

**Humanized diet and thermoneutral housing enable sex-aware dissection of the
MASLD-T2D-CVD triad**
Supplementary information

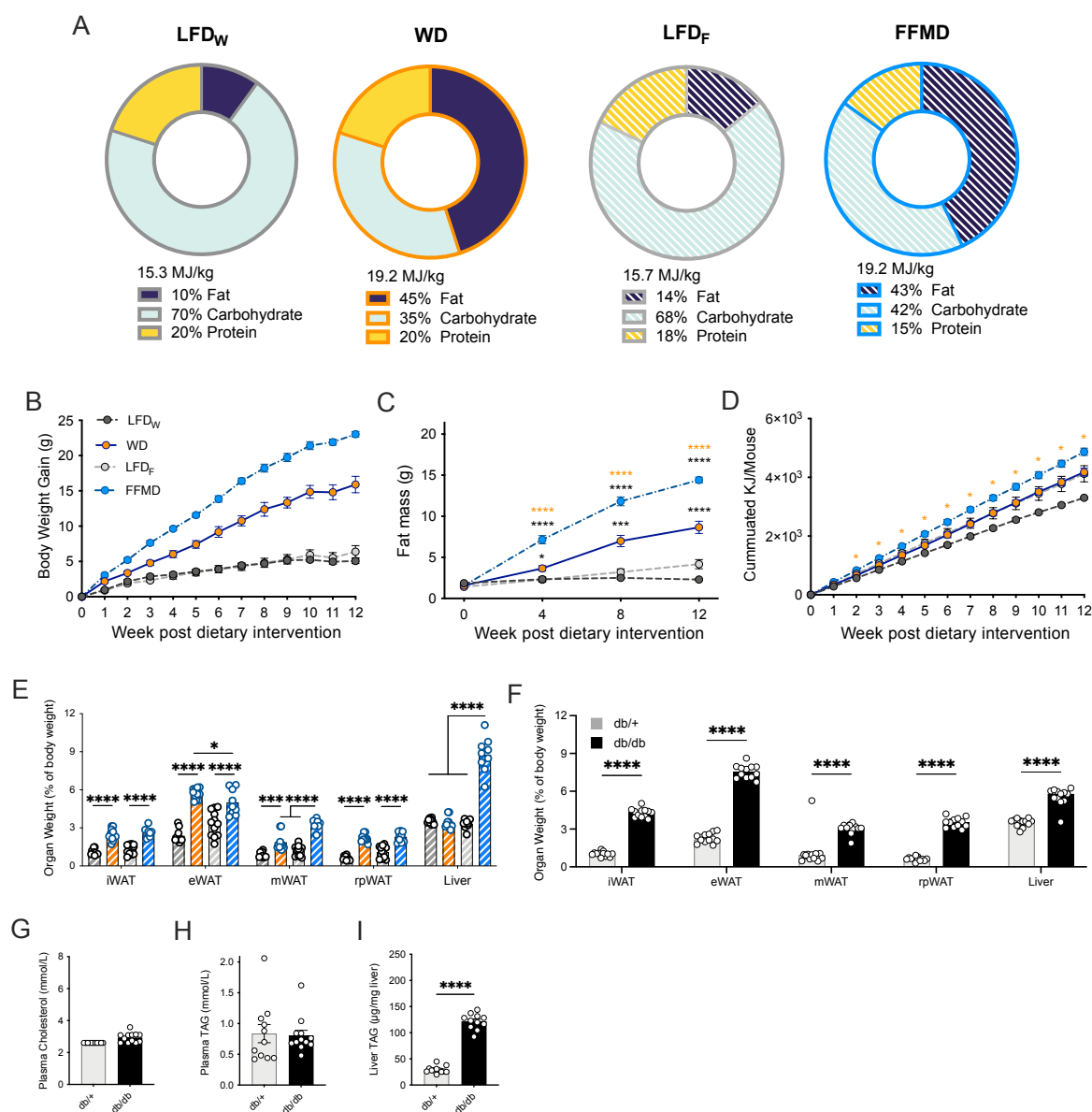


Figure S1. (A) Macronutrient distribution of experimental rodent diets. (B) Weight development of WT mice. (C) Fat mass development over time as assessed by magnetic resonance imaging (MRI). Black stars indicate significance from respective LFD group, coloured stars indicate significance from experimental group of that color. (D) Cumulative energy intake/mouse over the entire study. Relative organ weights of WT mice (E) and db/db mice (F). db/db plasma levels of cholesterol (G) and TAG (H), with liver TAG depicted in I. N = 8-12 per group, with individual data points representing each mouse. All data is presented as mean \pm SEM. When comparing more than two groups, data were analysed with a one-way ANOVA with Tukey's multiple comparison test, and when comparing two groups a two-tailed unpaired t-test was performed. Statistical significance was considered at $p < 0.05$, where * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$.

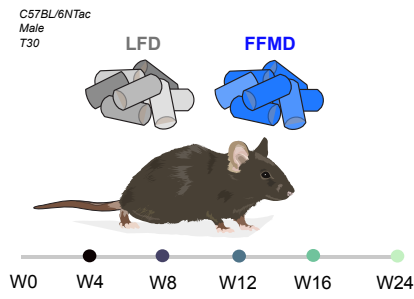
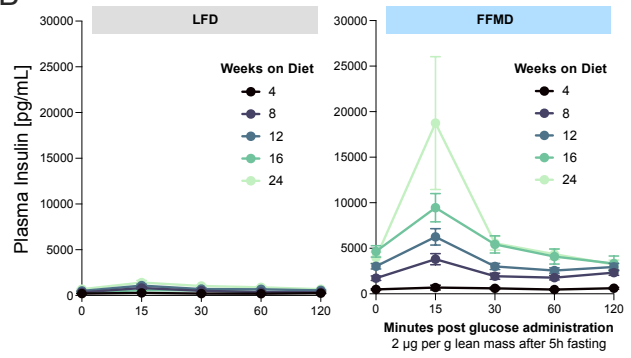
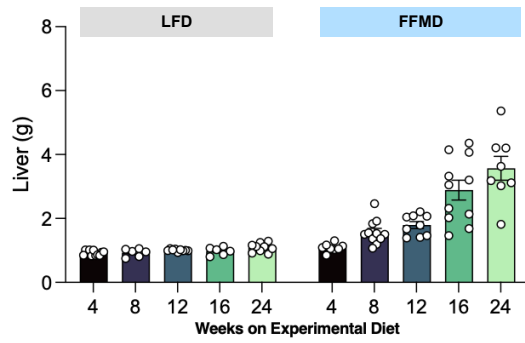
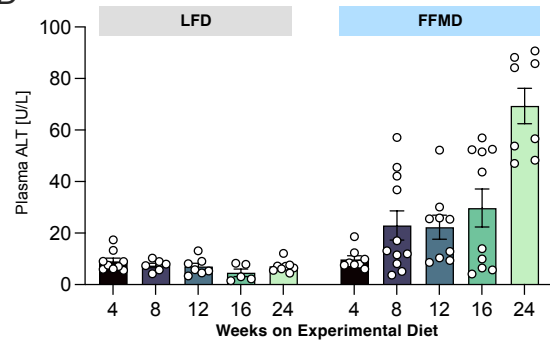
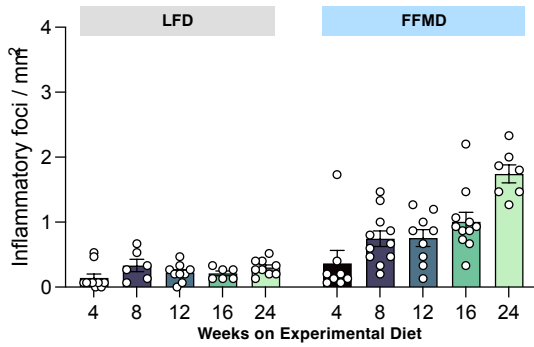
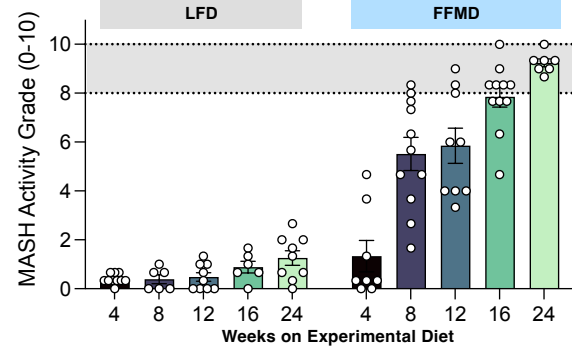
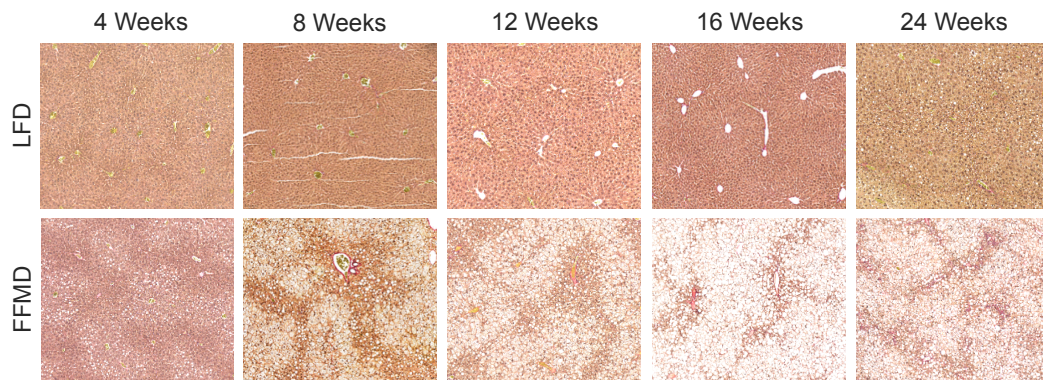
A**B****C****D****E****F****G**

Figure S2. (A) Schematic of study design. (B) Plasma insulin levels after an oGTT following 4-24 weeks of FFMD-feeding. (C) Liver wet weights over time. (D) Plasma ALT levels over time. (E) Hepatic inflammatory foci per HPF over time. (F) MASH scoring over time. The grey shaded area of 8-10 indicates frank MASH. (G) Representative histology pictures. N = 6-12 per group, with individual data points representing each mouse. All data is presented as mean \pm SEM. Data were analysed with a one-way ANOVA with Tukey's multiple comparison test. Statistical significance was considered at $p < 0.05$, where * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$.

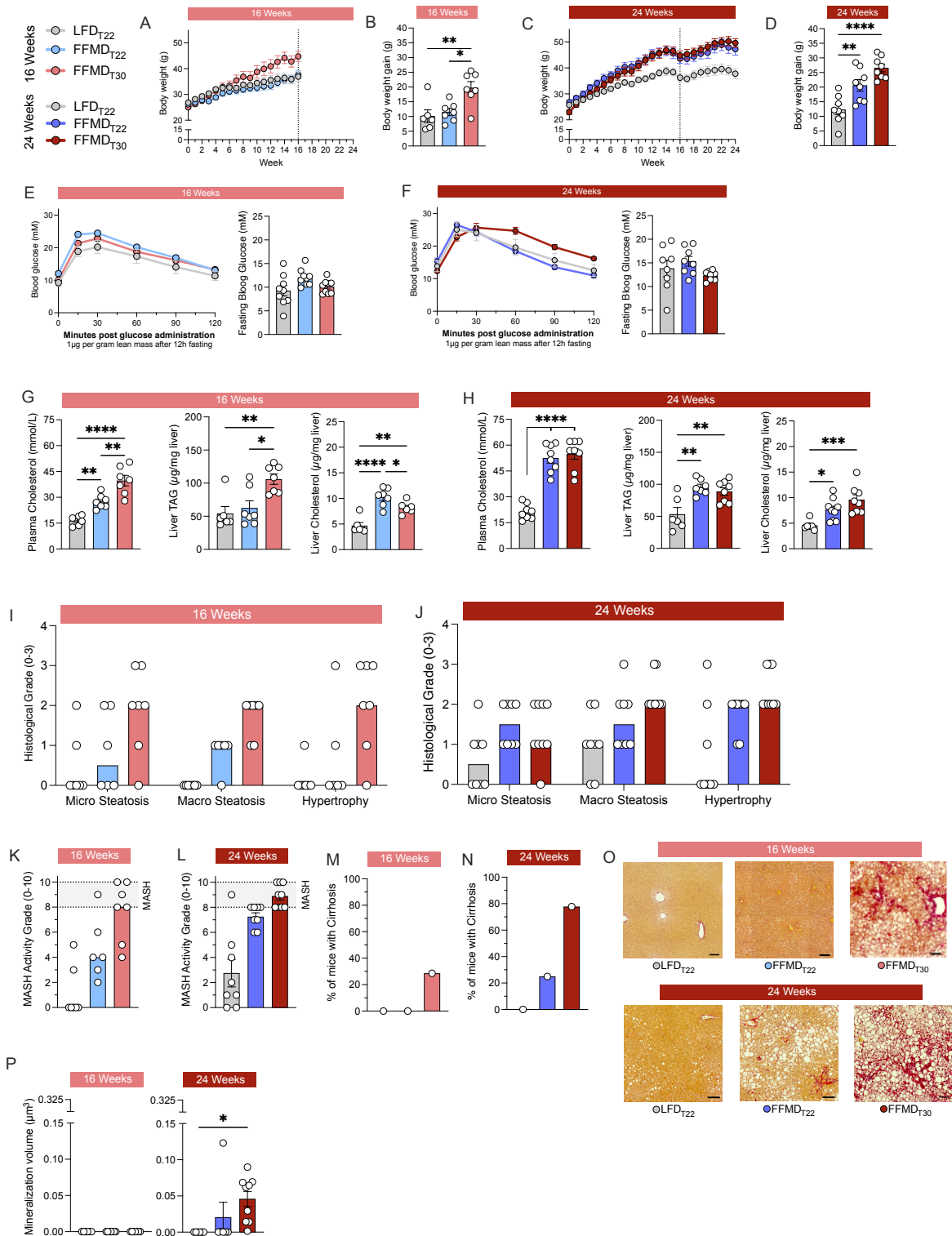


Figure S3. Body weight development (A) of male IGFI mice with total weight gain (B) after 16 weeks of feeding, with corresponding measurements after 24 weeks of feeding in C and D. Blood glucose levels during an oGTT at 16 weeks (E) and 24 weeks (F). Plasma cholesterol levels at 16 weeks (G) and 24 weeks (H). Histological grading of steatosis and hypertrophy shown as the median after 16 week (I) and 24 weeks (J) of feeding. MASH activity grading

after 16 weeks (K) and 24 weeks (L) - the grey shaded area of 8-10 indicates frank MASH. Fraction of mice with frank cirrhosis after 16 weeks (M) and 24 weeks (N). (O) Representative histology pictures. Scale bars = 100 μm . (P) Aortic mineralization volumes in μm^3 . N = 8-9 per group, with individual data points representing each mouse. All data is presented as mean \pm SEM unless otherwise indicated. Data were analysed with a one-way ANOVA with Tukey's multiple comparison test. Statistical significance was considered at $p < 0.05$, where * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$.

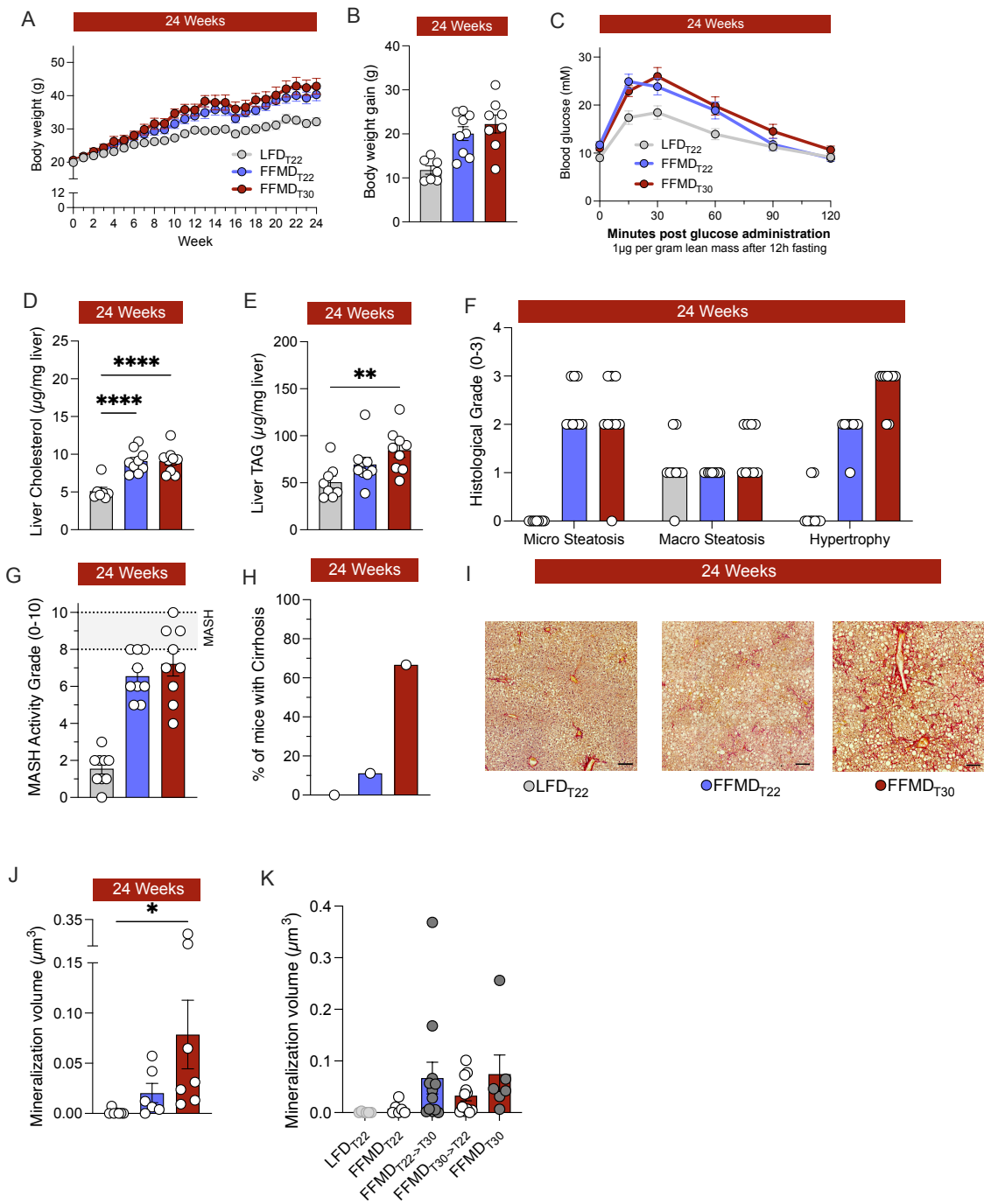


Figure S4. Body weight development (A) of female IGFII mice with total weight gain (B) after 24 weeks of feeding. Blood glucose levels during an oGTT at 24 weeks (C). Liver cholesterol (D) and TAG (E) levels at 24 weeks. (F) Histological grading of steatosis and hypertrophy shown as the median. (G) MASH activity grading 24 weeks - the grey shaded area of 8-10 indicates frank MASH. (H) Fraction of mice with frank cirrhosis. (I) Representative histology pictures. Scale bars = 100 µm. Aortic mineralization volumes in µm³ for the 24 week females (J) and the crossover cohort (K). N = 8-9 per group and 17-18 in temperature-swapping groups, with individual data points representing each mouse. All data is presented as mean ± SEM unless otherwise indicated. Data were analysed with a one-way ANOVA with Tukey's

multiple comparison test. Statistical significance was considered at $p < 0.05$, where * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$.

Table S1. Diet compositions

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	Protein	Fat		Low Fat Diet Control	Fast-Food Mimicking Diet	Western Diet
Ingredient	Content	Content	U	Mixed Protein	Mixed Protein	45 kcal% Fat
Product No.				S9552-E026	S9552-E024	S9552-E060
Casein	87	<0.6	%	3,000	3,000	
Poultry protein	71	10	%	8,500	8,500	25,000
Soyprotein isolate	88	0,4	%	4,900	4,900	
Wheat gluten, Vital	76,5	1,7	%	2,300	2,300	
Egg yolk	38	56	%	1,600	1,600	
Egg white	79	0,1	%	1,600	1,600	
Whey powder, 71% Lactose	11,9	1	%	2,9	2,900	
L-Cystine	72,2		%	0,300	0,300	0,300
Corn starch			%	45,260	5,200	7,000
Maltodextrin			%	8,000	8,000	11,000
Sucrose 1)			%	7,000	24,400	20,050
Fructose 1)			%		6,500	
Cellulose powder			%	5,000	5,000	5,700
Vitamin premixture 2)			%	1,000	1,000	1,000
Mineral premixture + additions 2)			%	4,300	4,300	6,000
Choline a (50%)			%	0,290	0,290	0,200
L-Carnitine			%	0,050	0,050	
Cholesterol			%		0,150	0,150
Butylated hydroxytoluene			%	0,010	0,010	
Butter fat, dehydrated			%	2,1	18,8	
Corn oil			%	1,62	1,100	
Linseed oil			%	0,270	0,100	
Pork lard			%			20,800
Soybean oil			%			2,800
Proximate contents						
Crude protein			%	17,2	17,2	22,100
Crude fat			%	5,8	21,8	23,600
Crude fibre			%	5,0	5,1	5,700
Crude ash			%	4,8	4,8	5,400
Starch			%	43,5	5,0	6,700
Dextrin			%	7,9	7,9	10,900
Sugar (total)			%	10,2	34,0	21,100
Energy (Atwater)			MJ/kg	15,7	19,2	19,300
kcal% Protein				18	15	20,000
kcal% Fat				14	43	45,000
kcal% Carbohydrates				68	42	35,000
Lysine			%	0,95	0,95	1,83
Methionine			%	0,43	0,43	0,78
Methionine & Cystine			%	0,94	0,94	1,18
Threonine			%	0,62	0,62	0,97
Tryptophan			%	0,19	0,19	0,29
Arginine			%	0,91	0,91	0,86
Histidine			%	0,37	0,37	0,67
Valine			%	0,86	0,86	1,54
Isoleucine			%	0,71	0,71	1,25
Leucine			%	1,23	1,23	2,19
Phenylalanine			%	0,74	0,74	1,14
Phe + Tyr			%	1,29	1,29	2,31
Glycine			%	0,96	0,96	0,47
Glutamic acid			%	3,00	3,00	4,97
Aspartic acid			%	1,36	1,36	1,64
Proline			%	1,20	1,20	2,53
Serine			%	0,82	0,82	1,32
Alanine			%	0,84	0,84	0,66
Fatty adds, % in the diet						
c 4:0				0,08	0,72	
c 6:0				0,05	0,47	
c 8:0				0,03	0,26	
c 10:0				0,06	0,56	0,03
c 12:0				0,07	0,64	0,05
c 14:0				0,24	2,00	0,29
c 16:0				1,15	5,50	5,34
c 18:0				0,36	1,97	2,92
c 20:0				0,02	0,04	0,07
c 16:1				0,13	0,43	0,62
c 18:1				1,68	5,16	9,42
c 18:2				1,21	1,21	3,45
c 18:3				0,17	0,17	0,37
Cholesterol			mg/kg	~440	~2340	~1580
Calcium			%	0,82	0,82	0,92
Phosphorus			%	0,58	0,57	0,64
Sodium			%	0,39	0,38	0,20
Magnesium			%	0,1	0,1	0,23
Potassium			%	0,8	0,8	0,97
Iron			mg/kg	72	72	168
Manganese			mg/kg	22	22	95
Zinc			mg/kg	42	42	65
Copper			mg/kg	13	13	13
Iodine			mg/kg	0,31	0,31	1,16
Selenium			mg/kg	0,20	0,20	0,18
Vitamin A			IU/kg	15.900	20.500	15.000
Vitamin D3			IU/kg	1.550	1.550	1.500
Vitamin E			mg/kg	156	159	150
Vitamin C			mg/kg	30	30	30
Vitamin K			mg/kg	20	20	20
Vitamin B1			mg/kg	26	26	25
Vitamin B2			mg/kg	18	18	16
Vitamin B6			mg/kg	17	17	16
Vitamin B12			µg/kg	32	32	30
Niacin			mg/kg	53	53	47
Pantothenic acid			mg/kg	59	59	55
Folic acid			mg/kg	16	16	16
Biotin			µg/kg	355	355	300
Choline			mg/kg	1.380	1.380	920

Table S2 – Echocardiography measurements for the cross over cohort

Table S2 - Echocardiography measurements

Measurement	Group	Timepoint	Mean	SEM
LVIDd	LFD22	Week 16	3.51611	0.07618
		Week 24	3.49806	0.08546
	FFMD 22	Week 16	3.49469	0.07916
		Week 24	3.73300	0.07665
	FFMD 22->30	Week 16	3.55239	0.07953
		Week 24	3.88222	0.08431
	FFMD 30->22	Week 16	3.55553	0.06048
		Week 24	3.76517	0.09172
	FFMD 30	Week 16	3.33806	0.11211
		Week 24	3.77576	0.10272
LV Mass	LFD22	Week 16	216.52600	3.10956
		Week 24	208.88769	2.57885
	FFMD 22	Week 16	221.82781	4.33744
		Week 24	217.92535	3.17026
	FFMD 22->30	Week 16	227.30017	5.44917
		Week 24	224.21967	4.03695
	FFMD 30->22	Week 16	214.07053	3.72951
		Week 24	225.00594	5.80649
	FFMD 30	Week 16	208.79188	4.52927
		Week 24	216.19353	4.43880
Rel. Wall Thickness	LFD22	Week 16	0.43583	0.02262
		Week 24	0.39169	0.01703
	FFMD 22	Week 16	0.45669	0.02136
		Week 24	0.36618	0.01287
	FFMD 22->30	Week 16	0.45389	0.02174
		Week 24	0.34756	0.01725
	FFMD 30->22	Week 16	0.38582	0.01717
		Week 24	0.38339	0.01599
	FFMD 30	Week 16	0.44700	0.03356
		Week 24	0.33565	0.01740
IVRT	LFD22	Week 16	19.56811	0.86396
		Week 24	22.27363	1.21101
	FFMD 22	Week 16	22.10106	1.73723
		Week 24	24.25381	1.08151
	FFMD 22->30	Week 16	21.65094	1.64829
		Week 24	23.73483	1.10349
	FFMD 30->22	Week 16	20.29469	1.55616
		Week 24	21.86772	0.79514
	FFMD 30	Week 16	20.94818	1.44525
		Week 24	23.28435	1.07163
VTI	LFD22	Week 16	19.79422	1.49440
		Week 24	22.30544	1.07186
	FFMD 22	Week 16	16.52706	1.25873
		Week 24	22.54282	1.02688
	FFMD 22->30	Week 16	16.88733	1.03026
		Week 24	23.92061	1.14198
	FFMD 30->22	Week 16	18.15135	1.42952
		Week 24	22.08017	0.94621
	FFMD 30	Week 16	18.40671	1.43910
		Week 24	21.06800	0.61014
VTI Peak Velocity	LFD22	Week 16	533.22378	28.02430
		Week 24	520.05969	23.13816
	FFMD 22	Week 16	462.80250	36.04892
		Week 24	516.83806	20.35915
	FFMD 22->30	Week 16	455.28072	23.67384
		Week 24	519.62800	21.33008
	FFMD 30->22	Week 16	504.68318	28.16347
		Week 24	538.95339	25.18737
	FFMD 30	Week 16	496.84053	31.98179
		Week 24	473.46276	14.95940
AET	LFD22	Week 16	44.53678	1.90371
		Week 24	51.63188	1.48961
	FFMD 22	Week 16	44.13856	1.59748
		Week 24	52.87588	1.31289
	FFMD 22->30	Week 16	45.04633	1.51698
		Week 24	55.47833	1.25816
	FFMD 30->22	Week 16	44.82018	1.58755
		Week 24	51.01867	1.05836
	FFMD 30	Week 16	45.03241	1.78460
		Week 24	54.31376	1.38299
SV	LFD22	Week 16	0.01950	0.00143
		Week 24	0.02194	0.00104
	FFMD 22	Week 16	0.01606	0.00111
		Week 24	0.02306	0.00145
	FFMD 22->30	Week 16	0.01789	0.00139
		Week 24	0.02383	0.00136
	FFMD 30->22	Week 16	0.01831	0.00132
		Week 24	0.02183	0.00136
	FFMD 30	Week 16	0.01806	0.00128
		Week 24	0.02047	0.00100