3 Results

3.1 Body Weight, Body Weight Gain and Liver Weight of Rats

Table 3.1: Body Weight, Body Weight Gain and Relative Liver Weight of Rats used in Experiment 1, 2 and 3

Groups		Body Weight at the start [g]	Body Weight at the end [g]	Body Weight gain [g]	Relative liver weight [g/100 g BW]
Exp 1	Casein	70 ± 4	205 ± 4^{a}	135 ± 4^{a}	3.96 ± 0.21^{a}
	Soy	70 ± 4	186 ± 5^{b}	116 ± 3^{b}	3.29 ± 0.20^{b}
Exp 2	Pork	72 ± 5	197 ± 6^{a}	125 ± 8^{ab}	3.63 ± 0.41^{ab}
	Beef	73 ± 6	194 ± 7^{a}	122 ± 11^{ab}	3.57 ± 0.51^{ab}
	Fish	72 ± 6	195 ± 7^{a}	122 ± 13^{ab}	3.78 ± 0.61^{a}
	Turkey	72 ± 6	202 ± 7^{a}	130 ± 9^{a}	3.32 ± 0.27^{b}
	Casein	72 ± 6	196 ± 7^{a}	123 ± 7^{ab}	3.54 ± 0.36^{ab}
	Soy	72 ± 6	177 ± 4^{b}	105 ± 8^{b}	3.23 ± 0.37^{b}
Exp 3	Fish	76 ± 5	202 ± 6^{a}	125 ± 6^{a}	4.08 ± 0.37^{ab}
	Casein	77 ± 5	191 ± 6^{ab}	115 ± 5^{ab}	4.51 ± 0.37^{a}
	Soy	77 ± 7	193 ± 6^{ab}	116 ± 6^{ab}	4.13 ± 0.28^{ab}
	Pea	77 ± 5	186 ± 6^{b}	109 ± 3^{b}	3.59 ± 0.25^{b}
	Lupin	76 ± 5	153 ± 10^{c}	77 ± 10^{c}	3.85 ± 0.75^{b}

Values are mean values ± SD. Different superscript letters within one column denote significant difference within one experiment. P< 0.05 (Fisher's multiple range test). BW = Body weight, Experiment 1 (n = 10), Experiment 2 and 3 (n = 12). Rats were food deprived for 12 h in experiment 1 & 2, while in experiment 3 rats were killed without prior food deprivation.

In table 3.1, the body weight, body weight gain and relative liver weights are summarized. All experimental rats completed their feeding period.

In Experiment 1, the average diet intake per rat was 13.5 g/day and the average body weight at the starting was 70.2 ± 4.3 g (n = 20). The amount of the diet was increased starting from 6 g/day to 14 g/day. The diet was fed for 21 days. The body weight gain and the relative liver weight of the rats fed diet containing soy protein were significantly lower (-10%, -17%, respectively) than that of the rats fed diet containing casein as protein source.

In Experiment 2, the average diet intake per rat was 13.5 g/day. The average body weight at the starting was 72.3 ± 5.6 g (n = 72). The amount of diet was increased starting from 8 g/day to 14 g/day. The diet was fed for 20 days. Rats fed diet containing soy protein had significantly lower body weight gain than rats fed diet containing turkey protein. Rats fed diets containing pork, beef, fish or casein had body weight gain values in between. The relative liver weight was significantly higher of the rats fed diet with fish protein compared to the rats fed diet with turkey or soy protein. Rats fed pork, beef, or casein diets had relative liver weight values in between.

In Experiment 3, average diet intake per rat was 10.3 g/day. The average body weight at the start was 76.5 ± 5.3 g (n = 60). The amount of the diet was increased starting from 6 g/day to 14 g/day. The diet was fed for 22 days. In this experiment the amount of diet provided to the rats was a bit lower because some rats from the lupin group did not ingest the entire amount of diet and to standardize the diet intake of all the groups, we provided a bit lesser diet to each group. Rats fed fish protein had significantly higher body weight gain compared to rats fed pea or lupin protein. Rats fed casein and soy protein had body weight gain values in between. Rats fed lupin protein had the lowest body weight gain. The relative liver weight was significantly higher of rats fed casein compared to rats fed pea protein. Rats fed fish or soy protein had values in between.

3.2 Cholesterol Concentrations in Plasma and Lipoproteins

In the table 3.2, the cholesterol concentrations in plasma, lipoproteins and the quotient from LDL-/HDL-cholesterol are specified. In Experiment 1, no difference was found in the concentrations of plasma cholesterol, LDL-cholesterol as well as in HDL-cholesterol, only VLDL-cholesterol concentration was significantly lower in rats fed soy protein (-53%) compared to rats fed casein. The quotient of LDL-/HDL-cholesterol was significantly lower in the rats fed soy protein (-12%) compared to rats fed casein.

Table 3.2 Cholesterol Concentrations in Plasma and Lipoproteins of the Rats used in Experiment 1, 2 and 3

Groups		Plasma	VLDL	LDL	HDL	LDL/HDL
		[mmol/L]				
Exp 1	Casein	2.17 ± 0.39	0.15 ± 0.04^{a}	0.66 ± 0.09	1.00 ± 0.17	0.66 ± 0.09^{a}
	Soy	2.08 ± 0.18	0.07 ± 0.03^{b}	0.60 ± 0.06	1.04 ± 0.10	0.58 ± 0.07^{b}
Exp 2	Pork	1.84 ± 0.28^{a}	0.29 ± 0.09^{a}	0.61 ± 0.16	1.08 ± 0.16^{a}	0.56 ± 0.13^{ab}
	Beef	1.92 ± 0.33^{a}	0.31 ± 0.14^{a}	0.61 ± 0.15	1.12 ± 0.24^{a}	0.55 ± 0.10^{ab}
	Fish	1.56 ± 0.23^{b}	0.35 ± 0.16^{a}	0.54 ± 0.14	0.80 ± 0.16^{b}	0.69 ± 0.17^{a}
	Turkey	1.71 ± 0.31^{ab}	0.33 ± 0.09^{a}	0.55 ± 0.12	1.04 ± 0.22^{a}	0.53 ± 0.10^{ab}
	Casein	1.80 ± 0.21^{a}	0.30 ± 0.13^{a}	0.58 ± 0.17	1.05 ± 0.14^{a}	0.54 ± 0.11^{ab}
	Soy	1.74 ± 0.32^{ab}	0.08 ± 0.06^{b}	0.58 ± 0.12	1.10± 0.16^{a}	0.46 ± 0.23^{b}
Exp 3	Fish	2.46 ± 0.25	0.53 ± 0.24^{a}	0.58 ± 0.12^{b}	1.11 ± 0.17^{b}	0.54 ± 0.10^{a}
	Casein	2.75 ± 0.63	0.47 ± 0.12^{ab}	0.44 ± 0.09^{c}	1.43 ± 0.31^{ab}	0.31 ± 0.05^{b}
	Soy	2.39 ± 0.33	0.23 ± 0.09^{b}	0.49 ± 0.09^{c}	1.48 ± 0.24^{ab}	0.33 ± 0.06^{b}
	Pea	2.70 ± 0.54	0.14 ± 0.05^{c}	0.78 ± 0.12^{a}	1.64 ± 0.22^{a}	0.48 ±0.06^{ab}
	Lupin	2.60 ± 0.26	0.06 ± 0.03^{d}	0.73 ± 0.11^{a}	1.77 ± 0.14^{a}	0.41 ± 0.08^{ab}

Values are mean values ± SD. VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, High density lipoproteins; Different superscript letters within one column denote significant difference within one experiment. $P < 0.05$ (Fisher's multiple range test). Experiment 1 (n = 10), Experiment 2 and 3 (n = 12). Rats were food deprived for 12 h in exp.1 & 2, while in experiment 3 rats were killed without prior food deprivation.

In Experiment 2, the concentration of plasma cholesterol was significantly lower in rats fed fish protein than in rats fed beef, casein and pork proteins. Rats fed turkey or soy protein had values in between. VLDL-cholesterol concentration was significantly lower in rats fed soy protein compared to rats fed casein, pork, beef, turkey or fish protein, which had similar concentrations. No difference was observed in the LDL-cholesterol concentration among the groups. HDL-cholesterol concentration was significantly lower in rats fed fish protein than rats fed any other experimental protein. The quotient of LDL-/HDL-cholesterol

was significantly lower in the soy protein fed rats compared to the rats fed fish protein, rats fed pork, beef, turkey or casein had values in between.

In Experiment 3, no significant difference was found in plasma cholesterol concentration among the groups. VLDL-cholesterol concentration was significantly lower in rats fed plant proteins (soy, pea and lupin proteins) than in rats fed animal proteins (fish and casein proteins). LDL-cholesterol concentration was highest in rats fed pea or lupin proteins. Casein and soy protein fed rats had lowest LDL-cholesterol concentrations and in the same range. Fish protein fed rats had values in between. HDL-cholesterol concentration was significantly lower in rats fed fish protein than in rats fed pea or lupin protein. Rats fed casein and soy protein had similar concentration of HDL-cholesterol and in the mean range. The quotient of LDL-/HDL-cholesterol was significantly higher in the fish protein fed rats compared to the casein and soy protein fed rats. The quotient of LDL-/HDL-cholesterol was similar in the soy protein fed rats and in the casein protein fed rats. The quotients of LDL-/HDL-cholesterol were similar in the pea protein fed rats and lupin protein fed rats, and the values were in between of the rats fed fish protein and soy protein.

3.3 Triglyceride Concentrations in Plasma and Lipoproteins

In table 3.3, concentrations of triglycerides in plasma and lipoproteins are presented

In Experiment 1, no significant difference was found in the concentrations of triglycerides in plasma, VLDL, LDL or HDL.

In Experiment 2, triglyceride concentration in plasma was lowest in rats fed soy protein. Rats fed pork and fish protein had significantly lower triglyceride concentration than rats fed casein. Rats fed beef and turkey proteins had values in between. Triglyceride concentration in the VLDL was lowest in rats fed soy protein. Rats fed pork, beef, or fish protein had significantly lower triglyceride concentration in the VLDL than rats fed casein and significantly higher concentration than rats fed soy protein. Rats fed turkey protein had significantly higher triglyceride concentration in the VLDL than rats fed soy protein, but did not differ from the other groups. Triglyceride concentration in the LDL was significantly lower in rats fed pork, beef or soy protein compared to rats fed casein. Rats fed turkey or fish proteins had triglyceride concentrations in between. Triglyceride concentration in HDL was

significantly lower in rats fed fish protein compared to rats fed casein, rats of the other groups had concentrations in between these two groups.

Table 3.3 Triglyceride concentrations in plasma and Lipoproteins of rats used in experiment 1, 2 and 3

Groups		Plasma	VLDL	LDL	HDL
		[mmol/L]			
Exp1	Casein	0.66 ± 0.17	0.38 ± 0.11	0.09 ± 0.02	0.10 ± 0.01
	Soy	0.63 ± 0.18	0.31 ± 0.11	0.08 ± 0.02	0.11 ± 0.02
Exp 2	Pork	1.10 ± 0.34^{b}	0.85 ± 0.33^{b}	0.21 ± 0.08^{b}	0.13 ± 0.04^{ab}
	Beef	1.25 ± 0.44^{ab}	0.89± 0.32^{b}	0.21 ± 0.06^{b}	0.15 ± 0.05^{ab}
	Fish	1.11 ± 0.57^{b}	0.86 ± 0.45^{b}	0.23 ± 0.10^{ab}	0.12 ± 0.03^{b}
	Turkey	1.30 ± 0.59^{ab}	1.07 ± 0.52^{ab}	0.23 ± 0.08^{ab}	0.14 ± 0.03^{ab}
	Casein	1.52± 0.21^{a}	1.24 ± 0.51^{a}	0.29 ± 0.09^{a}	0.17 ± 0.04^{a}
	Soy	0.75 ± 0.11^{c}	0.43 ± 0.10^{c}	0.18 ± 0.06^{b}	0.14 ± 0.01^{ab}
Exp 3	Fish	1.31 ± 0.67^{b}	1.23 ± 0.38^{b}	0.20 ± 0.06^{b}	0.08 ± 0.02^{b}
	Casein	2.34 ± 0.85^{a}	1.54 ± 0.44^{a}	0.32 ± 0.08^{a}	0.12 ± 0.05^{a}
	Soy	1.20 ± 0.79^{b}	1.02 ± 0.34^{b}	0.22 ± 0.07^{b}	0.08 ± 0.03^{b}
	Pea	0.80 ± 0.46^{bc}	0.64 ± 0.26^{bc}	0.21 ± 0.08^{b}	0.08 ± 0.03^{b}
	Lupin	0.58 ± 0.26^{c}	0.32 ± 0.26^{c}	0.19 ± 0.05^{b}	0.08 ± 0.03^{b}

Values are mean values ± SD. VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, High density lipoproteins; Different superscript letters within one column denote significant difference within one experiment. $P < 0.05$ (Fisher's multiple range test). Experiment 1 (n = 10), Experiment 2 and 3 (n = 12). Rats were food deprived for 12 h in exp.1 & 2, while in experiment 3 rats were killed without prior food deprivation.

In Experiment 3, triglyceride concentration in plasma was lowest in rats fed lupin protein. Rats fed fish, soy or pea protein had lower concentrations than rats fed casein. Triglyceride concentration in the VLDL was significantly lower in rats fed fish, soy or pea

protein compared to rats fed casein. All the groups had significantly higher VLDL triglyceride concentrations than rats fed lupin protein. Concentrations of triglycerides in the LDL and HDL were significantly higher in rats fed casein compared to rats of all other groups, which had similar concentrations.

3.4 Lipid Concentrations in the VLDL

Results of the lipid analysis of VLDL from rats fed casein or soy protein in experiment 3 analyzed by thin layer chromatography are shown in figure 3.1. Rats fed diet containing soy protein had significantly lower triglyceride (-30%) in the VLDL than rats fed diet containing casein. Rats fed diet containing soy protein tended to have lower phospholipid concentrations compared to rats fed diet containing casein ($P<0.10$). No significant difference was observed in the cholesterol concentrations between the groups. Rats fed diet containing soy protein had significantly lower cholesterol ester values (-54%) than rats fed diet containing casein. The core surface ratio (TG + CE)/ (FC + PL) which provides an indication of the relative differences in the average size of the VLDL, was not different between rats fed casein (2.66 ± 0.72) and soy protein (2.50 ± 1.10).

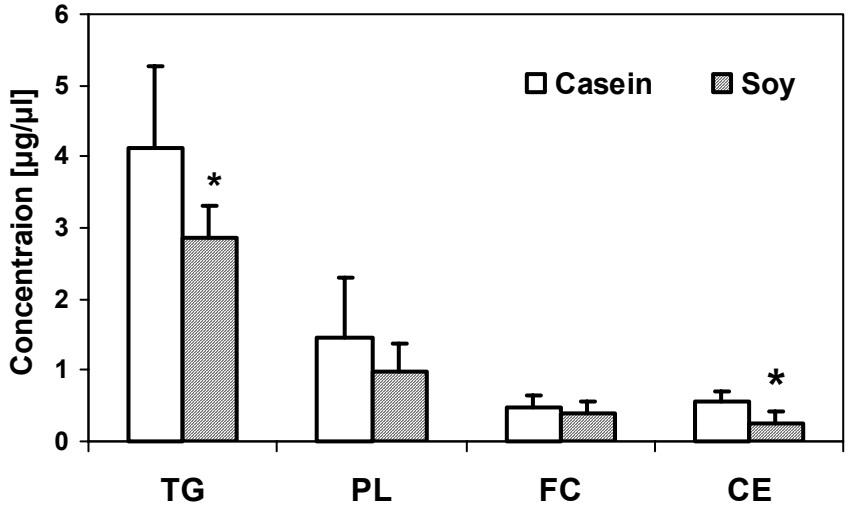

Figure 3.1: Concentrations of different lipids in the Very Low Density Lipoprotein fraction of rats fed either casein or soy protein determined by thin layer chromatography (Experiment 3)

Values are mean values ± SD. * significant difference between casein and soy group. $P<0.05$ (t-test). (n = 12). TG; triglycerides, PL; phospholipids, FC; free cholesterol, CE; cholesterol esters. Rats were killed without prior food deprivation.

3.5 Protein Concentrations in Lipoproteins and Plasma

Table 3.4 Protein Concentrations in Lipoproteins and Plasma of Rats used in Experiment 1, 2 and 3

Groups		VLDL protein [mg/L]	LDL protein [mg/L]	HDL protein [mg/L]	Plasma protein [g/L]
Exp 1	Casein	65.6 ± 13.2	173 ± 60	n.d.	47.3 ± 9.1
	Soy	57.6 ± 18.4	164 ± 35	n.d	41.6 ± 4.2.
Exp 2	Pork	345 ± 86[a]	n.d.	n.d.	n.d.
	Beef	348 ± 78[a]	n.d.	n.d.	n.d.
	Fish	357 ± 98[a]	n.d.	n.d	n.d.
	Turkey	372 ± 109[a]	n.d.	n.d.	n.d.
	Casein	394 ± 112[a]	n.d.	n.d.	n.d.
	Soy	206 ± 26[b]	n.d.	n.d.	n.d.
Exp 3	Fish	283 ± 111[b]	256 ± 101[b]	1338 ± 467	n.d.
	Casein	414 ± 125[a]	234 ± 46[b]	1671 ± 420	n.d.
	Soy	269 ± 147[b]	260 ± 43[b]	1706 ± 425	n.d.
	Pea	140 ± 43[bc]	382 ± 67[a]	1756 ± 386	n.d.
	Lupin	76 ± 33[c]	354 ± 59[a]	1802 ± 254	n.d.

Values are mean values ± SD. VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, High density lipoproteins; Different superscript letters within one column denote significant difference within one experiment. $P < 0.05$ (Fisher's multiple range test). Experiment 1 (n = 10), Experiment 2 and 3 (n = 12). (n.d., not determined). Rats were food deprived for 12 h in exp.1 & 2, while in experiment 3 rats were killed without prior food deprivation.

In the table 3.4, concentrations of proteins in the lipoproteins are presented. In Experiment 1, no significant difference was observed in VLDL, LDL and plasma protein concentrations between the groups.

In Experiment 2, rats fed soy protein diet had significantly lower protein concentration in the VLDL than rats fed diets containing pork, beef, fish, turkey or casein, which had similar concentrations.

In Experiment 3, rats fed lupin protein had lowest protein concentration and rats fed casein had highest protein concentration in the VLDL. Rats fed fish protein and soy protein had similar protein concentrations in the VLDL. Rats fed pea protein had VLDL protein concentration in between soy and lupin group. Rats fed lupin protein and rats fed pea protein had significantly higher protein concentration in the LDL than rats fed fish, casein and soy protein. No significant difference was observed in HDL protein concentration.

3.6 Apolipoprotein concentrations in the Lipoproteins

Relative amounts of selected apolipoproteins in different lipoprotein fractions are shown in figures 3.2 a and 3.2 b. Apo-B 100 and Apo-B 48 concentrations were significantly lower (-66% and -54%) in the rats fed diet containing soy protein compared to the rats fed diet containing casein ($P<0.05$). No significant difference were observed in the Apo-E concentrations between the two groups (Figure 3.2 a). No significant difference was observed in the concentrations of Apo-B 100 in LDL and Apo-AI in HDL fraction between the two groups (figure 3.2 b).

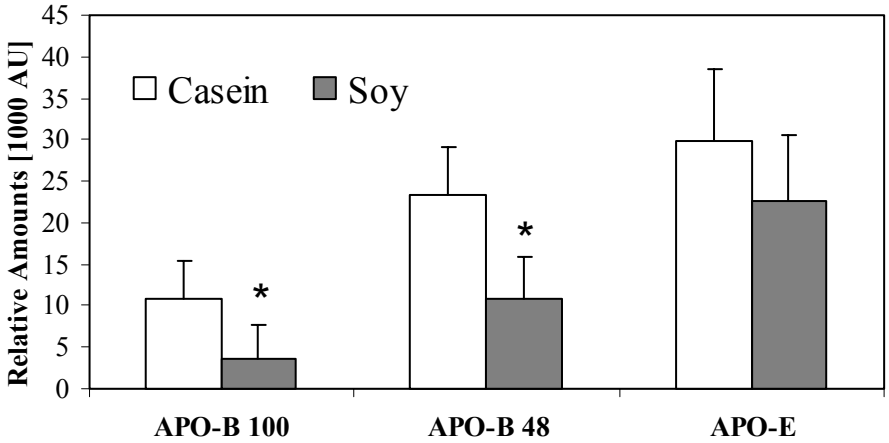

Figure 3.2 a: Apolipoprotein concentrations in the triglyceride rich lipoprotein (VLDL + Chylomicrones) of rats fed diets containing either casein or soy protein (Experiment 3)

Values are mean values ± SD. AU, Absorbance units. APO, Apolipoprotein. * Significant difference between casein and soy group ($P< 0.05$) (t-test). n = 12. Rats were killed without prior food deprivation.

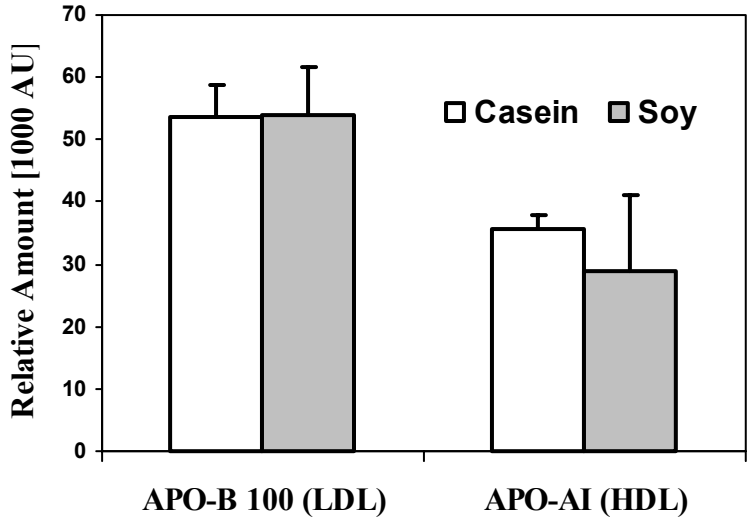

Figure 3.2 b: Apolipoprotein concentration in the LDL and HDL of rats fed diets containing either casein or soy protein (Experiment 3)

Values are mean values ± SD. AU, Absorbance units. Apo, Apolipoprotein. n = 12. Rats were killed without prior food deprivation.

3.7 Amino acid concentrations in Plasma

In table 3.5 a, the amino acid concentrations in plasma of rats used in experiment 1 are presented.

Plasma amino acid concentrations of isoleucine, leucine, lysine, methionine, threonine, valine, alanine, and phenylalanine were significantly higher in casein fed rats compared to soy protein fed rats. But no differences were found in the concentrations of arginine, histidine, tryptophan, asparagine, glutamine, glutamic acid, glycine, serine, tyrosine and taurine.

Table 3.5 a Amino Acid Concentrations in Plasma of Rats used in Experiment 1

Amino acids	Casein	Soy
	[µM]	
Arginine	125 ± 20	138 ± 20
Histidine	87 ± 9	96 ± 11
Isoleucine	58 ± 8[a]	49 ± 6[b]
Leucine	143 ± 25[a]	115 ± 17[b]
Lysine	432 ± 62[a]	370 ± 64[b]
Methionine	46 ± 4[a]	33.6 ± 5[b]
Threonine	619 ± 132[a]	429 ± 55[b]
Tryptophan	69 ± 9	59 ± 15
Valine	198 ± 29[a]	166 ± 25 [b]
Alanine	471 ± 56[a]	377 ± 41[b]
Asparagine	62 ± 9	53 ± 10
Glutamine	928 ± 82	852 ± 85
Glutamic acid	194 ± 24	181 ± 30
Glycine	329 ± 44	342 ± 40
Serine	424 ± 43	469 ± 46
Tyrosine	110 ± 20	80 ± 41
Phenyl alanine	78 ± 12[a]	61 ± 12[b]
Taurine	79 ± 45	78 ± 26

Values are mean values ± SD. Different superscript letters denote significant difference between casein and soy group. $P < 0.05$ (Fisher's multiple range test). n = 10. Rats were food deprived for 12 h.

In table 3.5 b, the amino acid concentrations in plasma of the rats used in experiment 2 are presented.

Table 3.5.b Amino Acid Concentrations in the Plasma of Rats used in Experiment 2

Amino acids	Pork	Beef	Fish	Turkey	Casein	Soy
	[µM]					
Arginine	128 ± 15^{ab}	131 ± 20^{ab}	146 ± 22^{a}	125 ± 18^{ab}	112 ± 18^{b}	117 ± 17^{b}
Histidine	51 ± 9^{ab}	43 ± 7^{b}	41 ± 6^{b}	41 ± 6^{b}	51 ± 8^{ab}	57 ± 9^{a}
Isoleucine	63 ± 11^{ab}	63 ± 8^{ab}	68 ± 12.4^{a}	61 ± 8^{ab}	67 ± 11^{a}	53 ± 10^{c}
Leucine	95 ± 17^{ab}	92 ± 13^{ab}	94 ± 17^{ab}	93 ±13^{ab}	105± 17^{a}	85± 14^{b}
Lysine	441 ± 124^{ab}	472 ± 79^{ab}	518 ± 85^{a}	402 ± 81^{b}	476 ± 81^{ab}	338 ± 63^{c}
Methionine	35 ± 5^{ab}	36 ±5^{ab}	42 ± 10^{a}	34 ± 3^{ab}	41 ± 5^{a}	28 ± 5^{b}
Threonine	434 ± 86^{ab}	430 ± 99^{ab}	313 ± 50^{b}	337 ± 90^{b}	527 ± 117^{a}	313 ± 49^{b}
Tryptophan	56 ± 19^{ab}	59 ± 10^{a}	61 ± 11^{a}	52 ± 7^{b}	56^{ab} ± 9	44^{c} ± 8
Valine	122 ± 16^{ab}	114 ± 12^{b}	107 ± 16^{b}	108 ± 17^{b}	130 ± 21^{a}	105 ± 20^{b}
Alanine	352 ± 76^{ab}	337 ± 53^{ab}	464 ± 198^{a}	321 ± 54^{b}	351 ± 67^{ab}	298± 52^{b}
Asparagine	51 ± 5^{b}	50 ± 4^{b}	54 ± 4^{ab}	49 ± 5^{b}	61 ± 10^{a}	48 ± 3^{b}
Glutamine	665 ± 62^{ab}	616 ± 43^{b}	614 ± 82^{b}	625 ± 57^{b}	689 ± 66^{a}	687 ± 39^{a}
Glycine	352 ± 64^{a}	315 ± 52^{ab}	354 ± 54^{a}	303 ± 50^{ab}	246 ± 25^{b}	274 ± 51^{b}
Serine	290 ± 29^{b}	263 ± 35^{b}	276 ± 26^{b}	265 ± 42^{b}	331 ± 33^{ab}	379 ± 36^{a}
Tyrosine	70 ± 11^{ab}	71 ± 10^{ab}	65 ± 10^{ab}	67 ± 13^{ab}	94 ± 21^{a}	58 ± 16^{b}
Phenyl alanine	46 ± 4^{a}	46 ± 4^{a}	46 ± 9^{a}	46 ± 5^{a}	49 ± 9^{a}	39 ± 6^{b}
Cysteine	159± 25^{a}	157 ± 25^{a}	147 ± 27^{b}	165 ± 34^{a}	156 ± 23^{a}	117 ± 23^{c}
Taurine	129 ± 29^{b}	113 ± 32^{b}	171 ± 30^{a}	97± 31^{b}	50 ± 10^{c}	55^{c} ± 8

Values are mean values ± SD. Different superscript letters within one row denote significant difference. $P<0.05$ (Fisher's multiple range test). n = 12. Rats were food deprived for 12 h.

Plasma concentration of arginine was higher in rats fed fish protein compared to rats fed casein or soy protein, rats fed pork, beef or turkey protein had values in between. Rats fed fish, turkey or beef protein had lower histidine concentration compared to rats fed soy protein. Rats fed pork and casein had values in between. Isoleucine concentration was significantly higher in rats fed fish protein and casein compared to rats fed soy protein, rats fed pork, beef and turkey protein had isoleucine concentrations in between. Leucine was present in significantly higher concentration in rats fed casein compared to rats fed soy protein, rats fed pork, beef, turkey or fish protein had concentrations in between. Lysine concentration was highest in the rats fed fish protein and lowest in the rats fed soy protein. Rats fed turkey protein had lower concentration then rats fed fish protein, rats fed pork, beef or casein had values in between. Methionine concentration was significantly higher in rats fed fish protein and casein compared to rats fed soy protein rats fed, pork, beef or turkey protein had concentrations in between these groups. Threonine concentration was significantly higher in rats fed casein compared to rats fed fish, turkey or soy protein. Rats fed pork and beef proteins had concentrations in between rats fed casein and soy protein. Tryptophan was present in significantly higher concentration in rats fed beef or fish protein compared to rats fed turkey or soy protein. Rats fed pork protein or casein had tryptophan concentrations significantly higher compared to rats fed soy protein. Valine concentration was significantly higher in rats fed casein compared to rats fed beef, fish, turkey or soy protein. Rats fed pork protein had concentration in between. Plasma concentration of alanine was significantly higher in rats fed fish protein compared to rats fed soy protein. Rats fed pork, beef, turkey or casein had plasma concentration of alanine in between. Asparagine concentration was significantly higher in rats fed casein compared to rats fed soy, pork, beef or turkey protein. Rats fed fish protein had concentrations in between. Rats fed casein and soy protein had similar glutamine concentrations and significantly higher than rats fed beef, fish or turkey protein. Rats fed pork protein had concentrations in between. Glycine concentration was significantly higher in rats fed pork or fish protein compared to rats fed casein or soy protein. Rats fed beef or turkey protein had concentrations in between. Serine was present in significantly lower concentrations in rats fed pork, beef, fish or turkey protein compared to rats fed soy protein. Rats fed casein had concentrations in between. Tyrosine and phenylalanine was present in significantly higher concentrations in plasma of rats fed casein compared to rats fed soy protein. Rats fed pork, beef, turkey or fish protein had tyrosine and phenylalanine concentrations in between.

Table 3.5.c Amino Acid Concentrations in the Plasma of Rats used in Experiment 3

Amino acids	Fish	Casein	Soy	Pea	Lupin
	[µM]				
Arginine	145 ± 22^{b}	85 ± 20^{c}	134 ± 23^{b}	177^{ab} ± 25	209 ± 59^{a}
Histidine	30 ± 9^{b}	43 ± 12^{a}	36 ± 9^{b}	47± 14^{a}	46 ± 14^{a}
Isoleucine	69 ± 11	81 ± 20	72 ± 13	71 ± 10	65 ± 21
Leucine	86 ± 17^{b}	123 ± 31^{a}	94 ± 17^{b}	96 ± 14^{b}	87 ± 29^{b}
Lysine	644 ± 124^{a}	487 ± 113^{b}	317 ± 192^{c}	444 ± 91^{b}	406 ± 193^{b}
Methionine	54 ± 4.7	55 ± 5.0	61 ± 8	64 ± 9	50 ± 18
Threonine	422 ± 81^{b}	526 ± 135^{a}	190 ± 32^{c}	252 ± 46^{bc}	315 ± 120^{bc}
Tryptophan	68 ± 14^{a}	66 ± 11^{a}	69 ± 8^{a}	29± 9^{b}	16 ± 6^{c}
Valine	107 ± 18^{b}	189 ± 54^{a}	103 ± 18^{b}	110^{b} ± 17	84 ± 26^{b}
Alanine	548 ± 133^{a}	539 ± 62^{a}	540 ± 78^{a}	510 ± 80^{a}	349 ± 80^{b}
Asparagine	337 ± 52^{c}	456 ± 101^{b}	569 ± 76^{a}	601 ± 112^{a}	450 ± 156^{b}
Glutamine	531 ± 77^{b}	663 ± 80^{a}	602 ± 49^{a}	621 ± 152^{a}	563 ± 86^{b}
Glycine	337 ± 66^{a}	152 ± 30^{c}	288 ± 47^{b}	238 ± 29^{b}	141± 19^{c}
Serine	250 ± 34	261 ± 38	236 ± 29	276 ± 36	252 ± 56
Tyrosine	55 ± 10^{b}	92 ± 21^{a}	63 ± 11^{b}	79 ± 22^{ab}	91 ± 45^{a}
Phenyl alanine	37 ± 8	43 ± 7	44 ± 7	46 ± 6	42 ± 16
Taurine	158 ± 26^{a}	62 ± 18^{c}	122 ± 38^{b}	103 ± 21^{b}	170 ± 70^{a}
Glutamic acid	93 ± 18^{a}	93 ± 11^{a}	98 ± 16^{a}	83 ± 20^{b}	78 ± 13^{b}

Values are mean values ± SD. Different superscript letters within one row denote significant difference. $P<0.05$ (Fisher's multiple range test). n = 12. Rats were killed without prior food deprivation.

Cysteine was present in significantly higher concentrations in rats fed pork, beef turkey or casein compared to rats fed soy protein. Rats fed fish protein had significantly higher concentration compared to rats fed soy protein, and lower compared to rest of the groups. Taurine concentration was significantly higher in rats fed fish protein compared to rats fed pork, beef, turkey, casein or soy protein. Rats fed pork, beef or turkey protein had

significantly lower taurine concentration than rats fed fish protein but significantly higher than rats fed casein or soy protein.

Amino acid concentrations in the plasma of rats used in experiment 3 are specified in table 3.5 c. Plasma concentration of arginine was significantly higher in rats fed lupin protein compared to rats fed casein, soy or fish protein. Rats fed pea protein had significantly higher plasma arginine concentration than rats fed casein. Rats fed fish and soy protein had significantly lower histidine concentration in plasma compared to rats fed casein, pea or lupin protein. Plasma isoleucine and methionine concentration were not different among the groups. Leucine concentration was significantly higher in rats fed casein compared to rats fed fish, soy, pea or lupin protein. Plasma lysine concentration was significantly higher in rats fed fish protein compared to rats fed casein, soy, pea or lupin protein. Rats fed soy protein had significantly lower lysine concentration compared to rats fed casein. Threonine concentration was significantly higher in rats fed casein or fish protein compared to rats fed soy, pea or lupin protein. Tryptophan concentration was similar among rats fed fish, casein or soy protein, but rats fed pea or lupin protein had significantly lower concentration compared to rats fed soy protein or casein. Plasma valine concentration was significantly higher in rats fed casein compared to rats fed fish, soy, pea or lupin protein. Alanine concentration was not different among rats fed fish, casein, soy or pea protein, only rats fed lupin protein had significantly lower concentration. Rats fed pea and soy protein had significantly higher asparagine concentration compared to rats fed fish, casein or lupin protein. Rats fed fish protein had significantly lower asparagine concentration compared to rats fed casein. Glutamine concentration was similar among rats fed casein, soy or pea protein, but rats fed fish and lupin protein had significantly lower concentration compared to rats fed casein or soy protein. Glycine concentration was significantly higher in rats fed fish protein compared to rats fed casein, soy, pea or lupin protein. Rats fed soy or pea protein had higher glycine concentrations compared to rats fed casein or lupin protein. No differences were observed in the serine and phenylalanine concentrations among the groups. Rats fed fish or soy protein had significantly lower plasma tyrosine concentration compared to rats fed casein or lupin protein, rats fed pea protein had plasma tyrosine concentration in between. Plasma taurine concentration was significantly higher in the rats fed fish or lupin protein compared to rats fed casein, soy or pea protein. Rats fed casein had plasma taurine concentration significantly lower compared to rats fed soy or pea protein. Plasma glutamic acid concentration was significantly higher in rats fed fish, casein or soy protein compared to rats fed pea or lupin protein.

3.8 Homocysteine concentrations in plasma

In figure 3.3, homocysteine concentrations in plasma of rats used in experiment 2 are shown. Homocysteine concentration was not different between rats fed casein and soy protein. Rats fed beef, fish or turkey protein had significantly lower homocysteine concentrations compared to casein and soy protein fed rats. Pork protein fed rats had homocysteine concentrations in between.

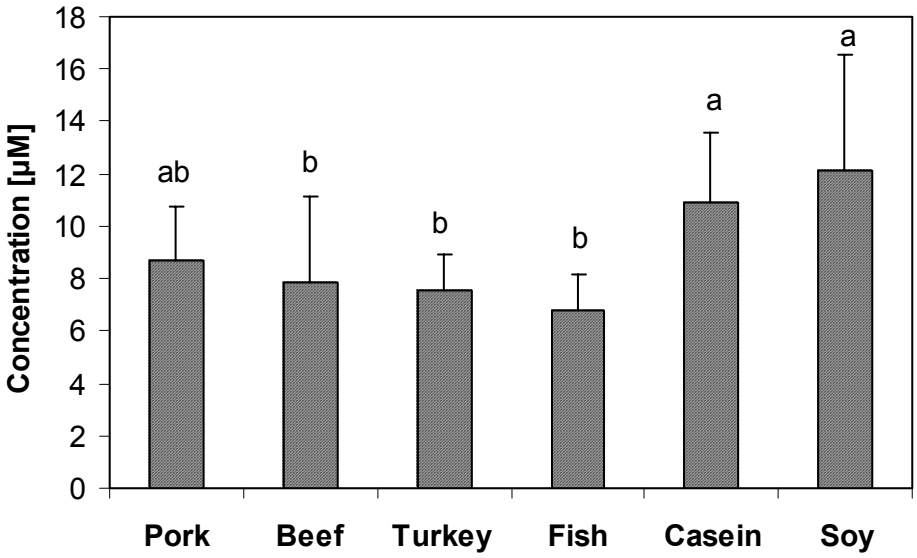

Figure 3.3 Homocysteine concentrations in plasma of rats used in experiment 2

Values are mean values ± SD. Different superscript letters denote significant difference. $P<0.05$ (Fisher's multiple range test). n = 12. Rats were food deprived for 12 h.

3.9 Cholesterol and Triglyceride in Faeces

In the table 3.6, cholesterol and triglyceride concentration in faeces and excretion values of rats used in experiment 1 and 2 are presented. In Experiment 1, no significant difference was found in the cholesterol concentration as well as in excretion between rats of the two groups. The triglyceride concentration as well as excretion was significantly higher in rats fed soy protein compared to rats fed casein (+192%, +256%), respectively.

In Experiment 2, cholesterol concentration in faeces was significantly higher in the rats fed casein compared to rats fed pork, beef, fish, turkey or soy protein, which had similar values. Cholesterol excretion was significantly higher in the rats fed casein or soy protein

compared to rats fed pork, beef, fish or turkey protein, which had similar values. Faecal triglyceride concentration as well as excretion was significantly higher in the rats fed soy protein compared to rats fed pork, beef, turkey, fish or casein protein.

Table 3.6 Cholesterol and triglyceride concentration in faeces and excretion of rats used in Experiment 1 and 2

Groups		Cholesterol		Triglyceride	
		Concentration [µmol/g]	Excretion [µmol/d]	Concentration [µmol/g]	Excretion [µmol/d]
Exp 1	Casein	6.8 ± 1.6	8.2 ± 2.0	0.4 ± 0.1^{b}	0.5 ± 0.1^{b}
	Soy	5.6 ± 1.0	8.4 ± 1.4	1.3 ± 0.5^{a}	1.9 ± 0.6^{a}
Exp 2	Pork	6.1 ± 1.7^{b}	7.2 ± 2.0^{b}	0.4 ± 0.2^{b}	0.5 ± 0.3^{b}
	Beef	6.2 ± 1.2^{b}	6.6 ± 2.7^{b}	0.5 ± 0.4^{b}	0.4 ± 0.3^{b}
	Fish	5.4 ± 0.7^{b}	6.5 ± 1.4^{b}	0.5 ± 0.3^{b}	0.6 ± 0.4^{b}
	Turkey	5.9 ± 1.5^{b}	6.5 ± 2.1^{b}	0.6 ± 0.4^{b}	0.4 ± 0.2^{b}
	Casein	10.7 ± 1.5^{a}	13.7 ± 3.1^{a}	0.2 ± 0.1^{b}	0.2 ± 0.1^{b}
	Soy	7.2 ± 2.7^{b}	10.5 ± 4.2^{a}	1.4 ± 0.8^{a}	1.9 ± 1.1^{a}

Values are mean values ± SD. Different superscript letters within one column denote significant difference within one experiment. $P<0.05$ (Fisher's multiple range test). Experiment 1, (n = 10), Experiment 2 (n = 12). Rats were food deprived for 12 h.

3.10 Bile Acids in Plasma and Faeces

In the table 3.7, bile acid concentration in plasma and bile acid concentration and excretion values in the faeces are presented.

In Experiment 1, bile acid concentration in the plasma was not different between the rats fed soy protein and rats fed casein. But bile acid concentration and excretion was

significantly higher (+120% and +170%) in rats fed soy protein compared to rats fed casein, respectively.

In Experiment 2, bile acid concentration was significantly higher in rats fed fish protein than in rats fed casein. Rats fed pork, beef turkey or soy protein had values in between. The bile acid excretion was significantly higher in rats fed fish or soy protein than in rats fed pork, beef, turkey or casein.

Table 3.7 Bile Acid Concentrations in Plasma and Faeces and Excretion in Rats used in Experiment 1 and 2

Groups		Bile acid in Plasma	Bile acid in Faeces	
		Concentration (µmol/g)	Concentration (µmol/g)	Excretion (µmol/d)
Exp 1	Casein	16.9 ± 7.0	0.9 ± 0.2^{b}	1.1 ± 0.3^{b}
	Soy	15.2 ± 8.3	2.0 ± 0.7^{a}	2.9 ± 0.8^{a}
Exp 2	Pork	n.d.	2.2 ± 0.4^{b}	2.6 ± 0.4^{b}
	Beef	n.d	2.1 ± 0.5^{b}	2.1 ± 0.6^{b}
	Fish	n.d.	2.9 ± 0.7^{a}	3.5 ± 0.9^{a}
	Turkey	n.d.	2.4 ± 0.4^{b}	2.7 ± 0.6^{b}
	Casein	n.d.	2.0 ± 0.3^{b}	2.6 ± 0.5^{b}
	Soy	n.d.	2.3 ± 0.2^{b}	3.4 ± 0.4^{a}

Values are mean values ± SD. Different superscript letters within one column denote significant difference within one experiment. $P<0.05$ (Fisher's multiple range test). Experiment 1 (n = 10), Experiment 2 (n = 12). Rats were food deprived for 12 h.

3.11 Cholesterol and Triglyceride Concentrations in the Liver

In the table 3.8, cholesterol and triglyceride concentration in the liver of rats used in experiment 1, 2 and 3 are specified.

In Experiment 1, cholesterol concentration was significantly lower (-30%) in rats fed soy protein compared to the rats fed casein and the triglyceride concentration in the liver was 24% lower in rats fed soy protein compared to rats fed casein.

In Experiment 2, rats fed fish protein had significantly higher cholesterol concentration in the liver compared to rats fed casein or soy protein. Rats fed soy protein had significantly lower cholesterol concentration compared to rats fed casein. Rats fed pork, beef and turkey protein had significantly higher values compared to rats fed soy protein. Rats fed pork protein or casein had similar cholesterol concentrations. The triglyceride concentration in liver was significantly higher in rats fed fish protein and casein compared to rats fed pork protein. Rats fed beef, turkey and soy protein had values in between.

Table 3.8 Cholesterol and Triglyceride Concentrations in the Liver of Rats used in Experiment 1, 2 and 3

Groups		Liver cholesterol	Liver triglyceride
		[µmol/g]	
Exp 1	Casein	8.7 ± 2.1^{a}	8.5 ± 3.5
	Soy	6.1 ± 1.0^{b}	6.5 ± 1.7
Exp 2	Pork	12.7 ± 4.3^{b}	7.5 ± 2.1^{b}
	Beef	14.3 ± 3.5^{b}	9.2 ± 2.5^{ab}
	Fish	20.9 ± 6.5^{a}	13.9 ± 4.8^{a}
	Turkey	16.8 ± 3.5^{ab}	11.1 ± 4.4^{ab}
	Casein	13.2 ± 1.8^{b}	13.1 ± 3.2^{a}
	Soy	9.4 ± 2.2^{c}	10.6 ± 1.7^{ab}
Exp 3	Fish	10.1 ± 2.1^{a}	8.3 ± 2.5^{b}
	Casein	8.4 ± 1.6^{ab}	12.1 ± 5.2^{a}
	Soy	4.3 ± 1.5^{b}	7.7 ± 2.4^{b}
	Pea	3.2 ± 1.2^{b}	7.0 ± 1.4^{b}
	Lupin	2.8 ± 1.0^{b}	7.0 ± 2.1^{b}

Values are mean values ± SD. Different superscript letters within one column denote significant difference within one experiment. P<0.05 (Fisher's multiple range test). Experiment 1, (n = 10), Experiment 2 and 3, (n = 12). Rats were food deprived for 12 h in exp.1 & 2, while in experiment 3 rats were killed without prior food deprivation.

In Experiment 3, rats fed fish protein had significantly higher liver cholesterol concentration and rats fed plant proteins had significantly lower cholesterol concentration, rats fed casein had cholesterol concentration in between. The triglyceride concentration in the liver was significantly higher in the rats fed casein compared to rats fed fish, soy, pea or lupin proteins which had similar triglyceride concentrations.

3.12 Free and Esterified Cholesterol in the Liver

Table 3.9 Concentrations of Free and Esterified Cholesterol in the Liver of Rats used in Experiment 1 and 2

Groups		Free Cholesterol (FC)	Cholesterol ester (CE)	CE/FC
		[µmol/g]		
Exp 1	Casein	4.3 ± 1.1[a]	25.6 ± 10.1[a]	6.4 ± 2.9
	Soy	1.9 ± 0.6[b]	11.9 ± 6.1[b]	6.7 ± 3.6
Exp 2	Pork	7.8 ± 3.4	25.1 ± 7.3[b]	3.5 ± 1.9[b]
	Beef	7.6 ± 3.5	23.0 ± 11.3[b]	3.5 ± 1.9[b]
	Fish	7.9 ± 2.9	40.8 ± 15.5[a]	6.4 ± 3.8[a]
	Turkey	8.5 ± 2.0	35.6 ± 14.3[a]	4.4 ± 1.9[b]
	Casein	6.8 ± 2.2	22.9 ± 12.6[b]	3.6 ± 1.5[b]
	Soy	6.0 ± 3.8	14.3 ± 9.4[c]	2.1 ± 1.2[c]

Values are mean values ± SD. Different superscript letters within one column denote significant difference within one experiment. P<0.05 (Fisher's multiple range test). Experiment 1 (n = 10), Experiment 2 (n = 12). Rats were food deprived for 12 h.

In table 3.9, concentrations of free and esterified cholesterol in the liver of rats used in experiment 1 and 2 are specified. In Experiment 1, free cholesterol as well as cholesterol ester

concentration in the liver was significantly lower (-56%, -54%)) in the rats fed soy protein compared to the rats fed casein. The esterified to free cholesterol ratio was not different between rats fed casein or soy protein.

In Experiment 2, no significant difference was found in the free cholesterol concentration among the groups. Esterified cholesterol concentration in the liver was significantly higher in the rats fed fish and turkey protein compared to rats fed pork, beef, casein or soy protein. Rats fed soy protein had significantly lower esterified cholesterol than rats fed casein. The esterified to free cholesterol ratio was significantly higher in rats fed fish protein compared to rats fed pork, beef, turkey, casein or soy protein. Rats fed soy protein had significantly lower esterified to free cholesterol ratio compared to rats fed casein.

3.13 HMG CoA Reductase in the Liver

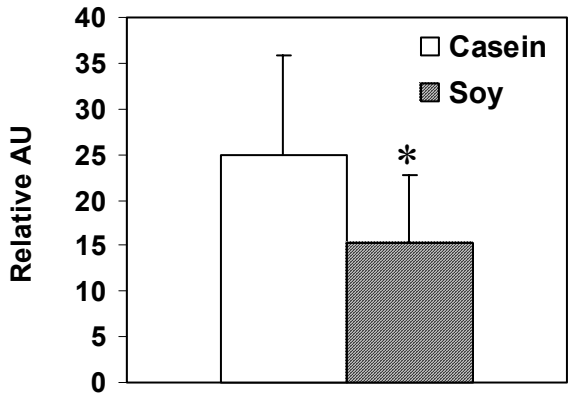

Figure 3.4: HMG CoA Reductase protein concentration in microsomal fraction of rats fed either casein or soy protein (Experiment 3)

Values are mean values ± SD. AU, Absorbance units, * Significant difference between casein and soy group. P<0.05 (t-test). Rats were killed without prior food deprivation, n= 12.

Rats fed soy protein diet had significantly lower (-38%) HMG CoA reductase protein concentrations in the microsomal fraction of the liver compared to rats fed casein protein diet (figure 3.4).

3.14 Enzyme activity in the Liver

In table 3.10, the activities of enzymes determined in experiment 1 and 2 are specified.

In experiment 1, rats fed soy protein had significantly lower MTP activity (-35%) in the liver compared to the rats fed casein. In experiment 2, rats fed turkey protein had significantly higher FAS activity in the liver compared to rats fed pork or soy protein. Rats fed beef protein, fish protein and casein had values in between. The activity of G6PDH was significantly lower in the rats fed pork and soy proteins compared to rats fed casein.

Table 3.10 Activities of Lipogenic Enzymes in the Liver Cytosol of Rats used in Experiment 1 and 2

Groups		MTP [ΔF/µg protein]	FAS	G6PDH
			[nmol/mg protein*min]	
Exp 1	Casein	43 ± 14[a]	n.d.	n.d.
	Soy	28 ± 7[b]	n.d.	n.d
Exp 2	Pork	n.d.	0.60 ± 0.23[b]	19.1 ± 4.4[b]
	Beef	n.d.	0.75 ± 0.35[ab]	n.d.
	Fish	n.d.	0.65 ± 0.24[ab]	n.d.
	Turkey	n.d.	0.99 ± 0.58[a]	n.d.
	Casein	n.d.	0.70 ± 0.45[ab]	31.5 ± 9.3[a]
	Soy	n.d.	0.52 ± 0.35[b]	16.4 ± 6.4[b]

Values are mean values ± SD. Different superscript letters within one column denote significant difference within one experiment. P< 0.05 (Fisher's multiple range test). Experiment 1 (n = 10), Experiment 2 (n = 12), n.d.: not determined. Rats were food deprived for 12 h. MTP, microsomal triglyceride transfer protein; FAS, fatty acid synthase; G6PDH, glucose 6 phosphate dehydrogenase.

3.15 Relative mRNA Concentrations in the Liver

Relative mRNA concentrations in the liver of rats fed diets containing casein or soy protein in experiment 1 are shown in figure 3.5. The mRNA concentrations of FAS and MTP were significantly lower (-30% and -27%) in rats fed soy protein than in rats fed casein (P<0.05). The mRNA concentrations of SREBP-1c and SREBP-2 tended to be lower (-30%

and -24%) in rats fed soy protein than in rats fed casein (P<0.10). No significant difference was found in the relative mRNA concentrations of HMG CoA reductase, LDL-receptor, and CYP7A1.

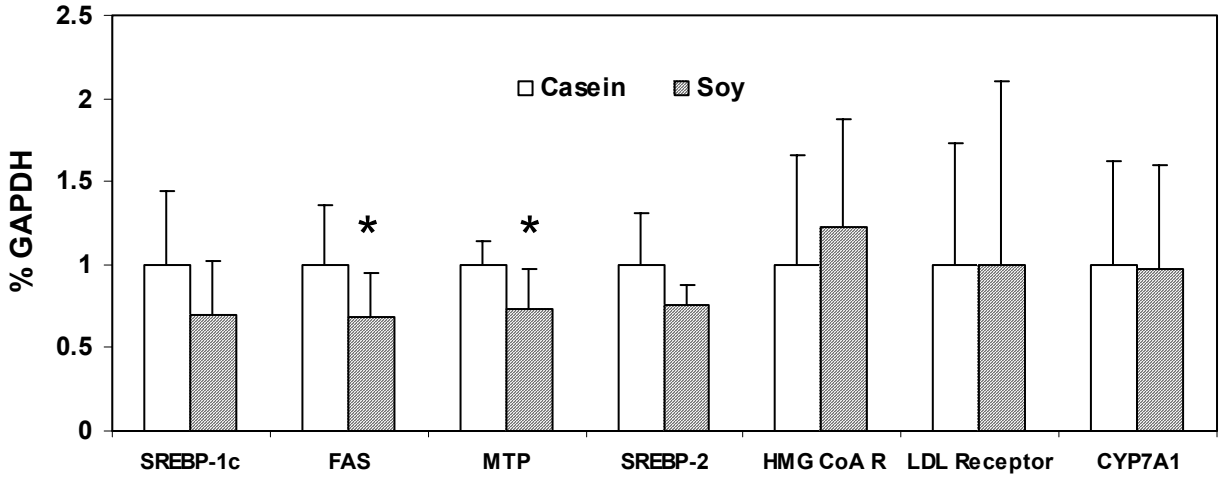

Figure 3.5: Relative mRNA concentrations in the liver of rats fed diets containing casein or soy protein in overnight food deprived state (Experiment 1)

Values are the mean values ± SD. n = 10. * denote significant difference between the two groups. P<0.05 (t-test). SREBP, sterol regulatory element binding protein; FAS, fatty acid synthase; MTP, microsomal triglyceride transfer protein; HMG CoA R, 3 hydroxy 3-methylglutaryl CoA reductase; LDL, low density lipoprotein; CYP7A1, cholesterol 7 α 1 hydroxylase.

Relative mRNA concentrations in the liver of rats fed diets containing casein or fish protein in experiment 2 are shown in figure 3.6. The mRNA expression of SREBP-2 was 65% higher in rats fed fish protein compared to rats fed casein (P<0.001) but however, we did not find significant difference in the mRNA concentration HMG Co A reductase. Although no significant difference was found in the mRNA concentrations of SREBP-1c, and G6PDH the mRNA concentrations of FAS and Delta 6-Desaturase were significantly higher 67% and 19% respectively, in the rats fed fish protein compared to rats fed casein.

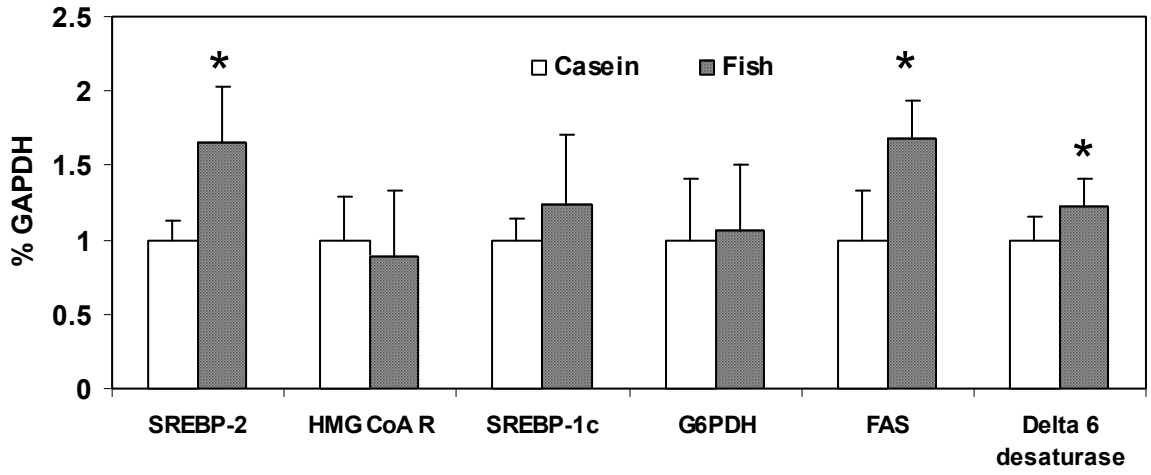

Figure 3.6: Relative mRNA concentrations in the liver of rats fed diets containing casein or fish protein in overnight food deprived state (Experiment 2)

Values are the mean values ± SD. n = 12. * denote significant difference between the two groups. P<0.05 (t-test). SREBP, sterol regulatory element binding proteins; HMG CoA R, 3 hydroxy 3 methyl glutaryl CoA reductase; G6PDH, glucose 6 phosphate dehydrogenase; FAS, fatty acid synthase.

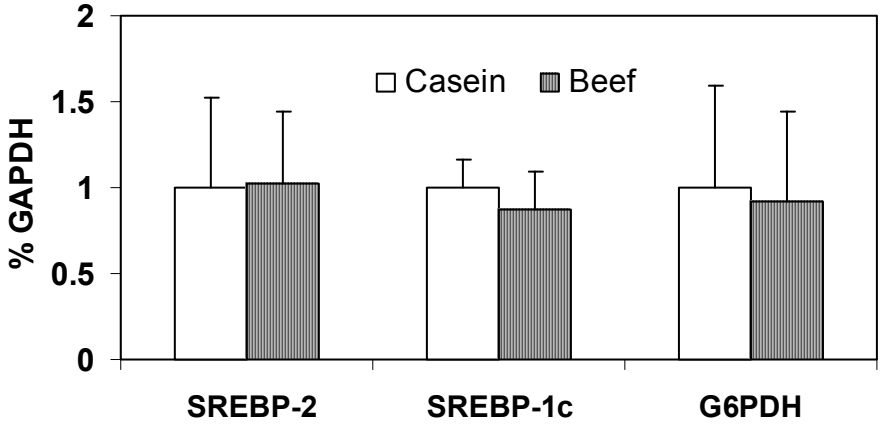

Figure 3.7: Relative mRNA concentrations in the liver of rats fed diets containing casein or beef protein in overnight food deprived state (Experiment 2)

Values are the mean values ± SD. n = 12, P<0.05 (t-test). SREBP, sterol regulatory element binding proteins; G6PDH, glucose 6-phosphate dehydrogenase.

There was no significant difference found in the relative mRNA expression of SREBP-1c, SREBP-2, or G6PDH in between the rats fed casein and beef protein (Figure 3.7).

3.16 cDNA Array data Soy Protein versus Casein:

Table 3.11: cDNA array data of selected proteins involved in lipid metabolism in the liver of rats fed diets containing soy protein compared with rats fed diet containing casein (Experiment 3)

GenBank Accession No.	Proteins/Genes	Ratio Soy/Casein group	Functions
D37920	Squalene epoxidase	0.8 ± 0.5	Cholesterol synthesis
M95591	Squalene synthetase	0.8 ± 0.4	Cholesterol synthesis
U12791	3-hydroxy-3-methylglutaryl-CoA synthase	0.9 ± 0.1	Cholesterol synthesis
X17595	Cholesterol 7-alpha-hydroxylase	0.8 ± 0.4	Bile acid metabolism
M38566	Sterol 27-hydroxylase	0.9 ± 0.1	Bile acid metabolism
M67465	3-beta hydroxy-5-ene steroid dehydrogenase type III (3beta-HSD III)	0.7 ± 0.2	Steroid metabolism
S63167	3-beta-hydroxysteroid dehydrogenase	0.8 ± 0.3	Steroid metabolism
P52233	11-beta-hydroxysteroid dehydrogenase 2	1.0 ± 0.5	Steroid metabolism
M00001	Apolipoprotein A-I precursor (APO-AI)	1.4 ± 0.4	HDL structural protein
U17697	Scavenger receptor class B type I	1.1 ± 0.5	Plasma HDL uptake
M00002	Apolipoprotein A-IV precursor (APO-AIV)	1.4 ± 1.0	Chylomicrones structural protein
U62803	Lecithin: cholesterol acyl transferase	0.9 ± 0.3	Extracellular cholesterol esterification
M35991	Fatty Acid-binding Protein	0.9 ± 0.2	Fatty acid binding, and transport
L46791	Liver Carboxylesterase 10 precursor	0.8 ± 0.3	Triglyceride degradation
D90109	Long chain acyl-CoA synthetase 2	0.9 ± 0.1	Fatty acid synthesis

J02791	Medium chain acyl-CoA dehydrogenase precursor	1.1 ± 0.4	Mitochondrial ß oxidation
U64451	Short chain acyl-CoA dehydrogenase precursor	1.6 ± 0.5	Mitochondrial ß-oxidation
M32801	3-ketoacyl-CoA thiolase A + B	1.6 ± 0.4	Paroxismal ß oxidation
J02752	Acyl-CoA Oxidase	0.7 ± 0.2	Paroxismal ß oxidation

Values are mean values ± SD. n = 4, For each probe RNA of 3 rats within same dietary treatment was pooled. Rats were killed without prior food deprivation. From 1176 gene/proteins spotted on the membrane about 575 gene/protein expressions were detectable. Only 127 were significantly different (at least 1.4 fold up or down regulated). HDL, high density lipoprotein.

In table 3.11, the cDNA array data of selected proteins involved in lipid metabolism in the liver of rats fed soy protein compared to rats fed casein are presented.

In the cDNA array analysis, soy protein fed rats showed a bit lower expressions of many proteins involved in cholesterol metabolism like squalene epoxidase, squalene synthetase and 3-hydroxy-3-methylglutaryl-CoA synthase, 3-beta-hydroxysteroid dehydrogenase III, 3-beta-hydroxysteroid dehydrogenase, cholesterol 7-alpha-hydroxylase, sterol 27-hydroxylase. apolipoprotein A-I precursor (APO-AI), apolipoprotein A-IV precursor (APO-AIV), and scavenger receptor class B type I were highly expressed in soy protein fed rats compared to casein fed rats. Medium chain acyl-CoA dehydrogenase precursor, short chain acyl-CoA dehydrogenase precursor, 3-ketoacyl-CoA thiolase A + B which are involved in fatty acid oxidation were highly expressed in rats fed soy protein.

Relative mRNA Concentrations Casein versus Soy protein in Experiment 3:

Relative mRNA expressions in the liver of rats fed diets containing casein or soy protein used in experiment 3 are shown in Figure 3.8. Relative mRNA expressions of APO-B (-37%), SREBP-2 (-35%), HMG CoA reductase (-38%), LDL receptor (-58%) and CYP7A1 (-38 %), were significantly lower in soy protein fed rats compared to casein fed rats. No difference was observed in the relative mRNA expressions of Insig-2. There was a tendency of higher Insig-1 (+ 49%) in rats fed soy protein compared to rats fed casein ($P<0.10$). The semiquantitative RT-PCR was performed to verify the results of cDNA arrays and these results prove the cDNA array findings (table 3.11).

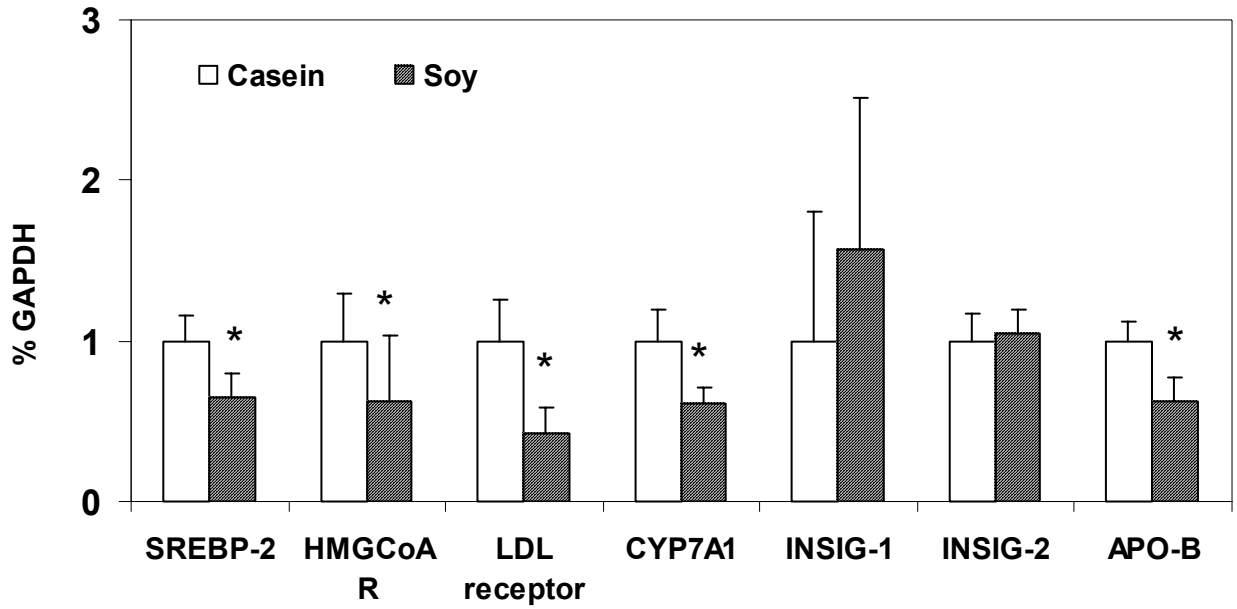

Figure 3.8: Relative mRNA concentrations in the liver of rats fed diets containing casein or soy protein in the postprandial state (Experiment 3)

Values are the mean values ± SD. * denote significant difference between the two groups. n = 12, P< 0.05 (t-test).). Rats were killed without prior food deprivation. SREBP, sterol regulatory element binding protein; HMG CoA R, 3 hydroxy 3-methylglutaryl CoA reductase; LDL, low density lipoprotein; CYP7A1, cholesterl 7 α 1 hydroxylase; INSIG, insulin induced gene; APO-B, apolipoprotein-B.

3.17 cDNA Array data Fish Protein versus Casein

In table 3.12, the cDNA array data of selected proteins involved in lipid metabolism in the liver of rats fed diets containing fish protein compared with rats fed diet containing casein are presented. The cDNA expression of genes involved in mitochondrial ß-oxidation of fatty acids like Carnitine palmitoyl transferase (CPT) I, CPT-II, short chain acyl-CoA dehydrogenase, medium chain acyl-CoA dehydrogenase, and long chain-specific acyl-CoA dehydrogenase were 1.1-2.0 times higher expressed in rats fed fish protein compared to rats fed casein. 3-ketoacyl-CoA thiolase A + B an enzyme involved in peroxisomal ß-oxidation of fatty acids was 1.6 times higher expressed in the liver of rats fed fish protein, CYP4A1, CYP4A3, CYP3A1, CYP4F6, which are involved in microsomal ω-hydroxylation of fatty acids were 1.3-2.2 times higher expressed in rats fed fish protein compared to rats fed casein.

Cholesterol 7-alpha-hydroxylase and sterol 27-hydroxylase which are involved in bile acid metabolism were 1.5 and 1.3 times higher expressed in rats fed fish protein. Acetyl-CoA carboxylase, a key enzyme of fatty acid synthesis was about twice expressed in rats fed fish protein compared to rats fed casein. The cDNA array expression of genes involved in HDL metabolism like Apo AI, and Lecithin: cholesterol acyl transferase were not different in between rats fed fish protein and casein, while SR-BI, otherwise known as HDL receptor was 1.5 times more expressed in rats fed fish protein compared to rats fed casein.

Table 3.12: Relative cDNA array expressions of proteins involved in lipid metabolism in the liver of rats fed diets containing fish protein compared with rats fed diet containing casein (Experiment 3)

GenBank Accession no.	Proteins/Genes	Ratio Fish/Casein group	Funcrions
L07736	Carnitine palmitoyl transferase I	2.0 ± 1.2	Mitochondrial ß oxidation
J05470	Carnitine palmitoyl transferase II	1.1 ± 0.16	Mitochondrial ß oxidation
U64451	Short chain acyl-CoA dehydrogenase	1.3 ± 0.5	Mitochondrial ß oxidation
J02791	Medium chain acyl-CoA dehydrogenase	1.5 ± 0.9	Mitochondrial ß oxidation
J05029	Long chain-specific acyl-CoA dehydrogenase	1.5 ± 0.9	Mitochondrial ß oxidation
J02752	Acyl-CoA oxidase	1.0 ± 0.8	Peroxisomal ß oxidation
M32801	3-ketoacyl-CoA thiolase A + B	1.6 ± 1.1	Peroxisomal ß oxidation
P00502	Glutathione S-transferase alpha	0.8 ± 0.3	Biotransformation
P08011	Glutathione S-transferase	0.8 ± 0.4	Biotransformation
K02422	Cytochrome P450 IA2	0.9 ± 0.2	Biotransformation
J02657	Cytochrome P450 2C11	0.9 ± 0.4	Biotransformation
M58041	Cytochrome P450 2C22	1.6 ± 1.3	Biotransformation
U39943	Cytochrome P450 2J3	1.2 ± 0.4	Biotransformation

X07259	Cytochrome P450 4A1	2.1 ± 1.7	Microsomal ω-hydroxylation
M33936	Cytochrome P450 4A3	2.2 ± 1.4	Microsomal ω-hydroxylation
M10161	Cytochrome P450 3A1	2.2 ± 1.6	Microsomal ω-hydroxylation
U39208	Cytochrome P450 4F6	1.3 ± 0.4	Microsomal ω-hydroxylation
X17595	Cholesterol 7-alpha-hydroxylase	1.5 ± 1.0	Bile acid metabolism
M38566	Sterol 27 Hydroxylase	1.3 ± 0.5	Bile acid metabolism
P19100	Steroid 17-alpha-hydroxylase	2.1 ± 0.5	Steroid metabolism
M55315	Acetyl-CoA carboxylase	1.9 ± 1.7	Fatty acid synthesis
M00001	Apolipoprotein A-I precursor	1.0 ± 1.1	HDL structural protein
M00002	Apolipoprotein A-IV precursor	1.4 ± 0.6	Chylomicrones structural protein
U17697	Scavenger receptor class B type I	1.5 ± 0.7	Plasma HDL uptake
P35952	Low density lipprotein (LDL) receptor	1.2 ± 0.6	LDL uptake
U62803	Lecithin: cholesterol acyl transferase	1.0 ± 0.3	Extracellular cholesterol esterification
U12791	3-hydroxy-3-methylglutaryl-CoA synthase	1.1 ± 0.1	Ketogenesis

Values are mean values ± SD. n = 4, HDL, High density lipoprotein; For each probe RNA of 3 rats within same dietary treatment group was pooled. Rats were killed without prior food deprivation. From 1176 gene/proteins spotted on the membrane about 590 gene/protein expressions were detectable. Only 154 were significantly different (at least 1.4 fold up or down regulated).

Relative mRNA Concentrations Casein versus Fish protein in experiment 3:

Relative mRNA concentrations in the liver of rats fed diets containing casein or soy protein used in experiment 3 are shown in Figure 3.9. The relative mRNA concentrations of PPARα regulated genes ACO and CYP4A1 were found significantly higher in rats fed fish protein compared to rats fed casein. The relative mRNA concentrations of glutathione-S-transferase α, and PPARα were not different between rats fed casein and fish protein. The cDNA array results and the RT-PCR performed to prove the array results suggest that fish

protein up regulated PPAR alpha activity because lots of genes up regulated by fish protein are PPAR alpha target genes.

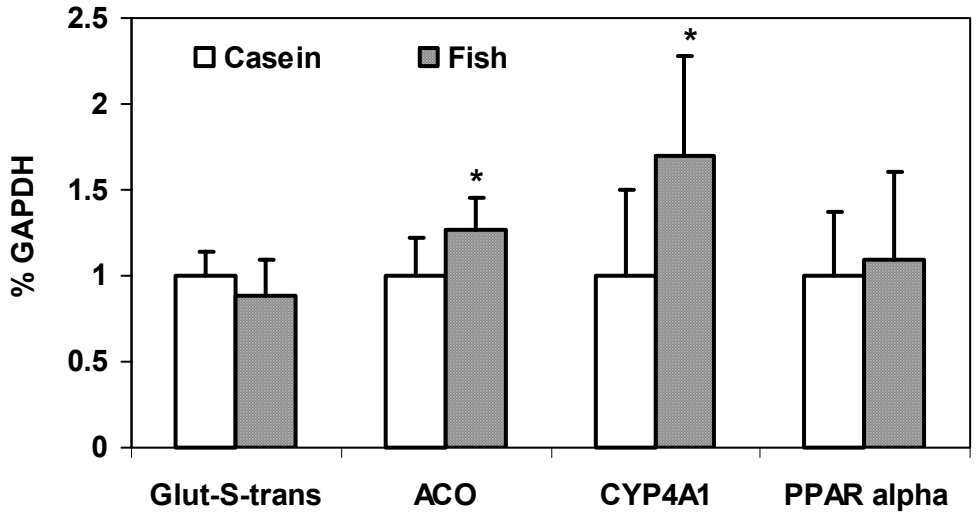

Figure 3.9: Relative mRNA concentrations of PPAR α down stream gene in the liver of rats fed diets containing casein or fish protein in the postprandial state (Experiment 3)

Values are the mean values ± SD. * denote significant difference between the two groups. $P<0.05$ (t-test). n = 12. Rats were killed without prior food deprivation. Glut-S-trans, Glutathione S transferase; ACO, Acyl CoA oxidase; CYP4A1, Cytochrome 450 4A1; PPAR, Peroxisome proliferator activated receptor.