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## Short Communication

# Dietary *n*-3 PUFA affect lipid metabolism and tissue function-related genes in bovine muscle

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**Abstract**

Gene expression profiles of bovine *longissimus* muscle as affected by dietary *n*-3 *v*. *n*-6 fatty acid (FA) intervention were analysed by microarray pre-screening of >3000 muscle biology/meat quality-related genes as well as subsequent quantitative RT-PCR gene expression validation of genes encoding lipogenesis-related transcription factors (CCAAT/enhancer-binding protein β, sterol regulatory element-binding transcription factor 1), key-lipogenic enzymes (acetyl-CoA carboxylase α (ACACA), fatty acid synthase (FASN), stearoyl-CoA desaturase (SCD)), lipid storage-associated proteins (adipose differentiation-related protein (ADFP)) and muscle biology-related proteins (cholinergic receptor, nicotinic, α1, farnesyl diphosphate farnesyl transferase 1, sema domain 3C (SEMA3C)). Down-regulation of ACACA ($P=0\cdot00$), FASN ($P=0\cdot09$) and SCD ($P=0\cdot02$) gene expression upon an *n*-3 FA intervention directly corresponded to reduced SFA, MUFA and total FA concentrations in *longissimus* muscle, whereas changes in ADFP ($P=0\cdot00$) and SEMA3C ($P=0\cdot05$) gene expression indicated improved muscle function via enhanced energy metabolism, vasculogenesis, innervation and mediator synthesis. The present study highlights the significance of dietary *n*-3 FA intervention on muscle development, maintenance and function, which are relevant for meat quality tailoring of bovine tissues and modulating animal production-relevant physiological processes.

**Key words: Gene expression: Microarray/quantitative RT-PCR methodology: Lipogenesis: Muscle function**

Continuously rising requirements of consumers, producers and nutritionists towards the sensory, techno-functional and tropho-functional attributes of animal origin foodstuffs have paved the way for novel meat quality tailoring strategies. Within the broad spectrum of methods to optimise meat quality traits, nutritional intervention of farm animals has been successfully exploited to modulate muscle tissue development, composition and characteristics[1,2].

Dietary intervention of ruminant and non-ruminant farm animals with exogenous fatty acid (FA) sources – such as linseed/linseed oil, rapeseed cake/oil, algae[3] as well as pasture *v*. concentrate[4] or grass-silage- *v*. maize-silage-based feeding regimens[5] – has been demonstrated to improve meat quality traits by affecting intramuscular fat development quantitatively in regard to meat tenderness, juiciness and flavour[6] and qualitatively with respect to FA composition and *n*-6:*n*-3 FA ratio[4,7]. As the underlying mechanisms, genetically[8,9] and physiologically[7] determined shifts in lipogenic gene expression, protein expression and enzyme activities were identified[10–12].

Concerning the dietary intervention of farm animals with *n*-3 FA sources, previous investigations of our research

**Abbreviations:** ACACA, acetyl-CoA carboxylase α; ADFP, adipose differentiation-related protein; CEBPB, CCAAT/enhancer-binding protein β; CG, control group; CHRNA1, cholinergic receptor, nicotinic, α1; FA, fatty acid; FASN, fatty acid synthase; FC, fold change; FDFT1, farnesyl diphosphate farnesyl transferase 1; IG, intervention group; qRT-PCR, quantitative RT-PCR; SCD, stearoyl-CoA desaturase; SEMA3C, sema domain 3C; SREBF1, sterol regulatory element-binding transcription factor 1.

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group obtained beneficially reduced *n*-6:*n*-3 FA ratios in *longissimus* muscle of German Holstein bulls via diminished sterol regulatory element-binding transcription factor 1 (SREBF1), acetyl-CoA carboxylase α (ACACA), fatty acid synthase (FASN) and stearoyl-CoA desaturase (SCD) gene expression, Δ6D and SCD protein expression as well as SCD enzyme activity[5,13,14]. Taking into account that an *n*-3 FA intervention affects in a complex manner the various metabolic pathways in tissues[15], the present study aimed at a characterisation of the impact of *n*-3 *v.* *n*-6 FA-based feeding regimens on the expression levels of several muscle biology and meat quality-related genes. In this context, bovine *longissimus* muscle samples were pre-screened for overall gene expression profiles via microarray analysis (regarding a limited number of ten animals) and subjected to quantitative RT-PCR gene expression validation (regarding the total population of twenty-seven animals), addressing selected genes for which either certain expression differences had been obtained via microarray analysis or distinct dietary *n*-3 FA intervention effects had been hypothesised.

## Methods and materials

### Animals, study design and tissue sampling

German Holstein bulls were assigned to a maize-silage/*n*-6 FA-based control diet (control group (CG); *n* 14) or a grass-silage/*n*-3 FA-based intervention diet (intervention group (IG); *n* 13) during a fattening period of 245 (SD 40) d until a live weight of 623 (SD 26) kg (CG) or 626 (SD 20) kg (IG) at an age of 475 (SD 39) d (CG) or 513 (SD 33) d (IG) was reached.

Experimental diets were isoenergetically formulated; actual energy intake ranged between mean values of 112·5 MJ (CG) and 108·5 MJ (IG) metabolisable energy per d. The control diet consisted of 0·153 g crude protein, 0·03 g crude fat (22·9 % SFA, 26·0 % MUFA, 51·1 % PUFA; 46·3 % *n*-6 FA, 4·8 % *n*-3 FA; *n*-6:*n*-3 FA ratio = 9·6) and 0·07 g crude ash/g DM. The intervention diet consisted of 0·149 g crude protein, 0·04 g crude fat (23·6 % SFA, 17·6 % MUFA, 58·8 % PUFA; 22·3 % *n*-6 FA, 36·5 % *n*-3 FA; *n*-6:*n*-3 FA ratio = 0·6) and 0·12 g crude ash. The FA composition of the control and intervention diets is given in Table 1.

Experimental animals were managed and slaughtered according to the national guidelines for animal welfare. Immediately after slaughter and exsanguination, *longissimus* muscle samples were taken from the right side of the carcass (between the thirteenth and fourteenth rib), snap-frozen in liquid $N_2$ and stored at −80°C until further analysis.

### Gene expression analysis

Gene expression profile pre-screening was performed by microarray gene expression analysis of *longissimus* muscle samples of a randomly selected subpopulation of ten animals (*n* 5 (CG), *n* 5 (IG)). Tissue samples of the total population of twenty-seven animals (*n* 14 (CG), *n* 13 (IG)) were subjected to quantitative RT-PCR gene expression validation, addressing genes (adipose differentiation-related protein (ADFP), CCAAT/enhancer-binding protein β (CEBPB), cholinergic receptor, nicotinic, α1 (CHRNA1), farnesyl diphosphate farnesyl transferase 1 (FDFT1), sema domain 3C (SEMA3C)) for which certain expression differences had been obtained via microarray analysis, or genes (ACACA, FASN, SCD, SREBP) for which distinct dietary *n*-3 FA intervention effects had been hypothesised.

**Table 1.** Fatty acid (FA) composition (% of the total FA) of the control and intervention diets

| | FA* | |
|---|---|---|
| | Control diet | Intervention diet |
| 12:0 | 0·2 | 0·2 |
| 14:0 | 0·5 | 0·4 |
| 14:1 | 0·0 | 0·0 |
| 16:0 | 15·8 | 16·7 |
| 16:1 | 0·4 | 0·3 |
| 18:0 | 2·7 | 2·6 |
| 18:1*cis*-9 | 24·7 | 15·7 |
| 18:2*n*-6 | 45·9 | 21·5 |
| 18:3*n*-3 | 4·5 | 35·5 |
| 20:0 | 0·9 | 0·5 |
| 20:4*n*-6 | 0·1 | 0·0 |
| 22:5*n*-3 | 0·2 | 0·4 |
| SFA† | 22·9 | 23·6 |
| MUFA‡ | 26·0 | 17·6 |
| PUFA§ | 51·1 | 58·8 |
| *n*-3 FA‖ | 4·8 | 36·5 |
| *n*-6 FA¶ | 46·3 | 22·3 |
| *n*-6:*n*-3 | 9·6 | 0·6 |

* Determined by GC-flame ionisation detection analysis of fatty acid methyl esters; see Hiller *et al.*[13]
† SFA = 10:0 + 11:0 + 12:0 + 13:0 + 14:0 + 15:0 + 16:0 + 17:0 + 18:0 + 20:0 + 21:0 + 22:0 + 23:0 + 24:0.
‡ MUFA = 14:1 + 15:1 + 16:1 + 17:1 + 18:1*n*-9 + 18:1*cis*-11 + 18:1 *trans*-9 + 18:1*trans*-10 + 18:1*trans*-11 + 22:1 + 24:1.
§ PUFA = *n*-3 PUFA + *n*-6 PUFA.
‖ *n*-3 PUFA = 18:3*n*-3 + 18:4*n*-3 + 20:3*n*-3 + 20:5*n*-3 + 22:5*n*-3 + 22:6*n*-3.
¶ *n*-6 PUFA = 18:2*n*-6 + 18:3*n*-6 + 20:2*n*-6 + 20:3*n*-6 + 20:4*n*-6 + 22:2*n*-6 + 22:4*n*-6.

### Gene expression analysis via microarray experiment

Expression of >3000 genes involved in muscle biology or meat quality of beef cattle was assessed with a custom-designed Agilent GENOTEND chip with several specific probes for each gene. The total number of probes was 10 257 of which 1614 were control probes for gene expression normalisation[16].

Total RNA was extracted from muscle samples and analysed for quantity and integrity (Agilent 2100 Bioanalyser; Agilent Technologies, Massy, France). RNA was amplified, labelled with Cy3 and purified[16]. Labelled complementary RNA was fragmented and hybridised to an Agilent 8 × 15K custom Oligo Microarray. After washing, microarrays were scanned and data extracted with Feature Extraction 9.1 Software (Agilent Technologies)[16]. Data of each array were normalised via the median of all 1614 control probes. Subsequently, data per probe were normalised via the median probe values of replicate arrays and log 2-transformed. Expression differences between the control and intervention groups were analysed by two-way ANOVA with fixed-factor diet and repeated-factor probes per gene, using proc MIXED of SAS (SAS Institute Inc., Cary, NC, USA)[17].

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### *Gene expression analysis via quantitative RT-PCR*

RNA was extracted from the muscle samples and analysed for RNA quantity, purity and integrity[13]. RNA was reverse-transcribed with the iScript™ complementary DNA Synthesis Kit (Bio-Rad Laboratories GmbH, Munich, Germany)[13]. Quantitative RT-PCR (qRT-PCR) analysis was performed by subjecting reaction mixes of 5 μl iQ SYBR® Green Supermix (Bio-Rad Laboratories GmbH), 4 μl forward/reverse primer solution (0·2 μmol/l) and 1 μl complementary DNA template (10 ng/μl) to a thermocycling program of 10 s at 94°C, 30 s at 60°C and 45 s at 70°C (forty-five cycles)[13]. Gene-specific oligonucleotides (Table 2) were designed with Primer3 (version 0.4.0; whitehead Institute for Biomedical Research, Cambridge, MA, USA). PCR analysis was performed in triplicate. Amplification specificity was confirmed by melt curve analysis, agarose gel electrophoresis and sequencing of PCR products[13].

Relative gene expression was calculated with the comparative, efficiency-corrected $\Delta\Delta C_t$ method, using splicing factor 3a, subunit 1, eukaryotic translation elongation factor 1, α2, ribosomal protein S18 and β2-microglobulin for gene expression normalisation. Differences in gene expression profiles between the control and intervention groups were tested for significance using REST© algorithm (REST© 2009, version 2.0.13; TUM, Munich, Germany)[18].

## Results

Expression of muscle biology and meat quality-related genes as affected by *n*-3 *v.* *n*-6 FA intervention was assessed via microarray gene expression pre-screening (regarding a limited number of ten animals) and qRT-PCR gene expression validation (regarding the total population of twenty-seven animals). Of an initial set of >3000 pre-screened genes, Tables 3 and 4 summarise the gene expression results derived for selected genes encoding lipogenesis-related transcription factors (CEBPB, SREBF1), key-lipogenic enzymes (ACACA, FASN, SCD), lipid storage-associated proteins (ADFP) and muscle biology-related proteins (CHRNA1, FDFT1, SEMA3C).

Microarray pre-screening of a limited number of ten animals indicated down-regulation of ACACA (fold change (FC) = 0·80), ADFP (FC = 0·69), FASN (FC = 0·82) and SCD (FC = 0·88) gene expression upon dietary *n*-3 FA intervention (Table 3), which was confirmed to be significant in the case of ACACA ($P$=0·00; FC = 0·78), ADFP ($P$=0·00; FC = 0·80) and SCD gene ($P$=0·02; FC = 0·83) by qRT-PCR analysis of the total population of twenty-seven animals (Table 4). Up-regulated CEBPB (FC = 1·41), CHRNA1 (FC = 1·75), FDFT1 (FC = 1·57) and SEMA3C (FC = 1·54) gene expression in the intervention than control group muscle samples such as that obtained by microarray pre-screening (Table 3) was

**Table 2.** Specifications of gene-specific oligonucleotides

| Gene and accession no. | Primer sequence (5′–3′) | Location on template | Exon/Exon | Amplicon length (bp) | Efficiency of primer pair (%)* |
|---|---|---|---|---|---|
| ACACA (NM_174224.2) | Fwd: GAGCTGAACCAGCACTCCCGA | 374–394 | – | 215 | 108·4 ($R^2$ 0·990) |
| | Rev: TGCAAGCCAGACATGCTGGATCTCA | 588–564 | 576/586 | | |
| ADFP (BT029909.1) | Fwd: GTCTGTCCTGGCTGGAGTGGAAGAG | 911–935 | – | 150 | 98·4 ($R^2$ 0·970) |
| | Rev: TGTTGGACAGGAGGGTGTGGCA | 1060–1039 | – | | |
| CEBPB (NM_176788.1) | Fwd: AGCTGGGTAGCATCGGAGAGCA | 185–206 | – | 202 | 110·3 ($R^2$ 0·992) |
| | Rev: TTGCCCCCGTAGTCGTCGGAG | 386–366 | – | | |
| CHRNA1 (NM_176664.2) | Fwd: AGGAGTCCAACAACGCGGCTG | 1256–1276 | 1269/1270 | 170 | 102·8 ($R^2$ 0·996) |
| | Rev: TGGAGGTTCAGCCCAGCTTTCCA | 1425–1403 | – | | |
| FASN (NM_001012669.1) | Fwd: GCCAGCGGGAAGCGTGTGAT | 4918–4937 | – | 235 | 107·8 ($R^2$ 0·998) |
| | Rev: CGATGGCAGCCTGGCCTACG | 5152–5133 | – | | |
| FDFT1 (NM_001013004.1) | Fwd: TGGATGGGGAAATGCGCCATGC | 282–303 | 297/298 | 164 | 104·5 ($R^2$ 0·984) |
| | Rev: CTTGCTCTCCGTGAACCGCCA | 445–425 | | | |
| SCD (NM_173959.4) | Fwd: CTACAAAGCTCGGCTGCCTCTGC | 525–547 | – | 202 | 100·5 ($R^2$ 0·990) |
| | Rev: TTTGACAGCTGGGTGTTTGCGC | 726–705 | – | | |
| SEMA3C (NM_001101082.1) | Fwd: TGCTCGCAGTGAGCTCATTCTGG | 1617–1639 | – | 150 | 109·2 ($R^2$ 0·991) |
| | Rev: GCTCCTCCGCTTTCCGGTTGG | 1866–1846 | 1859/1860 | | |
| SREBF1 (NM_001113302) | Fwd: TGGGCACCGAGGCCAAGTTGAAT | 1098–1120 | 1114/1115 | 170 | 105·10 ($R^2$ 0·996) |
| | Rev: TCCACTGCCACAAGCCGACA | 1267–1248 | – | | |
| RPS18 (NM_001033614.1) | Fwd: ACCAACATCGATGGGCGGCG | 96–115 | – | 150 | 101·1 ($R^2$ 1·000) |
| | Rev: CACACGTTCCACCTCATCCTCGG | 245–223 | 233/234 | | |
| B2M (NM_173893.3) | Fwd: TGGGTTCCATCCACCCCAGATTGA | 181–204 | – | 237 | 108·9 ($R^2$ 0·998) |
| | Rev: TGTTCAAATCTCGATGGTGCTGCT | 417–394 | 408/409 | | |
| SF3A1 (NM_001081510.1) | Fwd: GCCCCCAACTCCAGACCAGGT | 1248–1268 | – | 249 | 105·3 ($R^2$ 0·990) |
| | Rev: TCGATATCCAGACCTGGCGCGT | 1496–1475 | 1483/1484 | | |
| EEF1A2 (NM_001037464.1) | Fwd: GCTCTGGACTCACTGCTCAGCTTCC | 21–45 | 27/28 | 229 | 102·4 ($R^2$ 0·988) |
| | Rev: TCTCGGCCGCCTCCTTCTCAAA | 249–228 | – | | |

ACACA, acetyl-CoA carboxylase α; Fwd, forward; Rev, reverse; ADFP, adipose differentiation-related protein; CEBPB, CCAAT/enhancer-binding protein β; CHRNA1, cholinergic receptor, nicotinic, α1; FASN, fatty acid synthase; FDFT1, farnesyl diphosphate farnesyl transferase 1; SCD, stearoyl-CoA desaturase; SEMA3C, sema domain 3C; SREBF1, sterol regulatory element-binding transcription factor 1; RPS18, ribosomal protein S18; B2M, β2-microglobulin; SF3A1, splicing factor 3a, subunit 1; EEF1A2, eukaryotic translation elongation factor 1, α2

* Determined by plotting the $C_t$ values of 10·0, 1·0, 0·5, 0·1 and 0·01 ng complementary DNA *v.* the logarithm of the corresponding complementary DNA amount.

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**Table 3.** Microarray gene expression analysis ($n$ 10) results
(Fold changes and 95 % confidence intervals)

| | Gene expression data | | |
|---|---|---|---|
| | FC | 95 % CI | *P* |
| ACACA | 0·804 | 0·499, 1·291 | 0·319 |
| ADFP | 0·687 | 0·510, 0·927 | 0·020 |
| CEBPB | 1·408 | 0·989, 2·004 | 0·056 |
| CHRNA1 | 1·749 | 1·144, 2·676 | 0·016 |
| FASN | 0·822 | 0·318, 2·122 | 0·646 |
| FDFT1 | 1·569 | 1·009, 2·441 | 0·046 |
| SCD | 0·877 | 0·367, 2·097 | 0·738 |
| SEMA3C | 1·539 | 1·052, 2·252 | 0·031 |
| SREBF1 | 1·088 | 0·802, 1·476 | 0·543 |

FC, fold change; ACACA, acetyl-CoA carboxylase α; ADFP, adipose differentiation-related protein; CEBPB, CCAAT/enhancer-binding protein β; CHRNA1, cholinergic receptor, nicotinic, α1; FASN, fatty acid synthase; FDFT1, farnesyl diphosphate farnesyl transferase 1; SCD, stearoyl-CoA desaturase; SEMA3C, sema domain 3C; SREBF1, sterol regulatory element-binding transcription factor 1.

confirmed to be significant in the case of SEMA3C gene ($P$=0·05; FC = 1·10) by qRT-PCR analysis (Table 4). SREBF1 gene expression was not found to be affected by dietary intervention, via either microarray or qRT-PCR gene expression analysis (Tables 3 and 4).

## Discussion

Dietary FA intervention of farm animals has been successfully exploited to improve carcass quality attributes via diet-induced shifts in meat quality-related transcriptome, proteome and metabolome[10–12,19]. Preliminary investigations revealed that a dietary $n$-3 FA intervention of German Holstein bulls beneficially affected $n$-6:$n$-3 FA ratios and reduced SFA concentrations in *longissimus* muscle via diminished SREBF1, ACACA, FASN and SCD gene expression, Δ6D and SCD protein expression as well as SCD enzyme activity[5,13,14].

In addition to beneficial improvements of the FA composition of beef (see Hiller *et al.*[13]; Table 5), the present study indicated that an $n$-3 FA supplementation affects in a complex manner the expression of various muscle biology and meat quality-related genes in bovine *longissimus* muscle. Of an initial set of more than 3000 individual genes analysed by microarray gene expression pre-screening, three major classes of genes were confirmed to be modulated by dietary $n$-3 FA intervention via qRT-PCR gene expression validation: genes encoding (a) lipogenesis-related enzymes, (b) intracellular lipid storage-associated proteins and (c) cell function and signalling-associated proteins.

**Table 4.** Quantitative RT-PCR gene expression validation ($n$ 27) results
(Fold changes and 95 % confidence intervals)

| | Gene expression data | | |
|---|---|---|---|
| | FC | 95 % CI | *P* |
| ACACA | 0·780 | 0·452, 1·184 | 0·002 |
| ADFP | 0·804 | 0·557, 1·140 | 0·000 |
| CEBPB | 1·095 | 0·681, 2·096 | 0·255 |
| CHRNA1 | 0·973 | 0·634, 1·465 | 0·661 |
| FASN | 0·782 | 0·279, 2·281 | 0·094 |
| FDFT1 | 1·123 | 0·630, 1·901 | 0·177 |
| SCD | 0·833 | 0·554, 1·565 | 0·020 |
| SEMA3C | 1·102 | 0·696, 1·283 | 0·050 |
| SREBF1 | 0·949 | 0·350, 2·810 | 0·704 |

FC, fold change; ACACA, acetyl-CoA carboxylase α; ADFP, adipose differentiation-related protein; CEBPB, CCAAT/enhancer-binding protein β; CHRNA1, cholinergic receptor, nicotinic, α1; FASN, fatty acid synthase; FDFT1, farnesyl diphosphate farnesyl transferase 1; SCD, stearoyl-CoA desaturase; SEMA3C, sema domain 3C; SREBF1, sterol regulatory element-binding transcription factor 1.

Concerning (a) lipogenesis-related genes, diminished expression of genes encoding enzymes involved in FA *de novo* synthesis (acetyl-CoA carboxylase α, FA synthase) and monodesaturation (stearoyl-CoA desaturase) was obtained upon $n$-3 FA intervention, which was confirmed to be significant in the case of ACACA and SCD genes by qRT-PCR analysis.

These findings correspond to FA composition analyses of the control and intervention group muscle samples, indicating significantly lower amounts of ACACA and FASN gene products 12 : 0 (1·5 mg (CG) *v.* 1·1 mg (IG)/100 g), 14 : 0 (64·9 mg (CG) *v.* 43·8 mg (IG)/100 g), 16 : 0 (635·7 mg *v.* 460·2 mg (IG)/100 g) and total FA (2397·9 mg (CG) *v.* 1805·7 mg (IG)/100 g) as well as SCD gene products 16 : 1 (91·9 mg (CG) *v.* 59·4 mg (IG)/100 g) and 18 : 1$n$-9 (907·8 mg (CG) *v.* 629·6 mg (IG)/100 g) in the intervention group muscle samples (see Hiller *et al.*[13]; Table 5).

Although unsaturated FA intervention was already reported to cause tissue-, sex-[20], breed-[20] and developmental stage[22]-dependent decreases in ACACA, FASN and SCD gene expression[23], protein expression[5] and enzyme activities[14], the present results outline that – despite an extensive hydrogenation of exogenous unsaturated FA by ruminal microbiota[7] – $n$-3 FA-based diets exert significantly more restrictive effects on tissue-specific, lipogenesis-related transcriptome associated with SFA, MUFA and total FA synthesis than $n$-6 FA-based diets. The aspect that the gene expression levels of transcription factors CEBPB and SREBF1 controlling ACACA, FASN and SCD gene expression did not significantly differ between the control and intervention groups in the present study implies that either the diet-induced shifts in CEBPB and SREBF1 gene expression preceded the chosen biological endpoints or the CEBPB and SREBF1 expression levels were post-transcriptionally affected by an $n$-3 FA intervention. An involvement of further lipogenic transcription factors (e.g. PPAR) in the mediation of exogenous $n$-3 FA-induced effects may also be considered in this regard.

Concerning (b) genes encoding lipid storage-associated proteins, significantly reduced expression of the ADFP gene was obtained in the intervention than control group tissue samples. Considering adipophilin as an essential lipid droplet coating protein[24,25], it remains unclear whether reduced adipophilin gene expression results from an overall lower total FA amount in the intervention (1805·7 (SD 369·4) mg/100 g) than control (2397·9 (SD 932·0) mg/100 g) group muscle samples (see Hiller *et al.*[13]; Table 5) or whether an $n$-3 FA-based diet induces specific, restrictive transcriptomic control mechanisms towards adipophilin expression mediated via e.g. PPAR and peroxisome proliferator response elements (PPRE)[24]. Taking into account that adipophilin molecules associated with lipid droplet membranes sterically retard an

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**Table 5.** Fatty acids (FA, mg/100 g tissue) in *longissimus* muscle of experimental animals
(Mean values and standard deviations)

| | FA | | | | |
|---|---|---|---|---|---|
| | Control group | | Intervention group | | |
| | Mean | SD | Mean | SD | *P* |
| Fatty acid classes | | | | | |
| SFA† | 1088·1 | 428·2 | 826·7 | 188·5 | 0·05 |
| MUFA‡ | 1103·3 | 474·1 | 769·8 | 176·5 | 0·02 |
| PUFA§ | 188·7 | 22·0 | 193·0 | 22·5 | 0·62 |
| *n*-3 FA‖ | 27·5 | 4·1 | 57·0 | 6·4 | 0·00 |
| *n*-6 FA¶ | 158·5 | 18·3 | 132·1 | 15·4 | 0·00 |
| *n*-6:*n*-3 FA | 5·8 | 0·7 | 2·3 | 0·1 | 0·00 |
| ACACA/FASN gene products | | | | | |
| 12 : 0 | 1·5 | 0·7 | 1·1 | 0·3 | 0·03 |
| 14 : 0 | 64·9 | 30·5 | 43·8 | 12·8 | 0·03 |
| 16 : 0 | 635·7 | 263·6 | 460·2 | 112·1 | 0·04 |
| TFA | 2397·9 | 932·0 | 1805·7 | 369·4 | 0·04 |
| SCD gene products | | | | | |
| 14 : 1 | 16·9 | 9·7 | 11·1 | 3·9 | 0·06 |
| 16 : 1 | 91·9 | 40·5 | 59·4 | 15·8 | 0·01 |
| 18 : 1*n*-9 | 907·8 | 393·2 | 629·6 | 148·0 | 0·02 |
| 18 : 2*cis*-9, *trans*-11 | 6·5 | 3·4 | 5·5 | 1·2 | 0·34 |

ACACA, acetyl-CoA carboxylase α; FASN, FA synthase; TFA, total fatty acids; SCD, stearoyl-CoA desaturase.
* Determined by GC-flame ionisation detection analysis of fatty acid methyl esters; see Hiller *et al.*[13].
† SFA = 10 : 0 + 11 : 0 + 12 : 0 + 13 : 0 + 14 : 0 + 15 : 0 + 16 : 0 + 17 : 0 + 18 : 0 + 20 : 0 + 21 : 0 + 22 : 0 + 23 : 0 + 24 : 0.
‡ MUFA = 14 : 1 + 15 : 1 + 16 : 1 + 17 : 1 + 18 : 1*n*-9 + 18 : 1*cis*-11 + 18 : 1*trans*-9 + 18 : 1*trans*-10 + 18 : 1*trans*-11 + 22 : 1 + 24 : 1.
§ PUFA = *n*-3 FA + *n*-6 FA.
‖ *n*-3 FA = 18 : 3*n*-3 + 18 : 4*n*-3 + 20 : 3*n*-3 + 20 : 5*n*-3 + 22 : 5*n*-3 + 22 : 6*n*-3.
¶ *n*-6 FA = 18 : 2*n*-6 + 18 : 3*n*-6 + 20 : 2*n*-6 + 20 : 3*n*-6 + 20 : 4*n*-6 + 22 : 2*n*-6 + 22 : 4*n*-6.

enzymatic breakdown of intracellularly stored TAG by hormone-sensitive lipase (HSL) and adipose TAG lipase (ATGL)[26], the present findings may indicate that *n*-3 FA-based diets significantly improve muscle function by facilitating more rapid re-mobilisation and re-utilisation of stored lipids for energy homeostasis, organic syntheses and cell signalling processes than *n*-6 FA-based diets.

Regarding (c) genes encoding cell function and signalling-associated proteins, significant up-regulation of the SEMA3C gene was obtained by qRT-PCR analysis. In addition to acknowledged improvements of muscle function via increased cell membrane fluidity upon an *n*-3 FA supplementation[27], the present findings may indicate enhanced muscle function via accelerated vasculogenesis/angiogenesis, innervation, mediator synthesis and immune response[28]. These findings as well as literature data concerning the induction of genes/pathways related to cell signalling (arylalkylamine N-acetyltransferase; AANAT), thermogenesis and oxidative control (uncoupling protein 2; UCP2), energy homeostasis (activator of Hsp90 ATPase-1; AHA1)[29], insulin-mediated glucose utilisation[30,31], metabolic switching[31] and muscle protein synthesis[32] strongly outline the significance of *n*-3 FA supplementation on muscle development, maintenance and function.

Altogether, the present study indicates the superiority of *n*-3 over *n*-6 FA-based diets by beneficially affecting meat quality and muscle biology-related transcriptome, which may be relevant in regard to meat quality tailoring of animal tissues via dietary FA intervention, as also with respect to human primary and secondary nutritional intervention against insulin resistance, hyperglycaemia, type 2 diabetes[30] and muscle protein degradation syndromes[32].

## Conclusions

Of an initial set of >3000 genes pre-screened by microarray methodology, qRT-PCR expression validation of genes encoding lipogenesis-related transcription factors (CEBPB, SREBF1), key-lipogenic enzymes (ACACA, FASN, SCD), lipid storage-associated proteins (ADFP) and muscle biology-related proteins (CHRNA1, FDFT1, SEMA3C) revealed significant down-regulation of ACACA, ADFP and SCD gene as well as up-regulation of SEMA3C gene upon *n*-3 *v.* *n*-6 FA intervention. Reduced levels of ACACA, FASN and SCD gene expression directly corresponded to reduced SFA, MUFA and total FA concentrations in *longissimus* muscle, whereas changes in ADFP and SEMA3C gene expression indicated improved muscle function via enhanced energy metabolism, vasculogenesis, innervation and mediator synthesis.

This study highlights the significance of alimentary *n*-3 FA intervention on muscle development, maintenance and function, which are relevant for tailoring meat quality traits and modulating animal production-relevant physiological processes.

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