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Barley malt increases hindgut and portal butyric acid, modulates gene expression of gut tight junction proteins and toll-like receptors in rats fed high-fat diets, but high advanced glycation end-products partially attenuate the effects.

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Abstract
Barley malt, a product of controlled germination, has been shown to give high levels of butyric acid in the cecum and portal serum of rats and may therefore have anti-inflammatory effects. The aim of the study was to investigate how four barley malts, caramelized and colored malts, 50-malt and 350-malt, differing in functional characteristics concerning beta-glucan content and color, affect short-chain fatty acids (SCFA), barrier function and inflammation in the hindgut of rats fed high-fat diets. Male Wistar rats were given malt-supplemented high-fat diets for four weeks. Low and high-fat diets containing microcrystalline cellulose were incorporated as controls. All diets contained 70 g/kg dietary fiber. The malt-fed groups induced higher amounts of butyric- and propionic acids, in the hindgut and portal serum compared with controls, while their cecal succinic acid only increased to a small extent. Fat increased the mRNA expression of tight junction proteins and Toll-like receptors (TLR) in the small intestine and distal colon of the rats, as well as the concentration of some amino acids in the portal plasma, but malt seemed to counteract these adverse effects to some extent. However, the high content of advanced glycation end-products (AGE) in caramelized malt, tended to abolish the positive effects on occludin in the small intestine and plasma amino acids seen with the other malt products. In conclusion, malting seems to be an interesting process for producing foods with positive health effects, but part of these effects may be destroyed if the malt contains a high content of AGE.

Key word: barley malt; short-chain fatty acids; succinic acid; advanced glycation end-products; high-fat diet; amino acids; tight junction protein; toll-like receptor.
Introduction

Dietary fiber is an essential part of a healthy diet. According to Codex Alimentarius, dietary fiber includes edible polysaccharides and oligosaccharides with three or more monomeric units, which are neither digested nor absorbed in the human small intestine.\(^1\) Due to its indigestibility, dietary fiber is delivered to the colon, where it is a substrate for the microbiota, favoring bacterial metabolism and the end-products formed thereof, mainly short-chain fatty acids (SCFA). SCFA play an important role in maintaining colonic health, integrity and immunological homeostasis, where butyric acid has the most potent effects, followed by propionic- and acetic acids.\(^2\) Butyric acid is the major and preferred energy source for gut epithelial cells,\(^3\) and a defect in butyric acid metabolism has been associated with inflammatory bowel disease.\(^4\) At a disturbed microbial activity in colon, intermediates, such as succinic- and lactic acids may be formed in high amounts.\(^5\)

The physico-chemical properties of dietary fiber are crucial for its beneficial outcome, such as the SCFA profile formed, which depends on factors such as the fiber’s monomeric composition and degree of polymerization and can be changed by processing.\(^6-10\) Additionally, the dietary fiber’s properties are important for the effects of probiotics. Previous studies have shown that *Lactobacillus rhamnosus* increased butyric acid formation in rats but only when the fiber in barley malt was of high solubility (30% versus 8%) and low molecular weight.\(^11,12\) A difference in the molecular weight of the fiber polysaccharides can also lead to changes in microbiota composition. Arabinoxylan (AX) with a molecular weight of 0.7 × 10^5 g/mol are more potent to stimulate the growth of *Lactobacillus* and *Eubacteria* than AX with higher molecular weight (3.5 and 2.8 × 10^5 g/mol)\(^13\). Furthermore, beta-glucan with a high or medium molecular weight (22.1 × 10^5, 8.5 × 10^5 or 5.3 × 10^5 g/mol) is sufficient to lower plasma cholesterol concentrations,
while the efficacy is reduced by 50 percent with low molecular weight beta-glucan (2.1 × 10^5 g/mol).\(^{14}\)

Although the breeding technique can provide whole grains with desired dietary fiber properties, to a great extent, it is costly and time consuming. Furthermore, dietary fiber oligosaccharides, often associated with prebiotic effects and anti-inflammatory properties, cannot be obtained with breeding. Malting, a bioprocess mainly used in beer production utilizes internal hydrolytic enzymes to activate the germination of the seed, is an interesting way to obtain food products with specific physico-chemical properties.\(^{10}\) In the standard malting process, beta-glucan is extensively degraded into smaller molecules and simple sugars, and arabinoxylan is also hydrolyzed to some extent.\(^{15}\) Low molecular weight dietary fibers are believed to modulate the immune response and to have prebiotic effects.\(^{16-18}\) Thus, arabinoxylo-oligosaccharides (AXOS) are able to selectively stimulate the growth of certain colonic bacteria, such as *Bifidobacterium*\(^{19}\) and low molecular weight beta-glucan maturates dendritic cells and reverses the inflammatory progress induced by lipopolysaccharides in rodents.\(^{16, 17}\)

An impaired nutritional condition of the colonic mucosa may increase the risk of inflammation and mucosal barrier dysfunction. A leaky gut, in general characterized by down-regulation of tight junction proteins, such as occludin and zonulin in the intestinal mucosa, is common with high-fat diets.\(^{20-22}\) Furthermore, high-fat diet-induced changes in the gut microbiota can affect expression of Toll-like receptors (TLR) in the gut, triggering inflammatory processes.\(^{23}\) Administration of the mucin-degrading bacterium, *Akkermansia muciniphila*, has been shown to reduce the mucosal permeability and low-grade inflammation in mice.\(^{24}\) Prebiotic treatment may convert the effect of fat and increase the mucosal barrier function and abundance of *A. muciniphila*, as shown by fructo-oligosaccharides.\(^{24}\)
The aim of this study was to investigate whether barley malt products containing high amounts of beta-glucan, partially degraded by the malting process, lead to the formation of high amounts of butyric acid during colonic degradation, which in turn may improve barrier function, inflammation in the gut and affect the microbiota composition. For this purpose, four commercial malts with tailored functional properties were investigated. Thus, two normal malts, with different beta-glucan contents (50-malt and 350-malt), and also two special malts used to make dark beer, differing in color (caramelized and colored malt) were selected for the study. The colored products were included, since processing at high temperatures leads to the formation of advanced glycation end-products (AGE), a collective name for the process-induced chemicals in food containing amino groups and reducing sugars. These products are inflammatory and associated with several adverse health effects such as diabetes and Alzheimer’s disease.
Materials and methods

Materials

Four different types of barley malt, caramelized malt, colored malt, 50-malt and 350-malt, were kindly provided by Viking Malt AB (Halmstad, Sweden). The malts were produced by special malting programs, and are normally used for beer production (Supplemental Materials and Methods). All of the malts were milled to a particle size less than 0.5 µm before being incorporated into the rat diets. Microcrystalline cellulose (MCC; FMC BioPolymer, Cork, Ireland) was resistant to bacterial degradation, as revealed in a separate experiment (unpublished results), and was included as a control.

Diets and experimental design of animal studies

Each of the four test diets contained one of the malts and a high level of fat (240 g/kg, dry weight basis, dwb). Two control diets containing MCC were also included in the study, with either low- (LF control, 50 g/kg, dwb) or high-fat levels (HF control, 240 g/kg, dwb). Malt products and MCC were added to the diets at a level of 70 g dietary fiber per kg diet (dwb). The dry matter content in the diets was adjusted with wheat starch, which has been shown to be more or less completely digested in the upper gut, and therefore has a minimal contribution to the formation of SCFA. The composition of the six diets is listed in Supplemental Table 1.

Male Wistar rats (Taconic, Ejby, Denmark) with an initial weight of 90 ± 2 g were randomly divided into 6 groups of 7 rats and assigned to one of the six diets. The rats were housed in groups of 3 or 4 rats per cage in a controlled environment (22 °C, 12 h daylight circle) with free
access to water. The experiment lasted for 4 weeks. During the experiment, feed intake was restricted to 12 g (dwb) per rat and day in the first 2 weeks and increased to 20 g (dwb) for the following 2 weeks. Feed residues were collected daily and the body weight of each rat was recorded every week. At the end of the experiment, the rats were anesthetized by subcutaneous injection of a mixture (1:1:2) of Hypnorm (Division of Janssen-Cilag Ltd., Janssen Pharmaceutica, Beerse, Belgium), Dormicum (F. Hoffmann-La Roche AG, Basel, Switzerland) and sterile water at a dose of 0.15 ml per 100 g body weight. Blood was drawn from the portal vein, sampled in serum or EDTA plasma collection tubes (SST Advance, Plus Blood Collection Tubes, BD, Polymouth, UK), centrifuged and then stored at -40 °C until the analysis of plasma amino acids, ammonia, cholesterol and triglycerides and serum SCFA were performed. The full and empty cecum, liver, spleen, epididymal- and retroperitoneal- fat pads were weighed, and the pH of the cecal content was measured. Cecal content was collected and stored either at -40 °C or -80 °C for analysis of the carboxylic acids (CA, including SCFA, succinic acid and lactic acid) and microbiota (Akkermansia). The transverse colon containing fecal pellets was dissected and fixed in methanol-Carnoy’s solution [60% (v/v) dry methanol, 30% (v/v) chloroform, 10% (v/v) glacial acetic acid], to measure the mucus layer thickness. The jejunum and distal colon were removed, frozen quickly in liquid nitrogen and then stored at -80 °C until the gene expression assays were performed. The animal experiment was approved by the Ethics Committee for Animal Studies at Lund University (M56-12).
Characterization of raw materials

**Dietary fiber.** The amounts of soluble and insoluble dietary fibers in the test materials were determined by a gravimetric method and the composition of the isolated dietary fibers by an AOAC method. The beta-glucan content was analyzed according to McCleary. To cover all mixed-linkage beta-glucans, including the low molecular weight ones, the prior step of ethanol-washing was skipped.

**Molecular weight of beta-glucans.** The average molecular weight of beta-glucans was determined according to Rimsten *et al.*, with the modification that the concentration of the calcofluor solution was changed to 0.0025 (w/v).

**Amino acids.** The amino acids in the malt were analyzed according to Assveen and Nilsson *et al.*

**AGE.** AGE products are conveniently measured by using carboxymethyl lysine (CML) and carboxyethyl lysine (CEL) as markers according to a methodology developed by Tareke *et al.* Ultra high pressure liquid chromatography (UHPLC) coupled to tandem mass spectrometry was used instead of HPLC (Supplemental Material and Method). The test samples were first hydrolyzed for 12 h at 110 °C using 6 M HCl, together with isotope-labeled CML and CEL as internal standards (Larodan Fine Chemicals AB, Malmö Sweden).
Animal experiment

Carboxylic acids in cecum and portal serum. SCFA in cecum and portal serum were determined by gas-liquid chromatography according to Zhao et al.\textsuperscript{35} Succinic- and lactic- acids were determined using ion-exclusion chromatography by a method developed in our lab.\textsuperscript{36}

AGE in cecum and portal serum. In samples where sufficient amounts from cecum and serum remained, after other analyses, AGE were analyzed. CML and CEL were then extracted after hydrolysis using solid phase extraction, with the exception of serum, which was directly dried under N\textsubscript{2} after filtration, and then analyzed according to Tareke et al.\textsuperscript{34}

Amino acids in portal plasma. The free amino acids and ammonia in the portal plasma were analyzed according to Assveen and Nilsson et al.\textsuperscript{32, 33} To precipitate high-molecular-weight proteins, plasma samples (200 µl) were mixed with 50 µl 10% sulfosalicylic acid. The samples were centrifuged and the supernatant removed with a syringe and then filtered with a 0.45µm PTEE syringe filter (Sartorius Minisart SRP 4) into an Eppendorf tube.

Gene expression in the small intestine and distal colon. Total RNA was extracted and purified using the RNeasy Mini kit (Qiagen) according to the manufacturer’s instructions. TissueLyser II (Qiagen) was applied to disrupt and homogenize the tissue during extraction. The quality and quantity of the extracted RNA was assessed with SPECTROstar Nano (BMG LABTECH, Ortenberg, Germany).

cDNA was generated from extracted RNA according to the manufacturer’s instructions (Thermo Scientific, Waltham, USA). The cDNA was amplified for ZO-1, occludin, and TLR 3 and 4 coding genes, using the SsoAdvanced\textsuperscript{TM} SYBR Green Supermix (Bio-Rad) with a CFX96 Touch\textsuperscript{TM} Real-Time System (Bio-Rad) according to the manufacturer’s instructions. GAPDH
was used as a housekeeping gene (See primers in Supplemental Table 2). The relative quantification of mRNA was calculated using the \( \Delta\Delta^{CT} \)-method.\(^{37} \). The data are reported as the fold change compared with the value obtained from the randomly selected control samples.

**Akkermansia in the cecum.** Amplification and detection were achieved with the Bio-Rad real-time PCR system and software. RT-qPCR was performed in a total reaction volume of 20 µl including 2 µl of cecal DNA samples (QIAamp DNA stool kit, Qiagen), 300 nM each of the primers (Supplemental Table 2) and 10 µl of SsoAdvanced SYBR Supermix (Bio-Rad). The reaction conditions were 95 °C for 3 min, followed by 40 cycles of 95 °C for 10 s and 60 °C for 30 s. A melt curve analysis was performed at the end of the amplification to distinguish the targeted PCR product from the non-targeted PCR product. Genomic DNA from the type strain (Culture Collection, University of Gothenburg, Sweden) was used as a template for the standard, which was prepared according to Axling *et al.*\(^{38} \).

**Mucus layer thickness.** Colon samples, fixed in methanol-Carnoy’s solution, were sent to Histocenter (Västra Frölunda, Sweden) for paraffin embedding, sectioning (5 µm) and staining (alcian blue-periodic acid Schiff). Five to ten randomly selected sections were analyzed with a minimum of 20 measurements of the inner mucus layer for each colon using Image J software (W Rasband, National Institutes of Health, Bethesda, USA).

**Total cholesterol and triglycerides in portal plasma.** The total cholesterol and triglycerides in portal plasma were determined using the Infinity cholesterol/TAG Liquid Stable reagent (Thermo Trace, Noble Park, Vic., Australia) according to the manufacturer’s instructions.
Calculations and statistical analyses

The design of the experiment was completely randomized and resulted in two control diets with LF or HF content. The test diets contained the barley malts in a HF setting. Cecal pools of CA (µmol) were calculated as the concentration of each CA (µmol/g) multiplied by the amount of cecal content. Body weight gain per feed was calculated as the body weight gain (g) for each rat divided by the average intake of feed (g) consumed by each rat during the experiment. The polydispersity index (PDI) for beta-glucan was calculated as molecular weight average (stands for molecular weight in the following text) per molecular number average. The maximum error in the analyses was <5%.

The data are shown as means ± standard error of the mean (SEM). One-way ANOVA was used for individual means to assess the differences between different groups followed by Tukey’s procedure. The results were normally distributed with the exception of cecal content in Table 2, Table 3, alanine in Table 4 and the data in Figure 2. When the error was found to be heterogeneous, data were transformed by log transformation (Box Cox) before ANOVA was used. This is marked in the relevant tables and figures. The results were considered to be statistically significant when $P < 0.05$ or described as a tendency if $0.05 < P < 0.1$. Statistical analyses were performed using Minitab 17 statistical software (Minitab Inc. Pennsylvania, USA).

Physiological result overview and treatment classification were visually analyzed by Principal Component Analysis (PCA; SIMCA 13, Umetrics, Umeå, Sweden) to display the relationship between analyzed physiological parameters and diets.
Results

Raw materials

Dietary fiber. 50-malt contained lower amounts of total dietary fiber than the other three malts (P < 0.05), which was due to a lower content of the main components arabinose, xylose and glucose (Table 1). The composition of the dietary fiber was comparable between different malts and contained xylose (36-38%), glucose (31-36%) and arabinose (18-20%). Considerable amounts of mannose, galactose and uronic acids were also detected (1-6%).

All the malt products had rather low amounts of beta-glucan (0.5-1.2 g/100 g, dwb), but 350-malt and caramelized malt contained higher amounts (1.2 g/100 g and 0.9 g/100 g dwb, respectively), compared with the two other malts (mean 0.6 ± 0.03 g/100 g, dwb) (P < 0.05) (Table 1). Beta-glucan in caramelized malt and 350-malt had the highest molecular weights and the lowest PDI (P < 0.05) (Figure 1).

AGE. Caramelized malt contained higher (P < 0.05) amounts of CML and CEL (12.4 and 4.0 g/100 g, dwb, respectively) than colored malt (9.5 and 1.9 mg/100 g, dwb, respectively), which was higher (P < 0.05) than that in 50-malt (5.6 and 0.7 mg/100 g, dwb, respectively) and 350-malt (4.6 and 0.6 mg/100 g, dwb, respectively) (Table 1). Only minor amounts of these components were detected in MCC.

Amino acids. Colored malt contained higher (P < 0.05) amounts of amino acids, than caramelized malt and 350-malt, which was higher than that in 50 malt (not significant in relation to 350-malt). This order was generally also reflected in the specific amino acids (Supplemental Table 3). The caramelized malt was distinguished from other malts by containing low amounts
and proportions of lysine, an amino acid known to be involved in the reaction forming AGE products together with a reducing sugar, for example, glucose.

Animal experiment

Feed intake, weight gain, cecal content, pH and tissue weight. All rats were active and gained weight during the whole experiment (Table 2). Rats fed with the malt products, except for caramelized malt, had higher body weight, body weight gain in g per g feed, cecal content, cecal tissue weight, epididymal fat and retroperitoneal fat compared with rats given HF control diet. The values in the group fed caramelized malt were similar as those groups fed the HF and LF control diets, except the liver weights that were lower in the LF control. Furthermore, the pH values were lower for all malt products than the control groups, except 350-malt.

Carboxylic acids in cecum, distal colon and portal blood. Rats fed malts had higher cecal pool of total and specific SCFA (acetic- propionic- and butyric acids) than rats fed the HF and LF control diets \( (P < 0.01) \). This result was also observed in the distal colon and the portal serum of rats (Table 3). Furthermore, the malt products generally had higher proportions of butyric acid and lower of acetic- and propionic acids in cecum and portal serum (data not shown) compared with the control groups \( (P<0.01) \).

No differences in cecal SCFA could be seen between the different malt groups, while some differences were noticed in distal colon and portal serum. Thus, lower concentrations of propionic acid \( (P < 0.005) \) were found in the distal colon and the portal serum of rats consuming caramelized malt \( (P < 0.005) \) than in rats consuming 50-malt and 350- malt (only distal colon). No significant difference in the proportions of specific SCFA could be found between the malt groups at any site (data not shown).
A higher yield of succinic acid was found in the cecum of rats fed 50-malt \((P < 0.005)\) and 350-malt \((P < 0.005)\) than in cecum of rats fed the LF control diets, while the amount of lactic acid was similar between the different treatment groups.

**AGE in cecum and portal serum.** The cecal amount of CEL was higher in rats fed caramelized and colored malt than in rats consuming the control diets \((P < 0.01)\) (Figure 2).

The content of CML was much higher in portal serum than in the content of CEL \((0.4-0.8 \text{ mg/L for CML versus } 0.1-0.2 \text{ mg/L for CEL})\). The group fed caramelized malt had a higher value of CEL in portal serum than the group receiving 350-malt and HF control \((P < 0.05)\). CML was also higher with caramelized malt than 350-malt while colored malt was higher than both 50-malt and 350-malt \((P < 0.05 \text{ and } 0.01, \text{ respectively})\) (Figure 2).

**Amino acids in portal plasma.** Eighteen free amino acids and ammonia were quantified in the portal plasma, where alanine, glutamine, lysine, glycine, proline, serine and threonine were the most abundant in descending order (Table 4). The portal concentration of these amino acids, except that of glutamine and proline, was affected by the dietary treatment. Furthermore, histidine, present in relatively low amounts in plasma, was another amino acid that was dependent of the diet given to rats.

In rats fed with the control diets, the higher fat intake increased their plasma concentrations of glycine \((P < 0.001)\), lysine \((P < 0.001)\), histidine \((P < 0.001)\) and serine (only a tendency, \(P = 0.099)\), whereas the plasma concentration of threonine decreased \((P < 0.001)\). The malt products, except caramelized malt, decreased the portal concentrations of glycine, histidine and serine and increased the portal concentration of threonine compared with the HF control diet. Furthermore, the plasma concentrations of amino acids and ammonia were similar between rats fed
caramelized malt and HF control diet, while the concentration of amino acids with the other three malts were in the same range as those observed in rats fed the LF control. An exception was lysine, where the concentration was higher or tended to be higher for the malt-fed rats than for the LF control group.

*Gene expression in the small intestine and distal colon.* The expression of ZO-1 ($P < 0.05$) and TLR3 ($P < 0.01$) was higher in the HF control group compared to the LF control diet in the small intestine. There was also an increase in the expression of the occludin coding gene in the HF control and the caramelized groups compared with the LF control group and the 350-malt group ($P < 0.05$). There were no significant differences in the expression of TLR4 between the different dietary treatments, due to the high variance between individual samples (*Figure 3A-3D*). There was a significant correlation between butyric acid and toll-like receptors ($\text{TLR4: } r = -0.63, P < 0.001; \text{TLR3: } r = -0.45, P < 0.01$).

The HF control diet increased the expression of ZO-1 ($P < 0.05$), occludin ($P < 0.001$), TLR3 ($P < 0.05$) and TLR4 ($P < 0.001$) in the distal colon of rats compared with the LF diet. Furthermore, supplementing the diet with barley malt decreased the expression of the same genes ($P < 0.05$), except TLR3, to a similar level as that in rats fed the LF control diet. No difference was found between the different malt products (*Figure 3E-3H*). Furthermore, expression of tight junction proteins in the distal colon was associated with butyric acid (ZO-1: $r = -0.46, P < 0.01$; occludin: $r = -0.37, P < 0.05$).

Regarding amino acids, there was a positive correlation between some amino acids and occludin gene expression (glycine: $r = 0.41, P < 0.01$; histidine: $r = 0.43, P < 0.01$).
Akkermansia in cecum. No difference was found in the cecal abundance of Akkermansia between the different groups (data not shown).

Mucus layer thickness. The thickness of the mucus layer in transverse colon was similar between the rats fed various diets. The 350-malt group tended to have a thicker mucus layer than the HF control group (35.6 ± 5.2 µm versus 24.4 ± 2.0 µm, P = 0.08).

Total cholesterol and triglycerides in portal serum. The plasma cholesterol was higher in rats given HF diets (3.9 mmol/L to 4.1 mmol/L) compared with rats given the LF control (3.6 mmol/L, P < 0.05). However, there was a tendency to lower plasma cholesterol with 50 malt compared with HF control and the two colored products. No difference in the plasma triglyceride concentrations were observed between the groups.

PCA biplot. To visualize the relation between the different diets and physiological parameters analyzed a PCA biplot was performed (Figure 4). The biplot explained 41.1% of total variance (32.7% by principal component (PC1) and 10.6% by PC2). Based on the physiological data rats were clustered into 4 groups: 1) LF control group (lower left corner), 2) HF control group (upper left corner), 3) caramelized malt group (upper centre) and 4) colored, 50-malt and 350-malt groups (to the right). HF control group was distinguished from other groups by the high expression of occludin, ZO-1 and TLR4 in the distal colon and occludin in the small intestine. Colored malt, 50-malt and 350-malt groups were clustered together due to the high amounts of SCFA in the cecum, distal colon and portal serum. Rats fed caramelized malt generally differed from the other rats consuming malt due to their high value of occludin in the small intestine, some plasma amino acids and plasma cholesterol, as rats fed HF control.
Discussion

Previous studies have shown that malting of barley may lead to degradation of dietary fiber into smaller molecules and an increased butyric acid production in rats receiving low-fat diets.\textsuperscript{10} However, whole-grain barley varieties fed to rats consuming high-fat diets gave high amount of succinic acid at the expense of butyric acid, compared with rats fed the same varieties in a low fat setting.\textsuperscript{39} In the present study we show that supplementing different types of barley malts in high-fat diets, where the molecular weight of the beta-glucan was comparatively low and more widely distributed (indicated by PDI value), resulted in much higher concentrations of butyric acid throughout the hindgut and, interestingly, in the portal plasma of rats. Furthermore, administration of the malts that yielded high amounts of butyric acid, altered the expression of tight junction proteins (ZO-1 and occludin), indicating a lower permeability in the small intestine and distal colon, as judged by the lowered concentrations of amino acids in the portal blood. Moreover, malts decreased the expression of toll-like receptors in the distal colon (TLR3 and 4) and small intestine (TLR3). Something noteworthy is that all malt groups except the caramelized malt group had increased fat pad and liver weights compared to the low- and high-fat control rats, which may be regarded as a negative effect in terms of diet-induced obesity. However, the rats used in the study were in a growth phase, increasing in body weight between 134 and 189 g during the four-week experimental period. Thus, the increased fat and liver weights in three of the malt groups may be a reflection of improved nutritional status of these rats, but this needs further clarification in rats fed malts for a longer period of time.

When modifying barley to malt, components in the kernel such as beta-glucan are partially solubilized and degraded by endogenous enzymes, as confirmed by the changes in the molecular weights and the PDI.\textsuperscript{40} However, the soluble fraction of dietary fiber and the amount of beta-
glucan were rather low in the malt products, which is also the purpose of the process, compared
with whole-grain barley, where certain varieties may contain over 50% soluble fiber and up to 15
g/100 g beta-glucan. Furthermore, during malting there is a loss of dry substance (~10%) due
to respiration, leaching during steeping, and discarded culms or rootlets. Despite the lower
solubility of the fiber and lower proportion of beta-glucan, substantial amounts of SCFA,
including butyric acid, were generated by malt compared with whole-grain barley when fed high
fat diets to rats, which is interesting. Depolymerized dietary fibers, other than beta-glucan (all
beta-glucan was included in the analysis) could be a possible explanation, which may escape
from dietary fiber analysis, especially those having a degree of polymerization below 20 such as
AXOS. However, according to Voragen et al. AX most abundantly found in the cell walls
are not extensively degraded by endogenous enzymes during malting, only solubilized, but it
cannot be excluded that AX from the endosperm were degraded contributing to the high amounts
of butyric acid formed with malt. In a previous study, fructo-oligosaccharides with a lower
degree of polymerization, resulted in a higher hindgut formation of butyric acid in rats than did
fibers with a higher degree of polymerization. A greater amount of undetected dietary fiber may
occur in 50-malt, compared with the other malts, because this special malt contained less
amounts of measurable total dietary fiber (and beta-glucan), which may explain the higher cecal
formation of SCFA with this diet, especially compared with the caramelized diet. Nevertheless,
the rats fed caramelized malt had a somewhat lower feed intake than the other groups. Our
measurement should cover all the mixed-linkage beta-glucan in malt, but AXOS not measured in
this study may also be derived from malting and affect SCFA formation. More studies are
needed to explain these high concentrations of SCFA, especially butyric acid, in the hindgut and
portal serum, and should include the fermentability of insoluble dietary fiber and the
quantification of low molecular weight dietary fiber by recently developed methods of dietary fiber analysis, such as AOAC method 2011.25.

The high amounts and proportions of butyric acid in the hindgut and portal serum of rats fed malt is of particular interest per se because the abundance of butyric acid-producing bacteria and the formation of butyric acid are generally attenuated in a high fat setting. High fat seems to have similar effects as antibiotics and decrease the microbial activity in the colon. As the preferred fuel for epithelial cells, butyric acid is important for colonic health, especially in the distal region, where the availability is relatively low and colonic diseases such as ulcerative colitis and colorectal cancer take place. Indeed, lower fecal concentration of butyric acid has been correlated with inflammatory bowel disease, while butyrate irrigation, oral administration, and formation from dietary fiber in the gut attenuate inflammation. We observed that increased distal concentrations of butyric acid were associated with decreased mRNA expression of toll-like receptors, a group of proteins representing key mediators of innate host defense in the intestine that are expressed at high levels in inflammatory bowel disease. In addition, butyric acid plays a role in gut integrity and stimulates mucus release in the colon of rats and the thickness of the mucus layer has been shown to be proportional to butyric acid availability. However, the thickness of the mucus layer was similar between all the groups in this study. Interestingly, high AGE exposure attenuated the effect of malt on the mRNA expression of occludin, a marker of gut integrity, only in the small intestine, but not in distal colon (see discussion below), which may be due to the presence of high concentrations of butyric acid in the colon. Butyric acid was associated with the expression of tight junction proteins in the distal colon. From the PC analysis, we observed that the 50- and 350 malt groups were clustered together, differing from the LF and HF control groups with regards to their SCFA levels and
gene expression, while the caramelized malt was not able to skew the SCFA and gene expression profile and thus clustered with the HF control rats. This may be due to the high amount of AGE in the caramelized malt.

Together with the high cecal pool of butyric acid, the accumulation of the intermediate product succinic acid (a precursor of butyric acid) in the cecum was only increased moderately, compared with a previous study on whole-barley grains (1–7 µmol versus up to 90 µmol). An increased accumulation of succinic acid was also observed with other types of dietary fibers like pectin, guar gum and a mixture of the two substrates (up to 150 µmol) with the same model as in this study. This fact, together with the high formation of butyric acid, makes malt of particular interest and malt might be a potent food to counteract the effect of a HF diet on colonic health in terms of fermentation. In the present study we used malts with husk, which is common for beer and whiskey production, but this malt may pose a limitation for food of nutritional interest, as the husk layer has a low palatability. The use of hull-less malts for food production may solve this problem, but hull-less malts need to be investigated further to elucidate whether they have similar in vivo effects as malts with the husk layer intact.

Including malt in a HF diet decreased the portal concentration of some amino acids and mRNA expression of occludin in the small intestine of rats, compared with the HF control diet, to a level similar to rats receiving a LF control diet, except caramelized malt. The higher portal concentrations of amino acids can be associated with increased gut permeability with the HF diet. Thus, the high portal concentrations of some amino acids in the current study in the control group fed HF compared with the control group fed LF, might be due to an increased permeability of the small intestine, indicated by the correlation between portal concentrations of some amino acids and the mRNA expression of occludin in the small intestine. Decreased
expression of tight junction proteins is generally associated with increased gut permeability.\textsuperscript{20} However, increased localization of occludin in the cytoplasm of epithelial cells has also been reported in rats with high-fat diet-induced gut permeability.\textsuperscript{55} Caramelized malt was distinguished from other malt products by the higher levels of AGE. AGE both from endogenous and dietary sources, are known to induce inflammation.\textsuperscript{56, 57} They are also known to form crosslinks in proteins, thus affecting their structures and functions, which may explain the observed effect on tight junction zones.\textsuperscript{57} So far, high concentrations of AGE in circulation and tissue are associated with the development of chronic diseases such as diabetes, aging, and kidney disease, but no study has been performed on their effect on the absorption in the gastrointestinal tract. Furthermore, although colored malt contained high amounts of AGE (but less than caramelized malt), no effect of AGE was observed regarding occludin in the small intestine and the portal amino acids. The current results suggest that the high amount of AGE in caramelized malt counteracted the effect of malt on small intestinal integrity by promoting oxidative stress. However, more direct evidences, such as those from histological or \textit{in vivo} permeability tests, are needed to confirm our finding in this study. Interestingly, no difference on tested gene expressions in the distal colon was found between the malts, which could be due to the formation of butyric acid.

No difference in plasma concentration of glutamine could be seen between rats fed the control diets and the malt diets. This is in contrast to results in rats fed a barley by-product (germinated barley), where glutamine was linked to the dietary fiber and therefore protected until delivered to the cecum.\textsuperscript{58} Glutamine is, next to butyric acid, the most important fuel for the epithelial cells in colon. An increased formation of butyric acid, as seen in this study, may reduce the need of glutamine for the colonic mucosa, thereby increasing the levels of circulating glutamine and
having possible effects on the immune function. Glutamine enemas have been shown to have anti-inflammatory properties in rats with colitis.\textsuperscript{59} However, such an effect could not be seen in patients (with inflammatory bowel disease) receiving oral and parental glutamine.\textsuperscript{60, 61}
Conclusion

Barley malt, in HF diets, remarkably increased the hindgut formation and portal concentrations of SCFA in rats, while the formation of the intermediate metabolite succinic acid was only mildly increased. Furthermore, malt not only modulated the gene expression of toll-like receptors and tight junction proteins in the intestine, but also inhibited the HF-induced increase of some amino acids in the portal vein. Interestingly, high levels of AGE attenuated the effects of malt on small intestinal tight junction gene expression and some plasma amino acids, but not in the distal colon, suggesting a role for SCFA, especially butyric acid, in gut barrier function.
Acknowledgement

We would like to thank Dr. Ulf Nilsson and Dr. Greta Jakobsdottir for their technical assistance with the animal experiment. We also wish to give thanks to Viking malt AB for kindly providing the barley malt material. This project was funded by Albert Pahlsson Foundation, The Swedish Research Council Formas, Antidiabetic Food Centre (a VINNOVA VINN Excellence Center) and Chinese Scholarship Council. The authors’ contributions were as follows: Y.Z. and M. N. designed the study; Y. Z. performed the study; C.T. analyzed the content and chemical properties of beta-glucan and interpreted the results together with R.A.; N. M. conducted the analysis of Akkermansia; W.S. performed the measurement of gene expression; E.T. analyzed AGE and interpreted the results; Y.Z. evaluated the data statistically and was responsible for writing the manuscript; M. N. and F.F. took part in writing and revising the manuscript; Y.Z, F.F. and M.N. were responsible for the final content. All authors read and approved the final manuscript.
References

### Tables

#### Table 1. Composition of dietary fiber (g/100 g, dwb) and advanced glycated end-products (mg/100 g, dwb) in malts and MCC. ¹,²,³

<table>
<thead>
<tr>
<th></th>
<th>MCC</th>
<th>Caramelized malt</th>
<th>Colored malt</th>
<th>50-malt</th>
<th>350-malt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhamnose</td>
<td>tr³</td>
<td>tr</td>
<td>tr</td>
<td>tr</td>
<td>tr</td>
</tr>
<tr>
<td>Arabinose</td>
<td>tr</td>
<td>2.4 ± 0.0⁶ (13)</td>
<td>2.5 ± 0.0⁶ (11)</td>
<td>2.0 ± 0.0⁶ (8)</td>
<td>2.3 ± 0.0⁶ (10)</td>
</tr>
<tr>
<td>Xylose</td>
<td>2.8 ± 0.0</td>
<td>4.6 ± 0.0⁶ (10)</td>
<td>4.3 ± 0.0⁶ (7)</td>
<td>3.6 ± 0.1⁶ (3)</td>
<td>4.7 ± 0.0⁶ (6)</td>
</tr>
<tr>
<td>Mannose</td>
<td>2.7 ± 0.0</td>
<td>0.2 ± 0.0⁶ (15)</td>
<td>0.3 ± 0.0⁶ (15)</td>
<td>0.2 ± 0.0⁶ (13)</td>
<td>0.3 ± 0.0⁶ (16)</td>
</tr>
<tr>
<td>Galactose</td>
<td>tr</td>
<td>0.4 ± 0.0⁶ (24)</td>
<td>0.4 ± 0.0⁶ (20)</td>
<td>0.4 ± 0.0⁶ (23)</td>
<td>0.4 ± 0.0⁶ (21)</td>
</tr>
<tr>
<td>Glucose</td>
<td>94.5 ± 0.5</td>
<td>4.7 ± 0.1⁶ (21)</td>
<td>3.8 ± 0.0⁶ (7)</td>
<td>3.2 ± 0.0⁶ (11)</td>
<td>4.7 ± 0.0⁶ (14)</td>
</tr>
<tr>
<td>Beta-glucan</td>
<td>nd⁶</td>
<td>0.9 ± 0.0⁶</td>
<td>0.5 ± 0.0⁶</td>
<td>0.6 ± 0.0⁶</td>
<td>1.2 ± 0.0⁶</td>
</tr>
<tr>
<td>Uronic acids</td>
<td>nd</td>
<td>0.6 ± 0.0 (5)</td>
<td>0.6 ± 0.0 (7)</td>
<td>0.6 ± 0.1 (6)</td>
<td>0.6 ± 0.0 (8)</td>
</tr>
<tr>
<td>Dietary fiber</td>
<td>100 ± 0.4</td>
<td>12.9 ± 0.2⁶ (16)</td>
<td>11.9 ± 0.1⁶ (9)</td>
<td>10.0 ± 0.1⁶ (6)</td>
<td>13.0 ± 0.5⁶ (10)</td>
</tr>
<tr>
<td>polysaccharides</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total dietary</td>
<td>nd</td>
<td>15.5 ± 0.1⁶</td>
<td>13.8 ± 0.2⁶</td>
<td>13.3 ± 0.1⁶</td>
<td>15.9 ± 0.3⁶</td>
</tr>
<tr>
<td>fiber</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CML⁴</td>
<td>tr</td>
<td>12.4 ± 0.3⁶</td>
<td>9.5 ± 0.2⁶</td>
<td>5.6 ± 0.5⁶</td>
<td>4.6 ± 0.4⁶</td>
</tr>
<tr>
<td>CEL⁴</td>
<td>tr</td>
<td>4.0 ± 0.1⁶</td>
<td>1.9 ± 0.1⁶</td>
<td>0.7 ± 0.1⁶</td>
<td>0.6 ± 0.0⁶</td>
</tr>
</tbody>
</table>

¹ Values are means ± SEM. Means in a row with superscripts without a common letter are significantly different, P < 0.05.

² Values in brackets show the proportion found in the soluble fraction (%).

³ Microcrystalline cellulose (MCC, FMC BioPolymer, Philadelphia, USA).

⁴ Carboxymethyl lysine (CML), carboxyethyl lysine (CEL).

⁵ tr, trace amounts, less than 0.05 g/100 g for dietary fiber, and for CML and CEL, less than 0.01 mg/100 g.

⁶ nd, not determined.
### Table 2

Feed intake, body weight gain, cecal pH and weight of cecal content and tissue; fresh liver, spleen and epididymal and retroperitoneal fat pads in rats fed high-fat diets containing different malt products (caramelized and colored malt, 50-malt and 350-malt) for 4 weeks. Diets containing cellulose with low- and high fat content were used as controls.\(^1,2\)

<table>
<thead>
<tr>
<th></th>
<th>HF control</th>
<th>Caramelized malt</th>
<th>Colored malt</th>
<th>50-malt</th>
<th>350-malt</th>
<th>LF control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed intake, g/rat/day</td>
<td>13.4</td>
<td>12.9</td>
<td>14.2</td>
<td>15.0</td>
<td>15.4</td>
<td>15.2</td>
</tr>
<tr>
<td>CML intake, µg/rat/day</td>
<td>-</td>
<td>717</td>
<td>673</td>
<td>456</td>
<td>312</td>
<td>-</td>
</tr>
<tr>
<td>CEL intake, µg/rat/day</td>
<td>-</td>
<td>233</td>
<td>212</td>
<td>182</td>
<td>157</td>
<td>-</td>
</tr>
<tr>
<td>Body weight gain, g/g feed/rat</td>
<td>0.35</td>
<td>0.39</td>
<td>0.43</td>
<td>0.44</td>
<td>0.44</td>
<td>0.35</td>
</tr>
<tr>
<td>Body weight gain, g</td>
<td>134 ± 5(^b)</td>
<td>143 ± 3(^b)</td>
<td>172 ± 8(^a)</td>
<td>183 ± 6(^a)</td>
<td>189 ± 8(^a)</td>
<td>146 ± 5(^b)</td>
</tr>
<tr>
<td>Cecal pH</td>
<td>7.2 ± 0.1(^a)</td>
<td>6.5 ± 0.1(^b)</td>
<td>6.6 ± 0.1(^b)</td>
<td>6.6 ± 0.1(^b)</td>
<td>6.8 ± 0.1(^ab)</td>
<td>7.2 ± 0.1(^a)</td>
</tr>
<tr>
<td>Cecal content, g</td>
<td>2.0 ± 0.2(^bc)</td>
<td>3.0 ± 0.2(^a)</td>
<td>3.2 ± 0.3(^a)</td>
<td>3.4 ± 0.4(^a)</td>
<td>2.8 ± 0.1(^ab)</td>
<td>1.9 ± 0.2(^c)</td>
</tr>
<tr>
<td>Cecal tissue, g</td>
<td>0.57 ± 0.04(^b)</td>
<td>0.68 ± 0.03(^ab)</td>
<td>0.81 ± 0.06(^a)</td>
<td>0.69 ± 0.03(^ab)</td>
<td>0.83 ± 0.06(^a)</td>
<td>0.50 ± 0.03(^b)</td>
</tr>
<tr>
<td>Liver, g</td>
<td>10.8 ± 0.3(^c)</td>
<td>11.3 ± 0.4(^bc)</td>
<td>14.0 ± 0.9(^a)</td>
<td>13.6 ± 0.5(^ab)</td>
<td>14.2 ± 0.8(^a)</td>
<td>9.0 ± 0.3(^d)</td>
</tr>
<tr>
<td>Epididymal fat, g</td>
<td>2.7 ± 0.3(^c)</td>
<td>2.5 ± 0.2(^c)</td>
<td>4.2 ± 0.3(^ab)</td>
<td>5.3 ± 0.4(^a)</td>
<td>5.6 ± 0.3(^a)</td>
<td>3.4 ± 0.4(^bc)</td>
</tr>
<tr>
<td>Retroperitoneal fat, g</td>
<td>2.8 ± 0.3(^c)</td>
<td>2.3 ± 0.3(^c)</td>
<td>4.8 ± 0.5(^ab)</td>
<td>6.1 ± 0.5(^a)</td>
<td>6.7 ± 0.5(^a)</td>
<td>3.5 ± 0.4(^bc)</td>
</tr>
<tr>
<td>Spleen, g</td>
<td>0.54 ± 0.03</td>
<td>0.56 ± 0.03</td>
<td>0.63 ± 0.04</td>
<td>0.68 ± 0.04</td>
<td>0.68 ± 0.04</td>
<td>0.58 ± 0.05</td>
</tr>
</tbody>
</table>

\(^1\) Values are means ± SEM, n = 7. Means in a row with superscripts without a common letter are significantly different, \(P < 0.05\). HF, high fat; LF, low fat.

\(^2\) Carboxymethyl lysine (CML), carboxyethyl lysine (CEL).

\(^3\) Cecal contents and liver weights were log-transformed before further statistical analyses.
Table 3. Cecal pools and concentrations of carboxylic acids, and portal serum concentrations of SCFA in rats fed high-fat diets containing different malt products (caramelized and colored malt, 50-malt and 350-malt) for 4 weeks. Diets containing cellulose with low- and high fat content were used as controls.  

<table>
<thead>
<tr>
<th></th>
<th>HF control</th>
<th>Caramelized malt</th>
<th>Colored malt</th>
<th>50-malt</th>
<th>350-malt</th>
<th>LF control</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cecal pool, µmol</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetic acid</td>
<td>55 ± 4b</td>
<td>137 ± 15a</td>
<td>193 ± 28a</td>
<td>217 ± 35a</td>
<td>148 ± 13a</td>
<td>70 ± 10b</td>
</tr>
<tr>
<td>Propionic acid</td>
<td>11 ± 1b</td>
<td>24 ± 2a</td>
<td>31 ± 4a</td>
<td>39 ± 7a</td>
<td>28 ± 2a</td>
<td>13 ± 2b</td>
</tr>
<tr>
<td>Butyric acid</td>
<td>11 ± 1b</td>
<td>48 ± 6a</td>
<td>64 ± 13a</td>
<td>75 ± 13a</td>
<td>47 ± 5a</td>
<td>16 ± 2b</td>
</tr>
<tr>
<td>Minor SCFA</td>
<td>5 ± 0c</td>
<td>10 ± 1ab</td>
<td>13 ± 1a</td>
<td>12 ± 1ab</td>
<td>10 ± 1ab</td>
<td>7 ± 1bc</td>
</tr>
<tr>
<td>Total SCFAs</td>
<td>83 ± 6b</td>
<td>219 ± 24a</td>
<td>300 ± 46a</td>
<td>343 ± 55a</td>
<td>233 ± 19a</td>
<td>106 ± 15b</td>
</tr>
<tr>
<td>Succinic acid</td>
<td>1.4 ± 0.3ab</td>
<td>1.0 ± 0.4ab</td>
<td>5.4 ± 3.7ab</td>
<td>6.4 ± 2.8a</td>
<td>4.5 ± 1.9a</td>
<td>0.3 ± 0.1b</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>0.2 ± 0.1</td>
<td>0.3 ± 0.2</td>
<td>0.2 ± 0.0</td>
<td>0.8 ± 0.3</td>
<td>0.6 ± 0.3</td>
<td>0.6 ± 0.3</td>
</tr>
<tr>
<td>Acetic acid, %</td>
<td>71 ± 0a</td>
<td>66 ± 1b</td>
<td>67 ± 1ab</td>
<td>66 ± 1b</td>
<td>66 ± 2ab</td>
<td>71 ± 1a</td>
</tr>
<tr>
<td>Propionic acid, %</td>
<td>14 ± 0a</td>
<td>12 ± 1bc</td>
<td>11 ± 1c</td>
<td>12 ± 0bc</td>
<td>13 ± 1abc</td>
<td>13 ± 0ab</td>
</tr>
<tr>
<td>Butyric acid, %</td>
<td>15 ± 0c</td>
<td>22 ± 1a</td>
<td>22 ± 1a</td>
<td>23 ± 1a</td>
<td>20 ± 2ab</td>
<td>16 ± 1bc</td>
</tr>
<tr>
<td><strong>Cecal concentrations µmol/g</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total SCFAs</td>
<td>42 ± 2d</td>
<td>73 ± 4bc</td>
<td>91 ± 7ab</td>
<td>99 ± 5a</td>
<td>84 ± 5ab</td>
<td>55 ± 4cd</td>
</tr>
</tbody>
</table>

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### Tables

<table>
<thead>
<tr>
<th></th>
<th>Portal serum concentrations, µmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic acid</td>
<td>18 ± 1&lt;sup&gt;d&lt;/sup&gt; 24 ± 1&lt;sup&gt;bc&lt;/sup&gt; 31 ± 3&lt;sup&gt;ab&lt;/sup&gt; 34 ± 2&lt;sup&gt;a&lt;/sup&gt; 33 ± 1&lt;sup&gt;a&lt;/sup&gt; 18 ± 1&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>Propionic acid</td>
<td>4 ± 0&lt;sup&gt;c&lt;/sup&gt; 6 ± 0&lt;sup&gt;b&lt;/sup&gt; 7 ± 0&lt;sup&gt;ab&lt;/sup&gt; 8 ± 0&lt;sup&gt;a&lt;/sup&gt; 9 ± 0&lt;sup&gt;a&lt;/sup&gt; 4 ± 0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Butyric acid</td>
<td>5 ± 0&lt;sup&gt;b&lt;/sup&gt; 9 ± 1&lt;sup&gt;ab&lt;/sup&gt; 11 ± 2&lt;sup&gt;a&lt;/sup&gt; 11 ± 1&lt;sup&gt;a&lt;/sup&gt; 10 ± 1&lt;sup&gt;a&lt;/sup&gt; 5 ± 0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Minor SCFA</td>
<td>2 ± 0&lt;sup&gt;ab&lt;/sup&gt; 3 ± 0&lt;sup&gt;ab&lt;/sup&gt; 4 ± 0&lt;sup&gt;a&lt;/sup&gt; 3 ± 0&lt;sup&gt;ab&lt;/sup&gt; 3 ± 0&lt;sup&gt;a&lt;/sup&gt; 2 ± 0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total SCFAs</td>
<td>29 ± 2&lt;sup&gt;c&lt;/sup&gt; 42 ± 3&lt;sup&gt;b&lt;/sup&gt; 53 ± 5&lt;sup&gt;ab&lt;/sup&gt; 57 ± 3&lt;sup&gt;a&lt;/sup&gt; 55 ± 2&lt;sup&gt;a&lt;/sup&gt; 29 ± 1&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

1. Values are means ± SEM, n = 7; n = 5 for blood samples from the 350-malt group. Means in a row with superscripts without a common letter are significantly different, *P* < 0.05. HF, high fat; LF, low fat.

2. Cecal pools were log-transformed before further statistical analyses.
### Tables

**Table 4.** Concentrations of free amino acids and ammonia (µmol/L) in portal plasma of rats fed high-fat diets containing different malt products (Caramelized and Colored malt, 50-malt and 350-malt) for 4 weeks. Diets containing cellulose with low- and high fat content were used as controls.

<table>
<thead>
<tr>
<th></th>
<th>HF control</th>
<th>Caramelized malt</th>
<th>Colored malt</th>
<th>50-malt</th>
<th>350-malt</th>
<th>LF control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>790 ± 60(^a)</td>
<td>860 ± 75(^a)</td>
<td>735 ± 45(^b)</td>
<td>678 ± 36(^b)</td>
<td>670 ± 100(^ab)</td>
<td>603 ± 48(^b)</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>97 ± 11</td>
<td>117 ± 9</td>
<td>107 ± 8</td>
<td>97 ± 4</td>
<td>106 ± 11</td>
<td>94 ± 5</td>
</tr>
<tr>
<td>Glutamine</td>
<td>687 ± 51</td>
<td>737 ± 44</td>
<td>680 ± 35</td>
<td>606 ± 14</td>
<td>617 ± 30</td>
<td>663 ± 42</td>
</tr>
<tr>
<td>Glycine</td>
<td>434 ± 27(^a)</td>
<td>381 ± 25(^ab)</td>
<td>328 ± 18(^bc)</td>
<td>306 ± 23(^bc)</td>
<td>294 ± 13(^bc)</td>
<td>286 ± 17(^c)</td>
</tr>
<tr>
<td>Histidine</td>
<td>121 ± 9(^a)</td>
<td>119 ± 13(^a)</td>
<td>90 ± 3(^ab)</td>
<td>80 ± 2(^b)</td>
<td>75 ± 6(^b)</td>
<td>81 ± 6(^b)</td>
</tr>
<tr>
<td>Lysine</td>
<td>695 ± 31(^a)</td>
<td>694 ± 23(^a)</td>
<td>723 ± 32(^a)</td>
<td>690 ± 21(^a)</td>
<td>647 ± 15(^ab)</td>
<td>530 ± 24(^b)</td>
</tr>
<tr>
<td>Proline</td>
<td>319 ± 28</td>
<td>313 ± 33</td>
<td>333 ± 52</td>
<td>363 ± 44</td>
<td>339 ± 46</td>
<td>318 ± 25</td>
</tr>
<tr>
<td>Serine</td>
<td>350 ± 16(^a)</td>
<td>337 ± 15(^a)</td>
<td>320 ± 19(^ab)</td>
<td>265 ± 9(^b)</td>
<td>275 ± 28(^ab)</td>
<td>288 ± 14(^ab)</td>
</tr>
<tr>
<td>Threonine</td>
<td>157 ± 16(^b)</td>
<td>161 ± 7(^b)</td>
<td>276 ± 18(^a)</td>
<td>291 ± 25(^a)</td>
<td>296 ± 22(^a)</td>
<td>337 ± 31(^a)</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>138 ± 9</td>
<td>142 ± 8</td>
<td>140 ± 5</td>
<td>135 ± 17</td>
<td>128 ± 15</td>
<td>174 ± 7</td>
</tr>
<tr>
<td>Valine</td>
<td>175 ± 9</td>
<td>193 ± 6</td>
<td>218 ± 10</td>
<td>213 ± 19</td>
<td>190 ± 15</td>
<td>179 ± 10</td>
</tr>
<tr>
<td>Total</td>
<td>4517 ± 189</td>
<td>4587 ± 152</td>
<td>4555 ± 180</td>
<td>4312 ± 159</td>
<td>4180 ± 262</td>
<td>4090 ± 122</td>
</tr>
<tr>
<td>Ammonia</td>
<td>413 ± 32</td>
<td>377 ± 44</td>
<td>433 ± 19</td>
<td>396 ± 22</td>
<td>476 ± 59</td>
<td>339 ± 29</td>
</tr>
</tbody>
</table>

\(^1\) Values are means ± SEM, n = 7; n = 5 for 350-malt group. Means in a row with superscripts without a common letter are significantly different, P < 0.05. HF, high fat; LF, low fat.
Figure 1. Polydispersity index and molecular weight of water-extractable β-glucan in malts. n = 2, data are means ± SEM. Data with different letters are significantly different at $P < 0.05$. 
Figures

**Figure 2.** Amounts of advanced glycated end-products in the cecum and the portal serum of rats fed high-fat diets containing different malt products (caramelized and colored malts, 50-malt and 350-malt) for 4 weeks. Values are means ± SEM. Data with different letters are significantly different at $P < 0.05$. CEL, carboxyethyl lysine; CML, carboxymethyl lysine; HF, high fat; LF, low fat.

LF and HF diets containing cellulose were used as controls. A: Cecal pool (µg) ($n = 4$) (HF and LF controls and 350-malt groups, $n = 3$). B: Portal serum concentration (mg/L) ($n = 6$) (CEL in caramelized group and CML in 50-malt group, $n = 5$; CML in 350-malt group, $n = 4$; colored malt group and CEL in 350-malt group, $n = 3$).
Figure 3. Gene expression of tight junction proteins (ZO-1 and occludin) and toll-like receptors (TLR3 and TLR4) in the small intestine and the distal colon of rats fed high-fat diets containing different malt products (caramelized and colored malts, 50-malt and 350-malt) for 4 weeks. Low- and high-fat diets containing cellulose were used as controls. Data with different letters are significantly different at $P < 0.05$. HF, high fat; LF, low fat; TLR3, toll-like receptor 3; TLR4, toll-like receptor 4; ZO-1, zonula occludens-1.
Figures
**Figure 4.** Principal Component Analysis biplot based on the tested physiological parameters. Each colored symbol represents a rat included in the present study. 1) Green circle represents LF control group; 2) Dark blue rectangle represents HF control group; 3) Brown triangle represents caramelized malt group; 4) Yellow inverted triangle represents colored malt group; 5) Light blue diamond represents 50-malt group; 6) Purple hexagon represents 350-malt group. Physiological parameters, with Hotelling's T2 ellipse confidence > 50%, are shown as black four-point stars. CEL, carboxyethyl lysine; CML, carboxymethyl lysine; E fat, epididymal fat; HF, high-fat; LF, low fat; R fat, retroperitoneal fat; SCFA, short-chain fatty acids; SI, small intestine; TLR, toll-like receptor; ZO-1, zonula occludens-1.