

Gain and loss of subcutaneous and abdominal fat depot mass from late pregnancy to 100 days in milk in German Holsteins

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Short title: **Adipose tissue depots in dairy cows**

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Supplementary File

S1 Feeding

S1.1 Feeding regime

The feeding regime is described in detail by Tienken et al. (2015). In order to simulate different levels of energy disposability throughout transition period and early lactation, animals were divided into two basic feeding groups differing in the energy density supplied. From the time of dry off, 42 days prior to the expected calving date, the low concentrate group (LC; n = 22), received a ration containing a percentage of 30 % concentrate and 70 % roughage. This ration was fed until calving. After calving concentrate percentage was increased in this group up to 50 % within the time span until 16 DIM. The high concentrate group (HC, n = 25) started with 60 % concentrate and 40 % roughage at 42 days prior to expected calving. After calving, the ration in this group was suddenly changed to 30 % concentrate and 70 % roughage. The percentage of concentrate then was also increased up to 50 % but within a prolonged time span until 24 DIM. Feeding level of 50 % concentrate and 50 % roughage in both groups was maintained until the end of the trial at 100 DIM. Roughage included 50 % corn silage and 50 % grass silage on dry matter basis and was fed ad libitum via self - feeding stations (type RIC, Insentec B.V., Marknesse, The Netherlands). The LC and HC group were again subdivided into two groups. One of each as a control group (LCC, n = 12, HCC, n = 13) and one of each with a supplementation of 24 g powdered, not rumen

protected nicotinic acid (NA, Mianyang Vanetta Pharmaceutical Technology Co., Ltd, Sichuan, 119 China) included in 1 kg pelleted concentrate per day starting at the beginning of the trial until 24 DIM (LCN, n = 10, HCN, n = 12). Each of the four feeding groups contained heifers and cows (LCC: n = 5 heifers, 7 cows; LCN: n = 4 heifers, 6 cows; HCC: n = 4 heifers, 9 cows; HCN: n = 5 heifers, 7 cows).

S2 Data processing and statistical analysis

S2.1 Calculations of average daily gain, average daily loss and relative average daily gain and relative average daily loss of estimated depot mass during dry period, fresh cow period and early lactation period

average daily gain (adG) dry period = (eDM 3 DIM - eDM 42 days prior to expected calving date)/ actual days of dry period + 3 days

average daily loss (adL) fresh cow period = (eDM 21 DIM - eDM 3 DIM)/ 18

adL early lactation period = (eDM 100 DIM - eDM 21 DIM)/ 79

relative (rel) adG dry period = adG dry period/ eDM 42 days prior to expected calving * 100

rel adL fresh cow period = adL fresh cow period/ eDM 3 DIM * 100

rel adL early lactation period = adL early lactation period/ eDM 21 DIM * 100

* eDM = estimated depot mass

S2.2 Modelling of changes over time and effects of energy density, niacin supplementation and parity on the estimated mass of a single adipose tissue depot

The PROC MIXED of SAS 9.3 was used for the creation of a model with the factors time, energy density, niacin supplementation and parity as fixed factors and cow as a random factor. The model was run for the eDM of each depot separately.

S3 Results

Supplementary Table S1: Effects of feeding diets with adequate (LC) or high (HC) energy contents either with supplementation of 0 g (CON) or 24 g nicotinic acid (NA), time (-42 to 100 DIM) and parity (primiparous cows n = 18; multiparous cows n = 29) on estimated adipose depot mass (kg) in German Holsteins (main effects) (LSmeans ± SE)

Item ¹	Concentrate level (C)		Supplement (S)		Parity (P) ²		Time (T)				P-Value			
	LC	HC	CON	NA	PP	MP	-42	3	21	100	C	S	P	T
SCAT	15.2 ± 0.6	15.6 ± 0.5	15.3 ± 0.5	15.5 ± 0.6	16.0 ± 0.6	14.7 ± 0.5	16.1 ± 0.6 ^a	19.6 ± 0.6 ^b	14.7 ± 0.6 ^a	11.2 ± 0.6 ^c	0.656	0.836	0.107	<0.001
AAT	38.6 ± 1.5	40.2 ± 1.4	39.3 ± 1.4	39.5 ± 1.5	39.5 ± 1.5	39.3 ± 1.3	41.3 ± 1.6 ^a	51.8 ± 1.6 ^b	36.2 ± 1.6 ^a	28.2 ± 1.6 ^c	0.431	0.935	0.918	<0.001
RPAT	11.5 ± 0.5	11.5 ± 0.5	11.4 ± 0.5	11.5 ± 0.5	11.7 ± 0.5	11.3 ± 0.4	12.2 ± 0.5 ^a	14.8 ± 0.5 ^b	10.9 ± 0.5 ^a	8.1 ± 0.5 ^c	0.956	0.832	0.562	<0.001
OMAT	12.5 ± 0.4	13.1 ± 0.4	12.6 ± 0.4	12.9 ± 0.4	12.9 ± 0.4	12.7 ± 0.3	12.2 ± 0.4 ^a	15.9 ± 0.4 ^b	13.2 ± 0.4 ^a	9.9 ± 0.4 ^c	0.245	0.683	0.666	<0.001
MAT	10.9 ± 0.5	11.9 ± 0.5	11.5 ± 0.5	11.2 ± 0.5	11.2 ± 0.5	11.5 ± 0.5	11.7 ± 0.5 ^a	15.0 ± 0.5 ^b	10.2 ± 0.5 ^a	8.5 ± 0.5 ^c	0.175	0.759	0.727	<0.001

^{a-c} Means within a row with different superscripts differ (P < 0.05)

¹ SCAT = subcutaneous adipose tissue; AAT = abdominal adipose tissue; RPAT = retroperitoneal adipose tissue; OMAT = omental adipose tissue; MAT = mesenterial adipose tissue

² PP = primiparous cows; MP = multiparous cows

Supplementary Table S2a: Effects of feeding diets with adequate (LC) or high (HC) energy contents either with supplementation of 0 g (CON) or 24 g nicotinic acid (NA) and parity (primiparous cows n = 18; multiparous cows n = 29) on estimated adipose depot mass (kg) in German Holsteins (interactions) (LSmeans ± SE).

Item ¹	Concentrate level (C) x Supplement (S)				Concentrate level (C) x Parity (P) ²				P-Value	
	LC		HC		LC		HC		C x S	C x P
	CON	NA	CON	NA	PP	MP	PP	MP		
SCAT	15.3 ± 0.8	15.2 ± 0.8	15.4 ± 0.8	15.8 ± 0.8	16.4 ± 0.9	14.0 ± 0.7	15.7 ± 0.9	15.4 ± 0.6	0.753	0.208
AAT	39.4 ± 2.0	37.7 ± 2.2	39.2 ± 2.0	41.2 ± 2.0	39.3 ± 2.2	37.9 ± 2.0	39.7 ± 2.2	40.7 ± 1.7	0.379	0.570
RPAT	11.5 ± 0.7	11.5 ± 0.7	11.3 ± 0.7	11.6 ± 0.7	12.0 ± 0.8	11.4 ± 0.6	11.4 ± 0.8	11.5 ± 0.6	0.887	0.490
OMAT	12.4 ± 0.5	12.6 ± 0.6	13.0 ± 0.5	13.3 ± 0.5	13.0 ± 0.6	12.0 ± 0.5	13.0 ± 0.6	13.3 ± 0.5	0.889	0.249
MAT	11.3 ± 0.7	10.5 ± 0.8	11.7 ± 0.7	12.0 ± 0.7	10.9 ± 0.8	10.8 ± 0.7	11.6 ± 0.8	12.1 ± 0.6	0.445	0.651

¹SCAT = subcutaneous adipose tissue; AAT = abdominal adipose tissue; RPAT = retroperitoneal adipose tissue; OMAT = omental adipose tissue; MAT = mesenterial adipose tissue

²PP = primiparous cows; MP = multiparous cows

Supplementary Table S2b: Effects of feeding diets with adequate (LC) or high (HC) energy contents and time (-42 to 100 DIM) on estimated adipose depot mass (kg) in German Holsteins (interactions) (LSmeans \pm SE).

Item ¹	Concentrate level (C) x Time (T)								P-Value
	LC				HC				
	-42	3	21	100	-42	3	21	100	
SCAT	16.0 \pm 0.8	19.6 \pm 0.8	14.6 \pm 0.8	10.7 \pm 0.9	16.2 \pm 0.8	19.5 \pm 0.8	14.8 \pm 0.8	11.7 \pm 0.8	0.908
AAT	41.3 \pm 2.3	52.8 \pm 2.3	34.8 \pm 2.3	25.4 \pm 2.3	41.4 \pm 2.2	50.9 \pm 2.2	37.6 \pm 2.2	30.9 \pm 2.2	0.218
RPAT	11.9 \pm 0.7	15.3 \pm 0.7	11.2 \pm 0.7	7.6 \pm 0.7	12.4 \pm 0.7	14.4 \pm 0.7	10.5 \pm 0.7	8.5 \pm 0.7	0.314
OMAT	12.2 \pm 0.6	15.6 \pm 0.6	13.0 \pm 0.6	8.9 \pm 0.6	12.2 \pm 0.6	16.0 \pm 0.6	13.3 \pm 0.6	11.0 \pm 0.6	0.260
MAT	11.8 \pm 0.7	15.0 \pm 0.7	9.6 \pm 0.7	7.0 \pm 0.8	11.7 \pm 0.7	15.0 \pm 0.7	10.7 \pm 0.7	10.0 \pm 0.7	0.053

¹SCAT = subcutaneous adipose tissue; AAT = abdominal adipose tissue; RPAT = retroperitoneal adipose tissue; OMAT = omental adipose tissue; MAT = mesenterial adipose tissue

Supplementary Table S2c: Effects of feeding diets either with supplementation of 0 g (CON) or 24 g nicotinic acid (NA), time (-42 to 100 DIM) and parity (primiparous cows n = 18; multiparous cows n = 29) on estimated adipose depot mass (kg) in German Holsteins (interactions) (LSmeans ± SE).

Item ¹	Supplement (S) x Parity (P) ²				Supplement (S) x Time (T)								P-Value	
	CON		NA		CON				NA				S x P	S x T
	PP	MP	PP	MP	-42	3	21	100	-42	3	21	100		
SCAT	16.2 ± 0.9	14.4 ± 0.6	15.9 ± 0.6	15.0 ± 0.7	16.0 ± 0.8	19.0 ± 0.8	14.8 ± 0.8	11.4 ± 0.8	16.2 ± 0.8	20.0 ± 0.8	14.7 ± 0.8	11.0 ± 0.8	0.531	0.616
AAT	41.4 ± 1.9	37.1 ± 1.8	37.5 ± 2.3	41.4 ± 1.9	40.9 ± 2.2	51.6 ± 2.2	35.6 ± 2.2	29.0 ± 2.3	41.8 ± 2.2	52.1 ± 2.2	36.7 ± 2.2	27.3 ± 2.3	0.051	0.907
RPAT	12.0 ± 0.8	10.8 ± 0.6	11.3 ± 0.8	11.7 ± 0.6	12.0 ± 0.7	14.6 ± 0.7	10.6 ± 0.7	8.3 ± 0.7	12.3 ± 0.7	14.9 ± 0.7	11.1 ± 0.7	7.8 ± 0.7	0.223	0.955
OMAT	12.7 ± 0.6	12.6 ± 0.5	13.0 ± 0.6	12.7 ± 0.5	12.1 ± 0.6	15.2 ± 0.6	13.2 ± 0.6	10.2 ± 0.6	12.3 ± 0.6	16.6 ± 0.6	13.2 ± 0.6	9.6 ± 0.6	0.848	0.118
MAT	11.8 ± 0.8	11.7 ± 0.6	10.7 ± 0.8	11.8 ± 0.7	11.6 ± 0.7	14.9 ± 0.7	10.3 ± 0.7	9.1 ± 0.7	11.8 ± 0.7	15.1 ± 0.7	10.1 ± 0.7	7.9 ± 0.8	0.208	0.682

¹SCAT = subcutaneous adipose tissue; AAT = abdominal adipose tissue; RPAT = retroperitoneal adipose tissue; OMAT = omental adipose tissue; MAT = mesenterial adipose tissue

²PP = primiparous cows; MP = multiparous cow

Supplementary Table S3: Pearsons correlation (r) of average daily gain (kg/d) of estimated adipose depot mass during dry period (42 days prior to expected calving to calving; DP) and average daily loss (kg/day) of estimated adipose depot mass during fresh cow period (3-21 DIM; FCP) and early lactation period (22-100 DIM; ELP) in German Holsteins (primiparous cows n = 18; multiparous cows n = 29).

Item ¹	statistics	DCP vs. FCP	DCP vs. ELP	FCP vs. ELP
	r	-0.58	-0.06	-0.01
SCAT	P	<0.0001	0.68	0.93
	r	-0.49	-0.29	0.09
AAT	P	0.0007	0.063	0.57
	r	-0.52	-0.12	-0.19
RPAT	P	0.0003	0.4418	0.22
	r	-0.54	-0.34	0.00
OMAT	P	<0.0001	0.0241	0.98
	r	-0.56	-0.09	-0.25
MAT	P	<0.0001	0.5466	0.1114

¹ SCAT = subcutaneous adipose tissue; AAT = abdominal adipose tissue; RPAT = retroperitoneal adipose tissue; OMAT = omental adipose tissue; MAT = mesenterial adipose tissue

References

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