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Abomasal infusion of essential fatty acids and conjugated linoleic acid during late pregnancy and early lactation affects immunohematological and oxidative stress markers in dairy cows

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SUPPLEMENTARY FILES

Supplemental Table S1. Amounts of daily abomasally infused supplements[1].

Supplementation	Treatment						
	CTRL[2]	EFA		CLA	EFA+CLA		
	Coconut oil[3]	Linseed oil[4]	Safflower oil[5]	Lutalin®[6]	Linseed oil	Safflower oil	Lutalin®
Daily infused oils (g/d)							
Dosage lactation	76	78	4	38	78	4	38
Dosage dry period	38	39	2	19	39	2	19
Daily infused fatty acids (g/d) at the lactation dosage[7]							
18:3 cis-9, cis-12, cis-15	0.00	39.90	0.01	0.00	39.90	0.01	0.00
18:2 cis-9, cis-12	1.39	12.40	2.48	1.34	12.40	2.48	1.34
18:2 cis-9, trans-11	0.00	0.00	0.01	10.30	0.00	0.01	10.30
18:2 trans-10, cis-12	0.00	0.02	0.01	10.20	0.02	0.01	10.20

[1]Cows were supplemented daily with coconut oil (CTRL), or mixture of linseed, safflower oil (EFA), and Lutalin® (CLA, c9, t11 and t10, c12), (EFA+CLA).

[2]Addition of vitamin E (0.06 g/d), Covitol 1360 (BASF, Ludwigshafen, Germany), to compensate for the vitamin E in linseed oil (0.07%) and safflower oil (0.035%).

[3]Sanct Bernhard, Bad Ditzenbach, Germany

[4]DERBY, Derby Spezialfutter GmbH, Münster, Germany

[5]GEFRO, Memmingen/Allgäu, Germany

[6]BASF, Ludwigshafen, Germany

[7]The lactation dosage was halved during the dry period.

Supplemental Table S2. Ingredients and chemical compositions of the diets.

Item (g/kg of DM)	Diet	
	Dry period[1]	Lactation
Ingredients	421	457
Corn silage Straw	223	97
Compound feed DEFA[2] (granulated)	-	446
Dried sugar beet pulp	163	-
Extracted soybean meal	99	-
Grain of rye	75	-
Mineral-vitamin mixture[3]	10	-
Urea[4]	9	-
Chemical composition		
NEL (MJ/kg DM)[5]	6.2	7.1
Crude fat	21	23
Crude fiber	219	173
Crude protein	141	146
Utilizable protein[5]	141	143
NFC	379	432
NDF	423	346
ADF	249	197
RNB[5,6]	0.0	0.5

[1]The dry period diet was fed from wk 6 to wk 1 before calving.

[2]Ceravis AG, Malchin, Germany Ingredients: 46.5% dried sugar beet pulp, 25.3% extracted soybean meal, 23.8% grain of rye, 1.4% urea, 1.1% premix cow, 1.00% calcium, 0.37% phosphorus, 0.42% sodium, vitamins A, D3, E, copper, ferric, zinc, manganese, cobalt, iodine, selenium Chemical composition: 44.4% NFC, 24.1% crude protein, 21.6% NDF, 12.4% ADF, 9.3% crude fiber, 8.2% crude ash, 1.8% crude fat, 7.9 MJ NEL/kg DM

[3]KULMIN®MFV Plus (Bergophor Futtermittelfabrik Dr. Berger GmbH & Co. KG, Kulmbach, Germany): 8.5% magnesium, 7.5% phosphorus, 6.5% sodium, 3.5% HClinsoluble ash, 1.5% calcium, additives: vitamins A, D3, E, B1, B2, B6, B5, B3, B12, B9, H, zinc, manganese, copper, cobalt, iodine, selenium, and Saccharomyces cerevisiae

[4]Piarumin® (SKW Stickstoffwerke Piesteritz GmbH, Lutherstadt Wittenberg, Germany): 99% urea, 46.5% total nitrogen

[5]German Society of Nutrition Physiology (2001, 2008, 2009) and Deutsche Landwirtschafts-Gesellschaft (DLG, 2013)

[6]RNB = ruminal nitrogen balance

Supplemental Table S3. Performance data during late lactation, dry and transition periods, postpartum or early lactation, and over the entire study of cows supplemented abomasally daily with coconut oil (CTRL; n=9), linseed and safflower oil (EFA; n=9), Lutalin[1] (CLA; n=10), or the combination (EFA+CLA; n=10) from wk 9 antepartum until wk 8 postpartum

		Treatment				Fixed effect, Pvalue					
		CTRL	EFA	CLA	EFA+CLA	EFA	CLA	EFA+ CLA	Time	EFA *time	CLA *time
NEL intake, MJ NEL/d	Late lactation	120.2 ± 4.6	116.7 ± 4.2	114.2 ± 3.9	113.8 ± 3.9	0.7	0.3	0.7	0.12	0.6	0.05
	Dry period	80.6 ± 3.1	81.9 ± 1.9	81.4 ± 2.8	84.1 ± 2.8	0.5	0.6	0.8	0.001	0.7	0.5
	Transition period	93.9 ± 3.3	93.6 ± 3.1	91.2 ± 2.9	93.4 ± 2.9	0.8	0.6	0.7	0.001	0.9	0.6
	Postpartum	120.8 ± 3.8	119.4 ± 3.7	109.8 ± 3.5	115 ± 3.5	0.6	0.04	0.3	0.001	0.5	0.4
	Entire Study	106.6 ± 3.3	106.4 ± 3.1	101.2 ± 3.0	104 ± 2.9	0.6	0.2	0.6	0.001	0.9	0.05
FEMY, kg milk/kg DMI	Late lactation	0.96 ± 0.11	1.10 ± 0.10	1.11 ± 0.09	0.98 ± 0.09	0.9	0.9	0.19	0.001	0.06	0.01
	Early lactation	2.25 ± 0.1	2.25 ± 0.10	2.37 ± 0.09	2.43 ± 0.09	0.7	0.13	0.7	0.001	0.5	0.9
FEECM, kg ECM/kg DMI	Late lactation	1.08 ± 0.1	1.19 ± 0.09	1.05 ± 0.08	0.95 ± 0.09	0.9	0.13	0.2	0.001	0.04	0.001
	Early lactation	2.31 ± 0.11	2.28 ± 0.10a	1.84 ± 0.10b	1.95 ± 0.1	0.7	0.001	0.5	0.001	1.0	0.6
BW, kg	Late lactation	701 ± 21	666 ± 20	676 ± 19	670 ± 19	0.3	0.6	0.5	0.001	0.9	0.12
	Dry period	742 ± 22	7000 ± 21	710 ± 20	718 ± 20	0.4	0.7	0.2	0.001	0.3	0.4
	Transition period	690 ± 20	654 ± 19	664 ± 18	672 ± 18	0.5	0.8	0.3	0.001	0.7	0.4
	Postpartum	634 ± 18	604 ± 18	622 ± 17	621 ± 17	0.4	0.9	0.4	0.001	0.8	0.16
	Entire Study	685 ± 20	649 ± 19	663 ± 18	665 ± 18	0.4	0.9	0.3	0.001	0.8	0.04

		Treatment				Fixed effect, Pvalue					
		CTRL	EFA	CLA	EFA+CLA	EFA	CLA	EFA+ CLA	Time	EFA *time	CLA *time
BCS	late lactation	3.62 ± 0.11	3.5 ± 0.11	3.48 ± 0.10	3.29 ± 0.1	0.16	0.10	0.7	0.001	0.4	0.9
	Dry period	3.72 ± 0.12	3.73 ± 0.12	3.62 ± 0.10	3.62 ± 0.11	1.0	0.4	0.9	0.001	0.1	0.02
	Transition period	3.54 ± 0.12	3.55 ± 0.11	3.50 ± 0.11	3.5 ± 0.11	1.0	0.7	1.0	0.001	0.7	0.8
	Postpartum	3.12 ± 0.11	3.13 ± 0.11	3.15 ± 0.10	3.1 ± 0.1	0.8	1.0	0.8	0.001	0.7	0.19
	Entire Study	3.43 ± 0.11	3.41 ± 0.10	3.38 ± 0.10	3.31 ± 0.1	0.7	0.5	0.8	0.001	0.11	0.02
BFT, mm	late lactation	13.4 ± 1	12.2 ± 0.9	12.0 ± 0.9	11.3 ± 0.9	0.3	0.2	0.8	0.001	0.18	0.6
	Dry period	15.3 ± 1.1	14.3 ± 1.0	15.8 ± 1.0	14.6 ± 1	0.3	0.7	0.9	0.001	0.5	0.4
	Transition period	14.7 ± 1.1	14.2 ± 1.0	15.5 ± 1.0	14.5 ± 1	0.5	0.6	0.8	0.001	0.9	0.5
	Postpartum	12.1 ± 1	11.7 ± 0.9	13.5 ± 0.9	12.6 ± 0.9	0.5	0.2	0.8	0.001	0.9	0.001
	Entire Study	13.5 ± 1	12.7 ± 0.9	14.0 ± 0.9	13.0 ± 0.9	0.3	0.7	0.9	0.001	0.8	0.001

[a,b]Means within a row with different lowercase superscripts differ ($P<0.05$).

[1]Values are presented as the LSM ± SE.

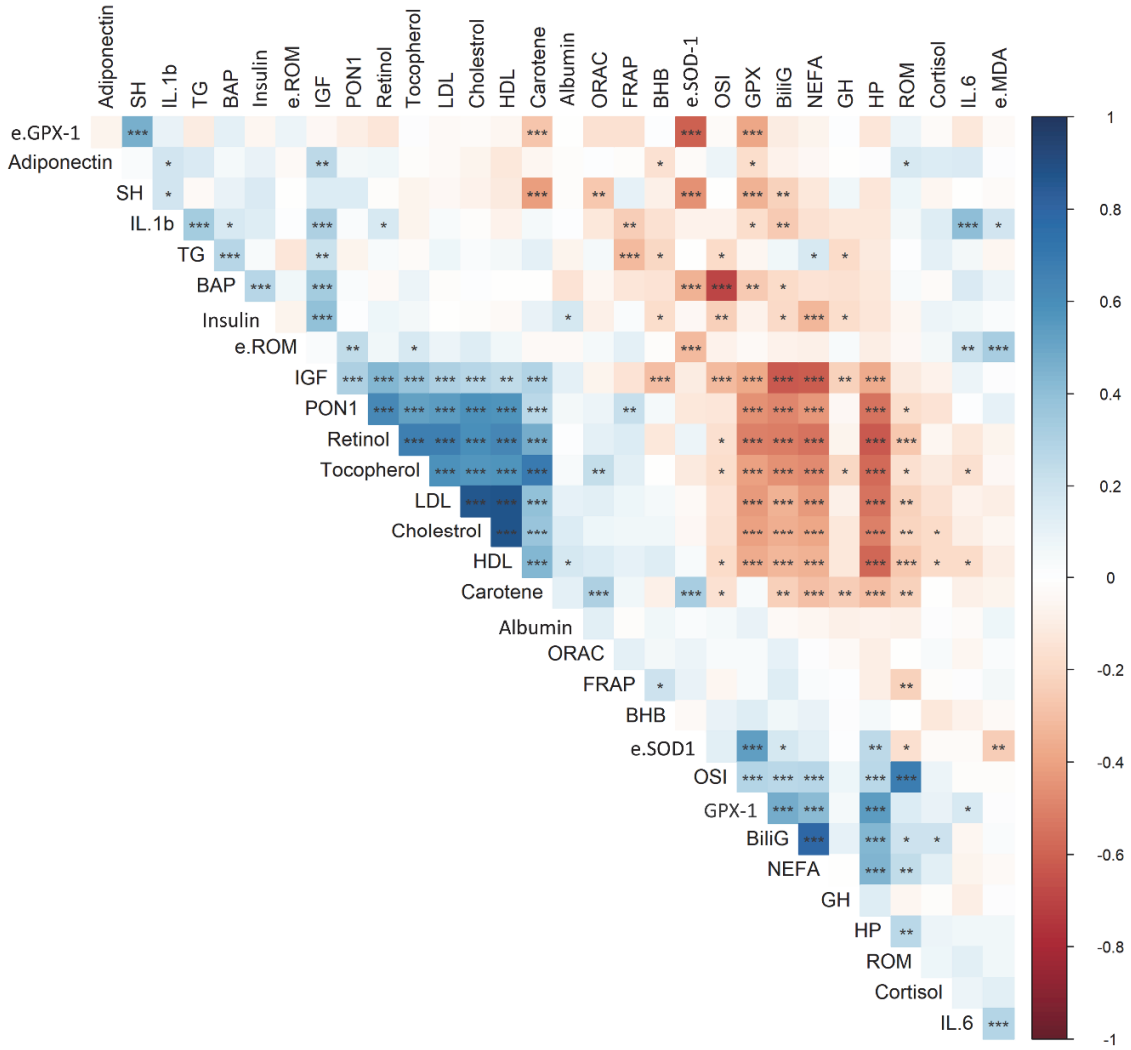
[2]FEMY = feed efficiency for milk production; FEECM = feed efficiency for ECM production; BFT = back fat thickness.

Supplemental Table S4. Characteristics of primers and real-time PCR conditions.

Primer	Sequence (5′-3′)	NIH Genebank accession number	bp	Mean		
				Cq[1]	Annealing, s/°C	Efficiency
SOD-1[2]						
	F: AAGGCCGTGTGCGTGCTGAA	NM_174615.2	240	20.54	10/60	1.85
	R: CAGGTCTCCAACATGCCTCT					
GPX-1[3]						
	F: CTTCCCCTGCAACCAGTTTG	NM_174076.3	62	22.20	10/60	1.86
	R: GGCAATTCAGGATCTCCTCGTT					
CAT[4]						
	F: TCACTCAGGTGCGGACTTTC	NM_001035386.2	162	17.35	10/60	1.87
	R: GGATGCGGGAGCCATATTCA					

[1]Quantification cycle
[2]*SOD-1* = superoxide dismutase 1
[3]*GPX-1* = glutathione peroxidase 1
[4]CAT = catalase

Supplemental Figure S1.



Supplemental Figure S1. Pearson correlation of plasma metabolites (adopted from Vogel et al., 2020) with plasma and erythrocytes (e.-) oxidative and inflammation markers. The color represent the correlation coefficient value and whether the correlation is negative (red) or positive (blue), respectively. Stars correspond to the P-values, *<0.05, **<0.01, and ***<0.001. BiliG = bilirubin, GH = growth hormone, IGF = Insulin-like growth factor-I.