at −80°C in de Man Rogosa Sharpe (MRS) broth [18] plus 25% (v/v) glycerol. Before inoculation into the oat-base product the cultures were propagated twice in MRS (Merck, Darmstadt, Germany) at 30°C (*P. damnosus* 2.6 and *Lactobacillus brevis* G-77) or 37°C (*Lactobacillus delbrueckii* subsp *bulgaricus* NCFB 2772). A 20%, reconstituted, oat-based medium, Adavena® G40 (Ceba Foods AB, Lund, Sweden) was first heat treated at 90°C for 5 min prior to inoculation. The bacterial strains, taken from a fresh culture grown in MRS medium were individually inoculated at a concentration of 5% (v/v) into the Adavena® G40 medium and incubated at 28°C for 18 h. The final pH of each fermented product was 4.5±0.2. All fermentations were carried out in a 150 L fermentor (Biostat®) (B. Braun Biotech International, Melsungen, Germany), with a 100 L working-volume. Two commercial, unfermented non-dairy drinks were used as controls, an oat product, Mill Milk™ (Ceba Foods AB, Lund, Sweden), (MM) and a rice product, Rice Dream® (Imagine Foods, London, UK) (RD). All five diets were freeze dried and sterilized by irradiation (β-rays).

2.2. Animals

35 adult GF AGUS rats and 35 CONV rats [19] (Gustafsson, 1959) of both sexes, weight 200-350 g, were randomly selected into five sub-groups of seven animals. The rats were kept in a room at 24 ± 2°C, a relative humidity of 55 ± 10% and controlled light (6 a.m. to 6 p.m.) for 21 days during which they had free access to respective diet and water. GF rats were kept in lightweight, stainless steel isolators and checked weekly for GF status [20]. These five sub-groups of GF and CONV rats, were fed five different diets consisting of three fermented, ropy oat-bases fermented by *P. damnosus* 2.6 (2.6), *L. brevis* G-77 (G-77) or *L. delbrueckii* subsp. *bulgaricus* NCFB 2772 (2772) and two control diets, a commercial oat product (MM) and a commercial rice product (RD). The composition of the diets is shown in Table 1 . Blood samples were collected before the animals entered the study (day 0) and after 21 days on the different experimental diets. Blood samples taken at day 0 were pooled for all GF and CONV rats, respectively. Faecal samples were collected at intervals. The

Table 1
Composition (g/100g) of the five experimental diets

	Diets				
	2.6[1]	G-77[2]	2772[3]	MM[4]	RD[5]
Dry matter	96.5	94.7	96.3	95.4	97.4
Protein	12.2	12.1	11.6	11.7	18.5
Dietary fibres	7.1	7.0	6.7	7.0	3.2
β-glucans	3.8	4.2	4.1	3.8	ND[6]
Carbohydrates	76.5	75.8	76.7	71.9	70.3
Fat	6.6	5.7	6.4	11.3	7.3

[1] G40 product fermented with *Pediococcus damnosus* 2.6.
[2] G40 product fermented with *Lactobacillus brevis* G-77.
[3] G40 product fermented with *Lactobacillus delbrueckii* subsp. *bulgaricus* 2772.
[4] Mill Milk™ product (oat-based control product).
[5] Rice Dream® product (rice-based control product).
[6] Not determined.

animals were weighed at the beginning and the end of the study. After 21 days the animals were killed using pentobarbital and blood and intestinal contents from each animal were sampled. The study was approved by the local ethical Committee, Stockholm Nord, Sweden (No. 249/00).

2.3. Serum cholesterol and triglyceride assays

Total serum cholesterol and triglycerides were assayed individually in a Monarch automated 2000 analyser (Instrumentation Laboratories Scandinavia AB, Sollentuna, Sweden) using enzymatic test kits, 181618-10 for cholesterol and 181610-60 for triglycerides (Instrumentation laboratory SpA, Milan, Italy).

2.4. Determination of coprostanol

All biochemical analyses were run within four weeks after sampling. Faecal samples were homogenised with 2 ml of a mixture of 10 M NaOH and 96% EtOH (1:2), and kept 60°C for 45 min. The mixture was extracted with n-hexane twice and the combined hexane phases were washed with ethanol (70% (v/v)) until neutral. The conversion of cholesterol to coprostanol was then analysed as described earlier by [21] using GLC (Perkin Elmer Autosystem XL, Perkin Elmer Corp., San Jose, US) equipped with a glass column packed with 3% OV-17 maintained at 290°C using nitrogen gas as carrier and with a flame ionization detector (Hewlett Packard, Avondale, US). Pure cholesterol and coprostanol were used as standards. By using this method, peak areas of less then 5% were regarded as impurities. GF animals do not excrete coprostanol in their faeces as they follow the germ free animal characteristic (GAC), whereas CONV animals do as they follow the microflora-associated characteristic (MAC) [22].

2.5. Determination of faecal SCFA

Faecal samples and sterile aliquots of (0.5 g) of mashed diets were taken and homogenised in 2 ml of distilled water containing 3 mmol/l of 2-ethylbutyric acid as an internal standard and 0.5 ml of H_2SO_4 (0.5 mol/l). All the homogenates were vacuum distilled for 20 min as described earlier by [23] with modifications by [24]. The amounts of SCFA in the distillates were analysed by GLC (Perkin Elmer Autosystem XL) on a packed column of 10% SP-1200/1% H_3PO_4 on 80/100 Chromosorb at 120°C using nitrogen gas as carrier. The chromatograms were recorded, the peak areas determined and the concentrations calculated with TurboChrom autoanalyzer system (Perkin Elmer). Each series of analyses started and ended with the injection of a standard solution. The total and individual SCFA concentrations were given in mmol/kg food or mmol/kg intestinal content (wet weight).

2.6. Statistical analyses

Values were expressed as means and standard deviation (SD). When statistically significant differences were found, the mean values of the treatments were compared by Student's t test. Differences were considered significant at $P < 0.05$.

3. Results

3.1. Animals

The groups fed on the rice-based diet (RD) generally ate a smaller amount of food and also looked somewhat slimmer in comparison with the other groups. It was also sometimes difficult to obtain a sufficient amount of faeces for the analytical procedures. The faeces from the GF group fed on the RD diet had very liquid faeces compared to the other GF groups. The faeces from the CONV rats fed on the RD diet had a hard, dark and dry in appearance compared to faeces from the other CONV groups. The body weights before and after the study are shown in Table 2. The body weight decreased in the CONV male rats fed on the RD diet and was almost similar in the females. For the other groups there was a moderate increase in body weight after 21 days on the various diets.

3.2. Serum cholesterol and triglyceride concentrations

Serum cholesterol and triglycerides were determined after 21 days on the diets (Table 3). The cholesterol levels in both GF and CONV rats were between 1.0–1.5 mmol/l and for the triglycerides 0.9–2.4 mmol/l. There was a significantly ($P < 0.01$) lower level of triglycerides in the GF rats fed on the G-77 diet. A somewhat lower level of triglycerides was also seen in the CONV rats fed on the G-77 diet. However, the differences were not significantly.

Table 2
Effect of diet on mean body weight[1] of germfree (GF) and conventional (CONV) male and female rats, after the 21-day diet

| Diet | Initial body weight (g) (day 0) | | | | | | | | Final body wieght (g) (day 21) | | | | | | | |
| | GF (male) | | CONV (male) | | GF (female) | | CONV (female) | | GF (male) | | CONV (male) | | GF (female) | | CONV (female) | |
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
2.6	338	11.8	309	37.7	184	6.9	250	13.4	369	3.6	328	51.1	202	13.6	270	9.1
G-77	275	4.2	289	1.4	220	8.6	245	8.5	277	2.8	290	17.7	217	8.9	271	21.7
2772	309	39.7	363	11.5	237	42.5	180	6.6	334	30.4	392	22.9	238	28.6	204	8.2
MM	292	7.6	321	37.0	215	8.2	185	12.2	315	4.2	340	45.5	213	10.0	191	14.9
RD	305	10.1	332	14.0	213	12.9	221	9.7	294	13.5	317	7.0	215	13.1	228	9.1

[1] Values are means ± SD, $n = 7$.

3.3. Conversion of cholesterol to coprostanol

The microbial conversion of cholesterol to coprostanol was measured on day 21 in the CONV rats. By definition the GF rats lack this fermentation. Females had a higher conversion of cholesterol to coprostanol (25–40%) than males (5–20%) (Fig. 1). This difference between the sexes was most obvious in the groups receiving the G-77 and the MM diets. The rats fed on the 2.6 diet were the only group in which the females showed a lower conversion of cholesterol to coprostanol than males.

3.4. Faecal short chain fatty acids (SCFA)

The total SCFA concentrations and concentrations of acetic, propionic and butyric acids in the diets are given in Fig. 2. Acetic acid was the predominant SCFA present in all diets.

Table 3
Serum cholesterol and triglycerides[1] after 21 days on a diet of three fermented, ropy, oat-based products (2.6, G-77, 2772) and two non-dairy products based on oats (MM) and rice (RD) in both germfree (GF) and conventional (CONV) rats, males and females combined

| Diet | Cholesterol (mmol/L)[2] | | | | Triglycerides (mmol/L)[3] | | | |
	GF Mean	SD	CONV Mean	SD	GF Mean	SD	CONV Mean	SD
2.6	1.2	0.10	1.2	0.17	2.4	1.33	2.4	0.43
G-77	1.3	0.07	1.2	0.09	0.9	0.35*	1.2	0.25
2772	1.2	0.29	1.1	0.20	1.3	0.66	1.7	0.83
MM	1.1	0.16	1.1	0.08	1.6	1.37	1.6	1.29
RD	0.9	0.11	1.2	0.17	1.5	0.80	1.6	0.37

[1] Values are means ± SD, $n = 7$.
[2] Initial values (day 0) were 1.4 ± 0.10 and 1.3 ± 0.10 for the GF and the CONV rats, respectively.
[3] Initial values (day 0) were 1.6 ± 0.12 and 1.4 ± 0.24 for the GF and the CONV rats, respectively.
* Significantly different from the initial value by Student's t test ($P < 0$).
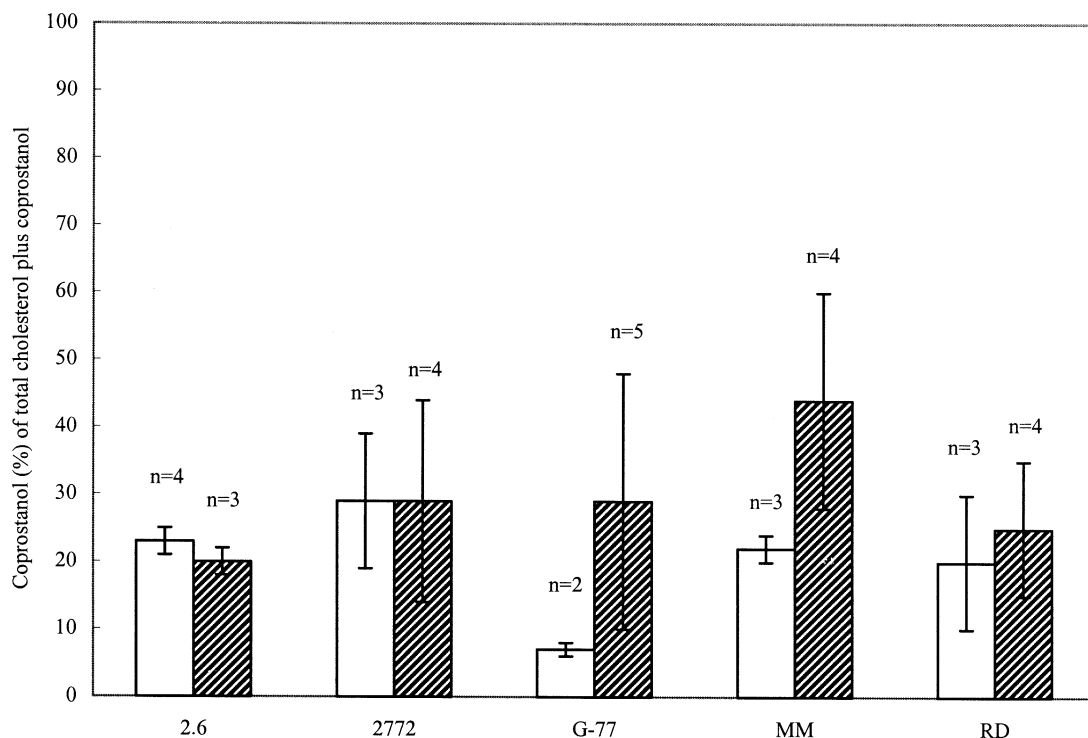
Fig. 1. Coprostanol in faeces from conventional (CONV) male (□) and female (▨) rats after 21 days on three different, fermented, oat-based diets (2.6, G-77, 2772) and two commercial, non-dairy products based on oats (MM) and (RD). The values are means ± SD.

Total faecal SCFA and concentrations of acetic, propionic and butyric acids from the GF and CONV rats are shown in Fig. 3a-b. The total concentration of SCFAs was between 12-22 mmol/kg in the faecal samples from the GF rats after 21 days on all diets (Fig. 3a). The highest amount of acetic acid was present in the faeces of the group fed on the G-77 diet. This was also the only group in which propionic acid was detected in the GF rat faeces. The amount of total SCFA from CONV rats was between 45-65 mmol/kg after 21 days on the diets (Fig. 3b). Acetic acid was lower in all the groups fed on the oat-based product in comparison with the group fed on the RD diet. Samples from this group had a lower amount of propionic acid than samples from the groups fed on the oat-based diets.

4. Discussion

The purpose of the present study was to evaluate the effects on serum lipids and faecal excretion of cholesterol and SCFA of three fermented, non-dairy, oat-based products, produced with EPS-producing LAB, in GF and CONV rats. The GF animals represent the animal *per se,* without the interference of the microflora [19]. This gives a base line of the microbial reactions, which is of importance to elucidate when introducing new diets as this
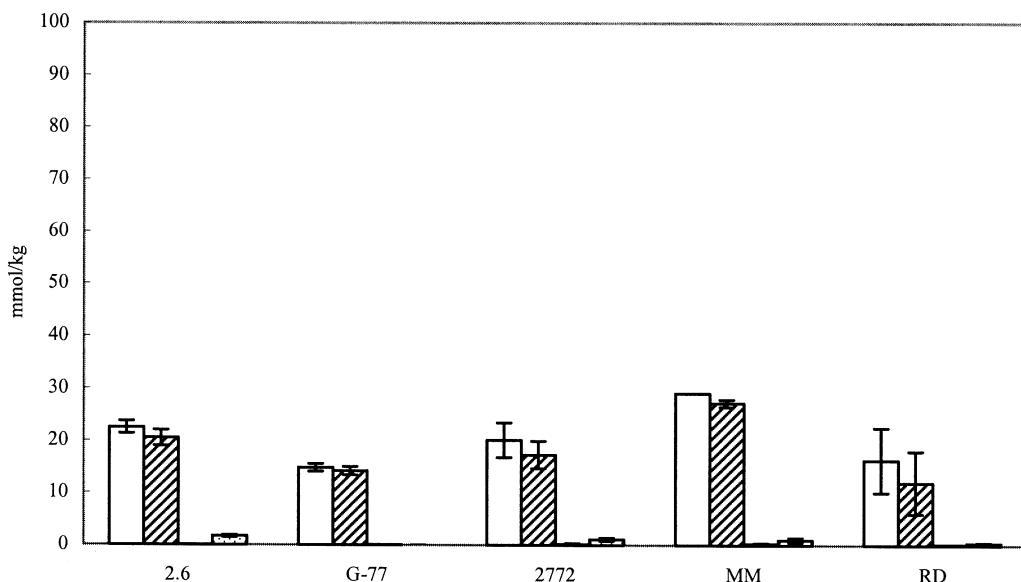
Fig. 2. Total concentrations of SCFAs (□) and of acetic (▨), propionic (▤) and butyric (⊡) acids in the three different fermented, oat-based diets (2.6, G-77, 2772), two commercial, non-dairy products based on oats (MM) and rice (RD). The values are means ± SD, $n = 7$.

study is a first step in the evaluation of these fermented, ropy, products and whether they have any positive effect on the above-mentioned physiological parameters.

In this study, the total concentrations of serum cholesterol were not significantly altered, either in the GF or the CONV rats after 21 days on any of the diets (Table 3). A significant difference ($P < 0.01$), however, was observed in the triglyceride levels of the GF rats fed on the G-77 diet compared to the initial value. The difference in cholesterol between the GF and the CONV rats was not significant as was reported in the study of [25]. However, the fact that a casein-based diet was used in that study may be of importance, since in our study only a cereal-based diet was used thorough the experiment. The oat-based control diet (MM) which has a documented cholesterol-lowering capacity in humans, showed no effect in reducing cholesterol levels in either the GF or the CONV rats used in this study. Generally, rats have a higher level of high-density lipoprotein (HDL) cholesterol in comparison to low-density lipoprotein (LDL) cholesterol in serum, whereas in humans, and particularly men, there is an opposite condition. Thus, rats may therefore not be the optimal model to use for the study of cholesterol-lowering properties of products that are intended to be used for human consumption. However, when studying several basic physiological parameters, among others parameters that are influenced by the microbiota in the GI- tract, the possibility to do comparisons between GF and CONV situations are of great importance and the use of rat models can therefore be appropriate.

The diets used in this study were all freeze-dried and irradiated prior to being fed to the rats. In the earlier study by [19] the products (MM and RD) were used in a water-solubilized form, with high water content and with the soluble fiber fraction totally dissolved. No significant differences in cholesterol levels were observed between the fermented, oat-base
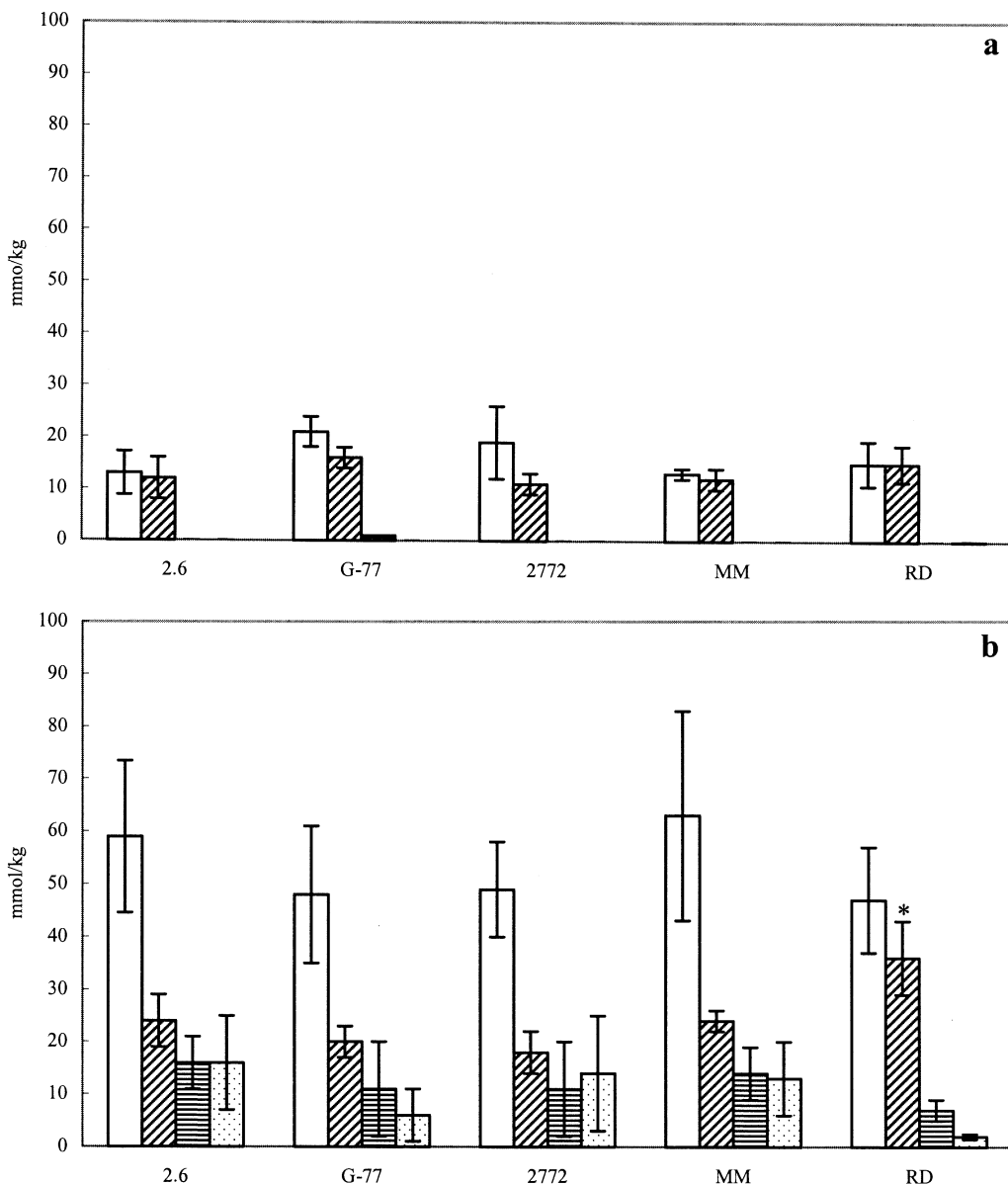
Fig. 3. Total faecal concentrations of SCFAs (□) and of acetic (▨), propionic (▤) and butyric (⊡) acids in faeces in germfree (GF) (a) and conventional (CONV) (b) rats after 21 days of intake of three different, fermented, oat-based diets (2.6, G-77, 2772) and two commercial, non-dairy products based on oats (MM) and rice (RD). The values are means ± SD, $n = 7$. *Significantly different by Student's t test ($P < 0.01$).

products mainly containing glucose (G40 product) and the product containing mainly maltose (MM product). Interestingly, in a recent study no significant cholesterol-lowering effect was seen in humans when β-glucan from oats was given to the subjects in a dried form [26]. It may be of importance that these soluble fibers need to be totally solubilized in the

water matrix of the food in order to have the expected physiological effect during the intestinal transfer. Thus, this can be one reason why no effect on serum lipid levels was seen in our study.

Microbial polysaccharides may have a water-holding capacity and anion exchange activity [27]. The physiological mechanism of these polysaccharides could therefore be the same as has been explained for dietary fiber of plant origin [5]. The cholesterol-lowering properties of milk fermented by different kinds of LAB have been studied in rats [28–30]. However, these studies used a diet containing high concentrations of cholesterol.

Intestinal cholesterol can either be absorbed or eliminated or undergo a microbial conversion with the major end metabolite being coprostanol [17]. This microbial transformation of cholesterol to coprostanol has been related, so far to only a few species, namely, *Eubacterium lentum* and *E. coprostanoligenes* [31,32]. GF rats lack this elimination of cholesterol by microbes, described as the microbial excretion-knife [33]. Higher levels of serum cholesterol have been determined in GF than in CONV rats [25]. It is generally stated that the microflora is active in metabolising sterols and steroid hormones, thus the microbial conversion of cholesterol to coprostanol is therefore of importance for the serum cholesterol level [25]. There was a decrease in the intestinal microbial conversion of cholesterol in the male rats fed on the G-77 or the MM diet. In the female rats this decrease was not evident. This difference in microbial conversion of cholesterol to coprostanol between male and female rats was not verified by the serum lipid analysis. More studies are needed, however, in order to ascertain that his difference between the sexes is due to the differences in diets.

Short-chain fatty acids (SCFA) are products of anaerobic microbial (anabolic and catabolic) metabolism in the intestine of carbohydrates and proteins, both of dietary and endogenous origin [34,35]. The different experimental diets had SCFA containing range from 15-30 mmol/kg. The same relative amount of SCFAs was seen in the GF rats after 21 days on the diet. The SCFAs measured in the GF rats can therefore be considered as being solely of dietary origin. The amounts of SCFA in faecal samples from the GF rats were significantly lower than in the CONV rats, which is in line with the fact that SCFA are products of microbial fermentation [36]. In the CONV rats the total SCFA concentrations were between 45-65 mmol/kg, which is in agreement with other findings of SCFA in faecal samples from rats [37]. The predominant SCFA were acetic, propionic and butyric acids. The spectra of the main SCFAs excreted in the faeces of the CONV rats showed that a larger amount of acetic acid was produced in rats eating the rice-based diet (RD) compared to rats eating the oat-based products. This shows the dietary influence on the faecal SCFA pattern. Earlier studies have shown that dietary changes have an effect on the faecal concentration of SCFA [38,39]. The liquid faeces that were observed in the GF rats may arise by osmotic phenomenon in the large intestine due to a high concentration of low molecular carbohydrates in combination with a low concentration of fibers in this diet. This was not observed in the CONV rats, as the intestinal microbes may use these carbohydrates as a source of energy. These rats had harder faeces in comparison to the CONV rats eating the fermented, oat-based products. This may be due to the low content of dietary fiber with good water-holding capacity in the RD diet.

In conclusion, no significant changes were seen between GF and CONV rats in terms of serum lipids. No difference in faecal concentrations of coprostanol was seen between the

groups of CONV rats. Differences in faecal SCFA patterns were observed in CONV rats that were on the different oat-based diets compared with the group on the rice diet. This indicates that different diets affect the formation of SCFAs in the colon and the faecal excretion of these SCFAs. Further research should include clinical studies on these products, before suggestions can be made concerning these fermented, ropy, non-dairy, oat-based products and their potential effects on serum lipid levels.

Acknowledgments

References

[1] Rosengren A, Hagman M, Wedel H, Willhelmsen L. Serum cholesterol and long-term prognosis in middle-aged men with myocardial infarction and angina pectoris: A 16-year follow-up of the Primary Prevention Study in Göteborg, Sweden. Eur Heart J 1997;18:754–61.

[2] Law MR, Wald NJ, WU T, Hackshaw A, Bailey A. Systemic underestimation of association between serum cholesterol concentration and ischaemic heart disease in observation studies: Data from BUPA Study. Br Med J 1994;308:363–66.

[3] Agerholm-Larsen L, Raben A, Haulrik N, Hansen AS, Manders M, Astrup A. Effect of 8 week intake of probiotic milk products on risk factors for cardiovascular diseases. Eur J Clin Nutr 2000a;54:288–97.

[4] Agerholm-Larsen L, Bell ML, Grunwald GK, Astrup A. The effect of a probiotic milk product on plasma cholesterol: a meta-analysis of short-term intervention studies. Eur J Clin Nutr 2000b;54:856–60.

[5] Anderson JW, Bridges S-R. Hypocholesterolemic effects of oat bran in humans. In: Wood PJ, editor. Oat bran. St. Paul: American Association of Cereal Chemists, US, 1993. p. 139–57.

[6] Asp N-G, Mattsson B, Önning G. Variation in dietary fibre, β-glucan, starch, protein, fat and hull content of oats grown in Sweden 1987-1989. Eur J Clin Nutr 1992;46:31–7.

[7] Lindahl L, Ahldén I, Öste R, Sjöholm I. Homogenous and stable cereal suspension and a method of making the same. 1997. United States Patent 5.686.123.

[8] Önning G, Åkesson B, Öste R, Lundquist I. Effects of consumption of oatmilk, soya milk or cow's milk on plasma lipids and antioxidative capacity in healthy subjects. Ann Nutr Metabol 1998;42:211–20.

[9] Mårtensson O, Öste R, Holst O. Lactic acid bacteria in an oat-based non-dairy milk substitute: Fermentation characteristics and EPS formation. Food Sci Technol/LWT 2000;33:525–30.

[10] Mårtensson O, Andersson C, Andersson K, Öste R, Holst O. Formulation of an oat-based fermented product and its comparison to yoghurt. J Sci Food and Agri 2001;81:1413–21.

[11] Mårtensson O, Öste R, Holst O. Texture promoting capacity and EPS formation by lactic acid bacteria in three different oat-based non-dairy media. Eur Food Res Technol 2002;214:232–36.

[12] Nakajima H, Suzuki Y, Kaizu H, Hirota T. Cholesterol lowering activity of ropy fermented milk. J Food Sci 1992;6:1327–29.

[13] Ruijssenaars HJ, Stingele F, Hartmans S. Biodegradability of food-associated extracellular polysaccharides. Curr Microbiol 2000;40:194–99.

[14] Cummings JH, Englyst HN. Gastrointestinal effects of food carbohydrate. Am J Clin Nutr 1995;61(Suppl): 938S–45S.

[15] Harris PJ, Feurgson LR. Dietary fibre: its composition and role in protection against colorectal cancer. Mut Res 1993;290:97–110.

[16] Gustafsson BE. The physiological importance of the colonic microflora. Scand J Gastroenterol 1982;77: 117–31.

[17] Midtvedt T. Microbial function activities. In: Hanson Å, Yolken RH, editors. Presentation at the 42nd Nestlé Nutrition Workshop in China May 11-15, 1997. Philadelphia: Lippincott-Raven Publishers, 1999. p. 79–96.

[18] De Man JC, Rogosa M, Sharpe MEA. Medium for the cultivation of lactobacilli. J Appl Bacteriol 1960;23:130–35.

[19] Gustafsson BE. Light weight stainless steel systems for rearing germfree animals. Ann NY Academ Sci 1959;78:17–28.

[20] Festing MFW. International index of laboratory animals. UK: University of Leicester, 1993.

[21] Midtvedt A-C, Carlstedt-Duke B, Norin K-E, Saxerholt H, Midtvedt T. Development of five metabolic activities associated with the intestinal microflora of healthy infants. J Ped Gastroenterol Nutr 1988;7:559–66.

[22] Midtvedt T., Bjørneklett A, Carlstedt-Duke B, Gustafsson BE. The influence of antibiotics upon microflora-associated characteristics in man and mammals. In: Wosmann BS, Pleasant JR, Pollard M, editors. Germfree research: microflora control and its application to the biomedical sciences. New York: Alan R. Liss Inc., 1985. p. 241–44.

[23] Zijlstra JB, Beukma J, Wolthers BG, Byrne BM, Groen A, Donkert L. Pretreatment methods prior to gas chromatography analysis of volatile fatty acids from faecal samples. Clin Chim Acta 1977;78:243–50.

[24] Høverstad T, Fausa O, Bjørneklett A, Bøhmer T. Short-chain fatty acids in the normal human faeces. Scand J Gastroenterol 1984;19:375–81.

[25] Danielsson H, Gustafsson B. On serum-cholesterol levels and neutral faecal sterols in germfree rats. Bile acids and steroids 59. Arch Biochem Biophys 1959;83:482–85.

[26] Lovegrove JA, Clohessy A, Milton H, Williams C. Modest doses of β-glucan do not reduce concentration of potentially atherogenic lipoproteins. Am J Clin Nutr 2000;72:49–55.

[27] Toba T, Nakajima H, Tobitani A, Adachi A. Scanning electron microscopic and texture studies on characteristic consistency of Nordic ropy sour milk. Int J Food Microbiol 1990;11:313–18.

[28] Tamai Y, Yoshimitsu N, Watanabe Y, Kuwabara Y, Nagai S. Effects of milk fermented by culturing with various kind of lactic acid bacteria and a yeast on serum cholesterol level in rats. J Fermentation and Bioeng 1996;2:181–82.

[29] Pulsani S, Rao DR. Whole body, liver and plasma cholesterol levels in rats fed *Thermophilus Bulgaricus* and *Acidophilus* milks. J Food Sci 1983;48:280–81.

[30] Grunewald KK. Serum cholesterol levels in rats fed skim milk fermented by *Lactobacillus acidophilus*. J Food Sci 1982;47:2078–79.

[31] Sadzikowski MR, Sperry JF, Wilkins TD. Cholesterol-reducing bacterium from human feces. Appl Environ Microbiol 1977;34:355–62.

[32] Eyssen HJ, Paramentier GGC, Compernolle FC, Pauw G, Piessens-Denef M. Biohydrogenation of sterols by *Eubacterium* ATCC 21.408-nova species. Eur J Biochem 1973;36:411–21.

[33] Midtvedt T. Metabolism of endogenous substances. In: De Clerque E, editor. Frontiers in Microbiology. Dordrecht, The Netherlands: Martinus Nijhoff Publishers, 1987. p. 79–87.

[34] Cummings JH. Short chain fatty acids in the human colon. Gut 1981;22:763–79.

[35] Fleming SE, Arce DS. Volatile fatty acids: their production, absorption, utilization and roles in human health. Clin Gastroenterol 1986;15:787–814.

[36] Høverstad T, Midtvedt T. Short-chain fatty acids in intestinal content of germfree mice and rats. J Nutr 1986;116:1772–76.

[37] Fleming SE, Choi SY, Fitch MD. Absorption of short-chain fatty acids from the rat cecum *in vivo*. J Nutr 1991;113:1613–26.

[38] Maczulak A E, Wolin MJ, Miller TL. Amounts of viable anaerobes, methanogenes, and bacterial fermentation products in the faeces of rats fed high-fiber or fiber-free diets. Appl Environ Microbiol 1993;59: 657–62.

[39] Spiller GA, Chernoff MC, Hill RAS, Gates JE, Nassar JJ, Shipley EA. Effect of purified cellulose, pectin, and a low-residue diet on faecal volatile fatty acids, transit time, and faecal weight in humans. Am J Clin Nutr 1980;33:754–75.