

Supplemental materials

Supplemental Table 1. Composition of diets, g/kg (dwb)¹

	HF control	Caramelized malt	Colored malt	50-malt	350-malt	LF control
Basal diet ²	280	280	280	280	280	280
Butter ³	180	180	180	180	180	-
Cholesterol ⁴	10	10	10	10	10	-
Wheat starch ⁵	460	80	24	3	91	650
MCC ⁶	70	-	-	-	-	70
Malt	-	450	506	527	439	-
<i>Dietary fiber</i>	70	70	70	70	70	70

¹ HF, high fat; LF, low fat.

² Containing (g/kg): 364 casein (Sigma Aldrich, St. Louis, MO, USA), 152 corn oil (Belgien, Solna, Sweden), 4 DL-methionine (Sigma Aldrich, St. Louis, MO, USA), 304 sucrose (Nordic Sugar, Copenhagen, Denmark), 24 vitamin mixture[†], 6 choline chloride (Sigma Aldrich, St. Louis, MO, USA), 146 mineral mixture[¶].

³ Skänemejerier, Malmö, Sweden.

⁴ Sigma Aldrich, St. Louis, MO, USA.

⁵ Norfoods Sweden AB, Malmö, Sweden, varied according to the MCC or barley content of the test materials.

⁶ Microcrystalline cellulose, FMC BioPolymer, Philadelphia, USA.

[†] Containing (g/kg): 0.62 menadione, 2.5 thiamin hydrochloride, 2.5 riboflavin, 1.25 pyridoxine hydrochloride, 6.25 calcium pantothenate, 6.25 nicotinic acid, 0.25 folic acid, 12.5 inositol, 1.25 p-aminobenzoic acid, 0.05 biotin, 0.00375 cyanocobalamin, 0.187 retinol palmitate, 0.00613 calciferol, 25 d- α -tocopheryl acetate, 941.25 corn starch (Lantmännen, Stockholm, Sweden)

[¶] Containing (g/kg): 0.37 CuSO₄·5H₂O, 1.4 ZnSO₄·7H₂O, 332.1 KH₂PO₄, 171.8 NaH₂PO₄·2H₂O, 324.4 CaCO₃, 0.068 KI, 57.2 MgSO₄, 7.7 FeSO₄·7H₂O, 3.4 MnSO₄·H₂O, 0.02 CoCl₂·6H₂O, 101.7 NaCl, 0.019 chromium(III)chloride and 0.011 sodium selenite.

Supplemental Table 2. Primers used in real time-polymerase chain reaction

Target gene	Forward primer	Reverse primer	Product size (bp)	Annealing (°C)		
				Small Intestine	Distal colon	Caecal content
Akkermansia	5'-CAGCACGTGAAGGTGGGGAC-3'	5'-CCTTGC GGTTGGCTTCAGAT-3'	372	-	-	60
ZO-1	5'-ATCTCCCGACCAACG-3'	5'-TGGCTGTCCGACTTGA-3'	244	58.2	60	-
Occludin	5'-TTGCTTCATCGCTTCCTTG-3'	5'-TCCATCTTCTTCGGGTTT-3'	375	60.2	58.8	-
TLR3	5'-TAAACTGAACCATGCACCT-3'	5'-TATGACGAAAGGCACCTATC-3'	101	60	60	-
TLR4	5'-ACTCTGCGCCTAAAACCCATT-3'	5'-TTGAGGTTAGAACGCCTCGTGC-3'	106	60	60	-
GAPDH	5'-ATGACTCTACCCACGGCAAG-3'	5'-CTGGAAGATGGT GATGGGTT-3'	89	-	-	-

Supplemental Table 3. Content (mmol/100 g, dwb) and composition (%) in bracket) of amino acids in malt

	Caramelized malt	Colored malt	50-malt	350-malt
Alanine	5.7 ± 0.1 ^b (8)	6.2 ± 0.0 ^a (8)	5.3 ± 0.1 ^b (8)	5.4 ± 0.1 ^b (8)
Arginine	2.5 ± 0.1 ^b (3)	3.2 ± 0.1 ^a (4)	2.8 ± 0.1 ^{ab} (4)	2.8 ± 0.1 ^{ab} (4)
Aspartic acid	5.5 ± 0.1 ^{ab} (8)	6.3 ± 0.2 ^a (8)	5.4 ± 0.2 ^b (8)	5.2 ± 0.1 ^b (7)
Cysteine	0.4 ± 0.0 (1)	0.4 ± 0.1 (1)	0.4 ± 0.0 (1)	0.4 ± 0.0 (1)
Glutamic acid	15.1 ± 0.5 ^a (21)	13.3 ± 0.4 ^{ab} (17)	11.8 ± 0.5 ^b (18)	13.8 ± 0.1 ^b (19)
Glycine	6.1 ± 0.2 ^a (8)	6.2 ± 0.1 ^a (8)	5.4 ± 0.1 ^b (8)	5.5 ± 0.1 ^b (8)
Histidine	1.5 ± 0.0 ^b (2)	1.7 ± 0.0 ^a (2)	1.5 ± 0.0 ^b (2)	1.5 ± 0.0 ^{ab} (2)
Iso-leucine	3.2 ± 0.1 ^a (4)	3.1 ± 0.1 ^a (4)	2.7 ± 0.0 ^b (4)	2.8 ± 0.1 ^b (4)
Leucine	5.5 ± 0.1 ^{ab} (8)	5.6 ± 0.1 ^a (8)	4.9 ± 0.0 ^c (7)	5.1 ± 0.1 ^{bc} (7)
Lysine	1.7 ± 0.1 ^b (2)	3.0 ± 0.0 ^a (4)	2.9 ± 0.0 ^a (5)	2.8 ± 0.1 ^a (4)
Methionine	0.7 ± 0.1 (1)	0.8 ± 0.1 (1)	0.7 ± 0.0 (1)	0.7 ± 0.1 (1)
Phenylalanine	3.2 ± 0.1 (4)	3.3 ± 0.1 (4)	2.9 ± 0.2 (4)	3.0 ± 0.1 (4)
Proline	9.1 ± 0.0 ^a (12)	8.9 ± 0.1 ^a (12)	8.2 ± 0.0 ^b (12)	8.8 ± 0.1 ^a (12)
Serine	3.7 ± 0.1 (5)	4.0 ± 0.1 (5)	3.6 ± 0.1 (5)	3.8 ± 0.1 (6)
Threonine	3.2 ± 0.1 ^{ab} (4)	3.5 ± 0.1 ^a (5)	3.1 ± 0.1 ^b (5)	3.0 ± 0.1 ^b (5)
Tyrosine	1.7 ± 0.0 ^{ab} (2)	1.8 ± 0.0 ^a (2)	1.6 ± 0.0 ^c (2)	1.7 ± 0.0 ^{bc} (2)
Valine	4.8 ± 0.1 ^{ab} (7)	5.0 ± 0.1 ^a (7)	4.2 ± 0.1 ^c (6)	4.3 ± 0.1 ^{bc} (6)
Total amino acids	73.6 ± 1.0 ^b	76.4 ± 1.6 ^a	67.4 ± 1.6 ^c	70.8 ± 1.6 ^{ab}
Total amino acids, g/100g	9.3 ± 0.0 ^b	9.7 ± 0.1 ^a	8.6 ± 0.2 ^c	9.0 ± 0.2 ^{ab}
Total amino acids intake, g ¹	35	39	37	40

¹Total intake of amino acids was calculated as the content of total amino acid (g/100 g) in malt multiplied with malt intake (100 g) during four weeks.

Test malts and their malting programs

Caramelized malt (barley source unknown; wort color, 25 °EBC), colored malt (mixture of Quench and Breamar barleys; wort color, 20 °EBC), 50-malt (Quench barley; wort color, 3 °EBC) and 350-malt (Tipple barley; wort color, 3 °EBC), were produced by special malting programs, and are normally used for beer production. The malting began with the standard conditions for steeping and germination followed by either roasting or kilning. The roasting process began with a saccharification phase and ended at 170 °C for total caramelization. Warm ingoing air was used in kilning for drying and ended either at 85 °C (50-malt and 350-malt) or 100 °C (colored malt).

Application of UHPLC-MS/MS in CML and CEL quantification

The analysis (quantification) of CML and CEL was performed by ultra-high-pressure liquid chromatography mass spectrometry (UHPLC-MS/MS). The instrument used was an Accela (Thermo Scientific) UHPLC pump with auto injector Detection was performed by a LTQ VelosPro Orbitrap mass spectrometer run in positive electrospray ionization ion trap MS/MS mode, detecting two SRM transitions for each analyte, and two for the internal standard. The ion source parameters, based on the Xcalibur software settings, were source voltage 4 kV, capillary and source temperatures 375°C, sheath gas flow 60, auxillary gas flow 320, sweep gas flow 5, collision energy of 35 mV and an S-len setting at 60%. The Xcalibur software (Thermo Scientific) was used both data acquisition and evaluation.