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Skeletal muscle lncRNA profile associated with fatty acids in Nellore beef cattle

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This study aimed to identify differentially expressed (DE) long non-coding RNAs (lncRNAs) in muscle tissue of Nellore cattle clustered by their fatty acid profile. *Longissimus thoracis* muscle samples from 48 young bulls were used to quantify fatty acid (FA) (myristic, palmitic, stearic, oleic, linoleic, conjugated linoleic (CLA), α-linolenic and the groups of saturated fatty acids (SFA), monounsaturated (MUFA), polyunsaturated (PUFA), ω3, ω6, PUFA/SFA ratio and ω6/ω3) and to generate RNA-Sequencing data for transcriptomic analyses. The *K*-means analysis was used to classify the 48 animals into three clusters based on their FA patterns. The C1 had significantly ($p \leq 0.05$) higher PUFA, ω3, ω6, linoleic and α-linolenic content. The proportion of SFA, myristic, palmitic and stearic were significantly ($p \leq 0.05$) higher in C3, while C2 presented an intermediate profile. DE analyses were performed on three different comparisons, C1 vs. C2, C1 vs. C3 and C2 vs. C3, and 22, 28 and 22 DE lncRNAs (fold change > | 2 |, *p*-value < 0.01 and false discovery rate (FDR) < 0.05) were found, respectively. For three comparisons, the novel DE transcripts, *lncRNA_15786.3*, *lncRNA_13894.1* and *lincRNA_17393.3* interacted with *CCN1*, *BNIP3*, and *CNOT2* genes, respectively, and appeared to contribute to a PUFA-enriched fatty acid profile. These genes are responsible for regulating the lipogenic genes, lipid metabolism, immune response and lipid synthesis. Meanwhile, the intergenic DE lncRNAs (*lincRNA_18394.1*, *lincRNA_2526.3* and *lincRNA_17681.1*) were associated with the genes *DDX1*, *EIF4E* and *APOL3*, and appeared to contribute to a SFA-enriched fatty acid profile. The gene *DDX1* was enriched by GO terms related to RNA splicing (GO:0008380), while the other genes (e.g., *EIF4E* and *APOL3*) were enriched to GO terms related to lipid transport (GO:0006869), localization (GO:0010876) and to cellular response to lipid (GO:0071396). These findings offer new insights into the biological mechanisms underlying the gene regulation of FA composition in beef and may provide a valuable foundation for further investigations regarding the interactions between lncRNAs and mRNAs, as well as their potential impact on meat quality.

Keywords Genic lncRNAs, lincRNA, Nellore cattle, RNA-Seq

Consumers' perception of meat quality products has evolved, becoming interested in the nutritional value of food, encompassing factors such as flavor, appearance, tenderness, food safety, health, social aspects, and sustainability[1]. Beef is a significant source of protein and is rich in essential polyunsaturated fatty acids (linoleic and linolenic acids), zinc, B vitamins, and iron, making it a unique and nutrient-rich food in the human diet to support healthy life, free from nutritional deficiencies[2,3].

Beef fatty acid profile plays a key role in beef 's quality traits, especially polyunsaturated fatty acids (PUFA), directly affecting sensory characteristics, such as taste, juiciness, and tenderness[4]. On the other hand, consumers are becoming more health-conscious and worried about the cholesterol and fats in food, mainly related to the

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concentration of certain FAs, such as oleic acid, α-linolenic acid, conjugated linoleic acid (CLA), as well as omega-3 (ω3) and omega-6 (ω6), by its impact on human health status and diseases prevention[5–8]. In addition, meat fat has a high concentration of monounsaturated fatty acids (MUFA) with a low melting point, which can help reduce low-density lipoprotein (LDL) cholesterol concentration, refers as "bad" cholesterol, in blood circulation[9]. Although improving the beef FA profile can significantly enhance the quality of meat products, it is essential to ensure that the content of these FAs is adjusted to support long-term human health.

Beef fatty acids are complex traits controlled by many genes and influenced by the environment[10,11]. The amount and type of fatty acids in beef meat depend on different factors, such as breed, nutrition, production system, sex, age, and carcass finishing level[12]. These factors influence the deposition and composition of FA, which limits the knowledge of the genetic mechanisms regulating these traits, hindering the genetic progress in producing healthier beef. Previous studies have reported that Nellore beef is nutritionally healthier than Angus beef due to higher amounts of ω3 and CLA[13–17]. In tropical production systems, Nellore cattle are widely used and exhibit considerable intra-breed variation in intramuscular fatty acid composition, particularly in the *Longissimus thoracis* (LT) muscle. This variability suggests the potential for genetic selection of animals with more favorable human health lipid profiles[18–24].

The deposition of FA in meat is a complex process involving the regulation of adipose metabolism (e.g., number and size of adipocytes) and the balance between lipogenesis and lipolysis[25,26]. This regulation involves multiple gene expressions, signal transduction, and network regulation, and new regulatory factors are constantly being identified, such as long non-coding RNAs (lncRNAs)[26–28]. Different authors have shown that lncRNA plays a crucial role in regulating protein-coding genes at different levels, such as epigenetic, transcriptional, and post-transcriptional regulation[26,29–31]. This regulation impacts the FA profile of meat by controlling the development of adipocytes, lipid metabolism, and fat-type conversion. However, there is a lack of information on lncRNA expression patterns, functions, complex gene networks, and molecular determinants related to different intramuscular FA profile deposition in the meat of cattle[25,27,32–36]. Hence, this study aims to identify differentially expressed lncRNAs from Nellore cattle muscle tissue with different FA profiles which may provide knowledge on the genetic and molecular mechanisms regulating FA profile to establish strategies for improving beef quality and directional selection.

Results

Phenotypic variation between groups

Based on *K*-means analysis, three clusters with distinct FA profiles were identified (Fig. 1) and the descriptive FA content for each cluster are shown in Table 1. Cluster 1 (C1; $n = 14$ young bulls) exhibited the highest content for PUFA/SFA ratio, PUFA, ω6, ω3, linoleic acid and α-linolenic acid. The C3 was significantly higher in levels of saturated fatty acids (SFA), including stearic acid, myristic acid, and palmitic acid ($p < 0.05$; Table 10; Fig. 1). While Cluster 2 (C2; $n = 24$) presented an intermediate profile FA.

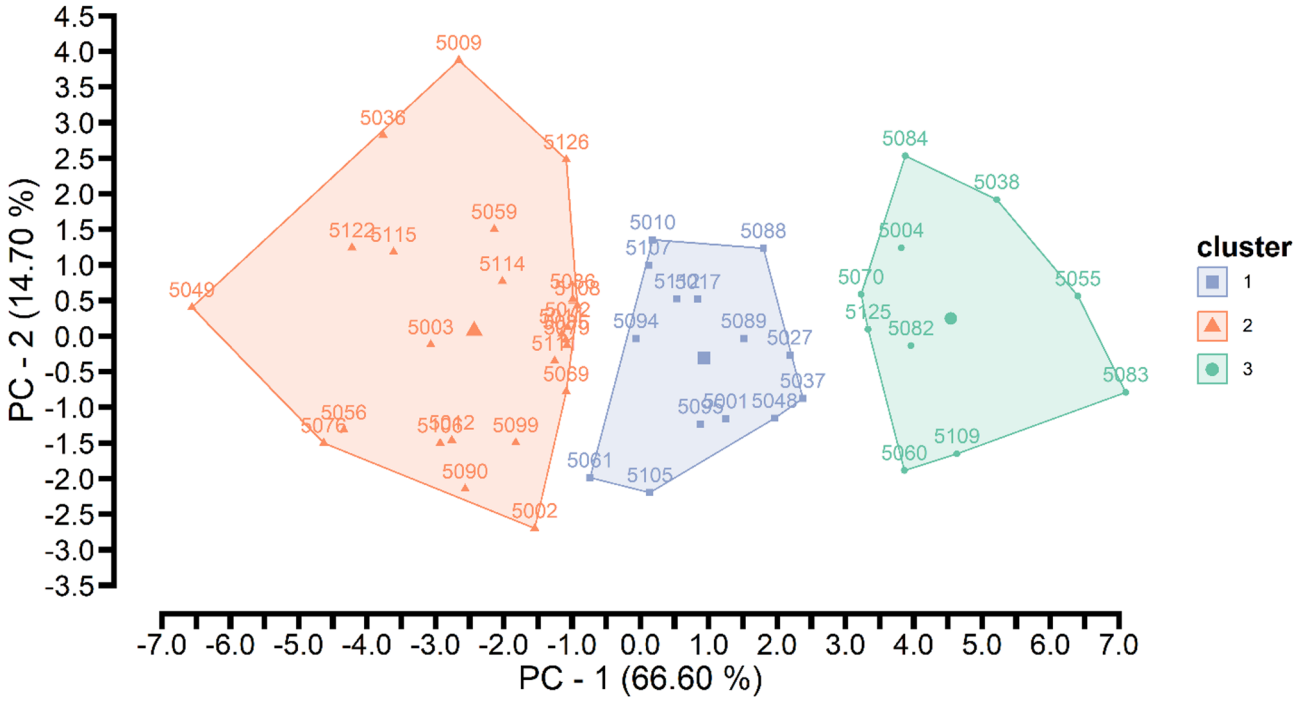

Fig. 1. The score plot showed individuals similarities for the fatty acid composition in *Longissimus thoracis* muscle of beef cattle.

Category	Fatty acids	Formula
SFA	Myristic	C14:0
SFA	Palmitic	C16:0
SFA	Stearic	C18:0
SFA	Sum of SFA	C4:0 + C6:0 + C8:0 + C10:0 + C11:0 + C12:0 + C13:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C21:0 + C24:0
MUFA	Oleic	C18:1 cis-9
MUFA	Sum of MUFA	C16:1 + C17:1 c10 + C18:1 t11 + C15:1 c10 + C20:1 c11 + C24:1 + C22:1 n9 + C18:1 c9 + C14:1 + 18:1n7 + C18:1 n9t
PUFA	Linoleic	18:2 cis-9,12
PUFA	Conjugated Linoleic Acid (CLA)	C18:2 cis9 trans11
PUFA	Alpha-Linolenic	C18:3 n3
PUFA	Sum of ω3	C18:3 n3 + C20:3 n3 c11, c14, c17 + C22:6 n3 + C20:5 n3
PUFA	Linoleic	C18:2 cis9 cis12 n6
PUFA	Sum of ω6	C18:3 n6 + C20:3 n6 c8, c11, c14 + C18:2 n6 + C20:4 n6
Ratio	PUFA/SFA	-
Ratio	ω6/ω3	-

Table 1. Classification and nomenclature of fatty acids analyzed in the study.

Differential expression analyses

Differentially expressed long non-coding RNAs (lncRNA) annotated in the bovine genome reference (ARS.UCD 1.2)

In the comparisons C1 vs. C2, C2 vs. C3, and C1 vs. C3, a total of 26 long non-coding RNAs were identified (Table 2). For the C1 vs. C2 comparison, one lncRNA, *ENSBTAG00000050891*, was upregulated, while five lncRNAs, *ENSBTAG00000052746*, *ENSBTAG00000054148*, *ENSBTAG00000048982*, *ENSBTAG00000048541*, *ENSBTAG00000051111*, *ENSBTAG00000054291*, and *ENSBTAG00000052038*, were downregulated in C1 relative to C2 (Table 2).

Comparing C1 vs. C3, seven lncRNAs, *ENSBTAG00000052922*, *ENSBTAG00000052100*, *ENSBTAG00000050348*, *ENSBTAG00000048918*, *ENSBTAG00000050739*, *ENSBTAG00000048899* and *ENSBTAG00000052078*, were upregulated, while three, *ENSBTAG00000052746*, *ENSBTAG00000049466* and *ENSBTAG00000048502*, were downregulated in C1 relative to C3 (Table 2).

In the C2 vs. C3 comparison, one lncRNA, ENSBTAG00000050891 was upregulated, whereas seven, *ENSBTAG00000051111*, *ENSBTAG00000052922*, *ENSBTAG00000054148*, *ENSBTAG00000052100*, *ENSBTAG00000048544*, *ENSBTAG00000048918* and *ENSBTAG00000052078* were downregulated in C2 relative to C3 (Table 2).

All these lncRNAs were annotated as novel transcripts in the reference genome (ARS.UCD 1.2), however their functional roles are poorly characterized in the literature.

Differentially expressed novel transcript length associated with long genic noncoding RNA annotated in the genome reference (ARS.UCD1.2).

C1 vs. C2, 14 differentially expressed novel lncRNA were identified, whereby three were upregulated and eleven were downregulated for animals in C1 group compared with C2 group (Table 3). The DE lncRNAs were related to genes with functions on DNA repair, cellular response to lipid, RNA splicing, phosphorylation, regulation of MAPK cascade and regulation of B cell activation (Table 4).

Comparing the group C1 vs. C3, 18 novels genic lncRNAs were found to be differentially expressed (Table 3), from those, eight were upregulated and ten were downregulated. The upregulated lncRNAs were associated with biological processes such as response to interleukin-1, central nervous system neuron differentiation, mitochondrion organization, and response to hypoxia (Table 5). Conversely, the downregulated lncRNAs were associated with genes involved in response to oxygen levels, mitochondrial transport, and transcription from RNA polymerase II promoters (Table 5).

In comparing C2 vs. C3, 14 novel lncRNAs were pointed out as DE in the animals of the C2 group compared to the animals of the C3 group (Table 3). Among these 14 lncRNAs, five were upregulated lncRNA and were associated with activation of protein kinase activity, response to organic cyclic compound, lipid localization, lipid transport and regulation of cell proliferation (Table 6). While 9 lncRNAs were downregulated in C2 animals compared to C3 FA profile (Table 3). These were associated with genes with functions in transcription factor binding, actin cytoskeleton and immune response (Table 6).

Differentially expressed novel long intergenic noncoding RNA (LincRNAs)

In the C1 vs. C2 comparison, a total of 53 novel lincRNAs were identified, of which 17 were upregulated and 36 downregulated in C1 animals (Table 7; Supplementary Table S1;). The majority of these lincRNAs (56.60%) were in the antisense orientation relative to protein-coding transcripts. The upregulated lincRNAs were associated with genes involved in transcription factor binding, cellular response to lipids, positive regulation of cell proliferation, and mitochondrial transport (Table 4). Conversely, the downregulated novel lincRNAs were linked

Feature ID	Position	Length[a]	*p*-Value	FDR [b]	FC (log2) [c]
Comparison C1 vs. C2					
ENSBTAG00000050891	16:55234389–55,238,149	341	1.41E-06	9.44E-05	11.17
ENSBTAG00000052746	21:32832866–32,857,979	647	6.44E-13	1.26E-10	-10.55
ENSBTAG00000054148	25:2533791–2,538,010	1840	3.25E-11	4.88E-09	-9.91
ENSBTAG00000048982	17:53500021–53,509,696	447	3.46E-07	2.66E-05	-8.40
ENSBTAG00000048541	10:2284385–2,291,785	564	6.59E-07	4.76E-05	-7.70
ENSBTAG00000051111	21:33076908–33,116,941	1347	1.31E-04	5.30E-03	-6.50
ENSBTAG00000054291	17:68750858–68,758,525	375	1.45E-04	5.78E-03	-11.63
ENSBTAG00000052038	23:50961431–50,974,711	2044	9.63E-04	2.68E-02	-4.47
Comparison C1 vs. C3					
ENSBTAG00000052922	18:65702277–65,710,756	1048	1.27E-12	2.27E-10	10.60
ENSBTAG00000052100	18:65720235–65,729,032	4306	9.27E-10	9.68E-08	6.29
ENSBTAG00000050348	4:49799680–49,987,158	682	7.83E-07	4.48E-05	11.33
ENSBTAG00000048918	19:50079818–50,082,870	922	1.47E-04	4.98E-03	3.31
ENSBTAG00000050739	10:30457617–30,654,451	1039	3.71E-04	1.05E-02	11.41
ENSBTAG00000048899	7:21895844–22,018,311	1026	3.79E-04	1.07E-02	11.38
ENSBTAG00000052078	25:34334336–34,338,041	2032	8.26E-04	2.01E-02	4.39
ENSBTAG00000052746	21:32832866–32,857,979	647	3.14E-12	5.15E-10	-10.63
ENSBTAG00000049466	21:67740943–67,754,089	791	2.54E-05	1.02E-03	-9.30
ENSBTAG00000048502	9:99053414–99,081,437	923	8.51E-04	2.06E-02	-3.31
Comparison C2 vs. C3					
ENSBTAG00000050891	16:55234389–55,238,149	341	1.52E-08	1.17E-06	14.82
ENSBTAG00000051111	21:33076908–33,116,941	1347	1.39E-14	3.19E-12	-8.79
ENSBTAG00000052922	18:65702277–65,710,756	1048	5.42E-11	6.97E-09	-8.92
ENSBTAG00000054148	25:2533791–2,538,010	1840	9.60E-09	7.61E-07	-9.75
ENSBTAG00000052100	18:65720235–65,729,032	4306	4.75E-08	3.35E-06	-4.92
ENSBTAG00000048544	23:36224038–36,465,322	1253	1.40E-06	7.11E-05	-10.90
ENSBTAG00000048918	19:50079818–50,082,870	922	4.39E-05	1.61E-03	-2.99
ENSBTAG00000052078	25:34334336–34,338,041	2032	3.23E-04	8.82E-03	-4.52

Table 2. Differentially expressed long non-coding RNAs identified in *Longissimus thoracis* muscle of Nellore cattle with different fatty acid profiles. C - Cluster, [a] Transcript Length in bases pair; [b] False Discovery Rate; [c] FC (log2) = Fold change (log2).

to genes associated with the positive regulation of kinase activity, regulation of B cell activation, and DNA repair (Table 4).

In the comparison between C1 and C3, a total of 85 DE lincRNAs were identified, of which 55 were upregulated and 30 downregulated in C1 (Table 8; Supplementary Table S2). Notably, the majority of these lincRNAs (65.88%) were located in the antisense orientation relative to their interacting genes. The upregulated lincRNAs were associated with genes involved in mRNA binding, lipid localization and transport, fatty acid metabolic processes, fat cell differentiation, transcription factor binding, and the negative regulation of cellular macromolecule biosynthetic processes (Table 5). Conversely, the downregulated lincRNAs were linked to genes associated with RNA biosynthetic processes, peptidyl-lysine modification, and ribonucleoside diphosphate metabolic processes (Table 5).

Fifty-six DE lincRNA were identified in the C2 vs. C3 comparison, with 9 upregulated and 47 downregulated in C2 (Table 9; Supplementary Table S3). The majority of these lincRNAs (67.85%) were positioned in the antisense orientation relative to their interacting genes. The upregulated DE lincRNAs were linked to transcription factor binding, nuclear body organization, and the negative regulation of cell proliferation (Table 6). Conversely, the downregulated DE lincRNAs were associated with lipid localization, transport, positive regulation of cytoskeleton organization, actin cytoskeleton, and the regulation of cellular component size (Table 6).

Functional analyses

The genes associated with differentially expressed lncRNAs were functionally classified into molecular function, biological process, and cellular component categories. This classification was done for three cluster comparisons, namely C1 vs. C2, C1 vs. C3, and C2 vs. C3. For the C1 vs. C2 comparison, 41 significant GO terms (p-value < 0.05) were identified, which included 4 molecular functions (MF), 32 biological processes (BP), and 5 cellular components (CC) terms (Table 4). Similarly, for the C1 vs. C3 comparison, 145 significant GO terms were identified (p-value < 0.05), which included 27 molecular functions (MF), 98 biological processes (BP), and 20 cellular components (CC) terms (Table 5). For the C2 vs. C3 comparison, 61 significant GO terms

Feature ID	Positon	Length [a]	*p*-Value	FDR [b]	FC (log2)[c]	lncRNA annotated [d]	Gene Interaction [e]
Comparison (C1 vs. C2)							
lncRNA_18429.11	6:42982543–43,153,637	2562	5.44E-05	2.48E-03	2.48	ENSBTAT00000077875	*ENSBTAG00000033327*
lncRNA_5876.5	16:55234389–55,238,149	615	1.67E-04	6.49E-03	2.66	ENSBTAT00000081813	*KLHL20*, *DARS2 ZBTB37*, *CENPL*, *RC3H1*, *SERPINC1*
lncRNA_15786.3	3:58491541–58,579,144	480	2.14E-04	7.94E-03	13.91	ENSBTAT00000066768	*CCN1*, *ZNHIT6*
lncRNA_16505.6	4:41775570–41,794,763	1241	1.38E-12	2.55E-10	-7.55	ENSBTAT00000087015	-
lncRNA_6086.4	16:79121509–79,142,756	3624	3.81E-12	6.57E-10	-6.54	ENSBTAT00000069212	*ZNF281*, *KIF14*
lncRNA_5039.5	8:97477285–97,504,848	5829	5.33E-12	9.08E-10	-4.94	ENSBTAT00000081266	-
lncRNA_18428.3	6:42982543–43,153,637	692	3.00E-10	4.00E-08	-9.39	ENSBTAT00000077875	*ENSBTAG00000033327*
lncRNA_3567.2	13:37839904–37,856,732	8398	3.49E-10	4.59E-08	-4.66	ENSBTAT00000085977	*ENSBTAG00000049520*, *ENSBTAG00000006043*, *BFSP1*, *PCSK2 DSTN*
lncRNA_12348.9	23:50961431–50,974,711	5505	7.12E-10	8.81E-08	-7.44	ENSBTAT00000076672	*MYLK4*, *WRNIP1*
lncRNA_7981.4	19:8790623–8,795,920	812	3.46E-07	2.66E-05	-8.40	ENSBTAT00000068745	*VEZF1*, *SRSF1*, *DYNLL2*, *CUEDC1*
lncRNA_19922.3	7:92888299–93,014,993	1228	1.98E-06	1.28E-04	-9.38	ENSBTAT00000067459	*NR2F1*, *FAM172A*
lncRNA_10726.5	21:33076908–33,116,941	1161	1.73E-05	9.05E-04	-4.12	ENSBTAT00000082173	*ENSBTAG00000024311*, *CSPG4*, *ODF3L1*
lncRNA_17096.3	4:118297039–118,325,584	462	2.33E-05	1.17E-03	-9.11	ENSBTAT00000066814	*LMBR1*, *MNX1*, *NOM1*
lncRNA_16456.3	4:31551686–31,554,697	706	1.66E-04	6.47E-03	-11.45	ENSBTAT00000072688	*ENSBTAG00000033806*, *IL6*, *TOMM7*, *FAM126A*
Comparison (C1 vs. C3)							
lncRNA_5876.4	16:55234389–55,238,149	615	8.24E-14	1.84E-11	8.03	ENSBTAT00000081813	*KLHL20*, *DARS2*, *ZBTB37*, *CENPL*, *RC3H1*, *SERPINC1*
lncRNA_6442.5	17:53500021–53,509,696	6591	3.05E-11	4.14E-09	5.71	ENSBTAT00000074572	*SETD1B*, *CFAP251*, *PSMD9*, *ENSBTAG00000052582*, *TMEM120B*
lncRNA_6037.8	16:72109237–72,128,769	2734	6.70E-07	3.90E-05	10.85	ENSBTAT00000067279	*RCOR3*, *TRAF5*
lncRNA_5415.4	16:1072072–1,076,246	764	4.89E-06	2.28E-04	5.23	ENSBTAT00000086621	*BTG2*, *CHI3L1*
lncRNA_13894.1	26:51237158–51,241,924	3237	5.63E-06	2.59E-04	2.90	ENSBTAT00000073957	*BNIP3*
lncRNA_843.3	1:148950230–148,972,709	1213	4.96E-05	1.90E-03	3.05	ENSBTAT00000074316	*SIM2*
lncRNA_5415.2	16:1072072–1,076,246	985	9.50E-04	2.26E-02	3.27	ENSBTAT00000086621	*BTG2*, *CHI3L1*
lncRNA_12348.11	23:50961431–50,974,711	7845	1.34E-03	3.01E-02	10.18	ENSBTAT00000076672	*MYLK4*, *WRNIP1*
lncRNA_16505.6	4:41775570–41,794,763	1241	6.35E-13	1.21E-10	-8.52	ENSBTAT00000087015	-
lncRNA_20382.10	8:70592566–70,718,220	1741	3.97E-12	6.38E-10	-10.54	ENSBTAT00000068948	*SLC25A37*, *NKX3-1*, *LOXL2*, *ENTPD4*
lncRNA_18428.3	6:42982543–43,153,637	692	5.38E-12	8.49E-10	-10.43	ENSBTAT00000077875	*ENSBTAG00000033327*
lncRNA_7981.4	19:8790623–8,795,920	812	1.49E-09	1.52E-07	-11.27	ENSBTAT00000068745	*VEZF1*, *SRSF1*, *DYNLL2*, *CUEDC1*
lncRNA_19922.3	7:92888299–93,014,993	1228	5.45E-06	2.51E-04	-10.36	ENSBTAT00000067459	*NR2F1*, *FAM172A*
lncRNA_10726.5	21:33076908–33,116,941	1161	1.31E-05	5.59E-04	-4.63	ENSBTAT00000082173	*ENSBTAG00000024311*, *ODF3L1*, *CSPG4*
lncRNA_17096.3	4:118297039–118,325,584	462	2.10E-05	8.64E-04	-9.43	ENSBTAT00000066814	*LMBR1*, *MNX1*, *NOM1*
lncRNA_12504	23:36224038–36,465,322	604	2.41E-04	7.46E-03	-6.70	ENSBTAT00000083528	*SOX4*
lncRNA_20062.10	8:22841637–22,873,487	2286	3.72E-04	1.06E-02	-2.20	ENSBTAT00000070622	*ENSBTAG00000048891*, *ENSBTAG00000054099*, *ENSBTAG00000055152*, *ENSBTAG00000050194*, *ENSBTAG00000052859*, *KLHL9*, *IFNAG*, *IFN-TAU*
lncRNA_15244.2	3:12245784–12,253,963	3500	1.76E-03	3.77E-02	-2.71	ENSBTAT00000072176	*ENSBTAG00000024960* *KIRREL1*
Comparison (C2 vs. C3)							
lncRNA_20382.10	8:70592566–70,718,220	1741	2.96E-16	8.88E-14	10.38	ENSBTAT00000068948	*SLC25A37*, *NKX3-1*, *LOXL2*, *ENTPD4*
lncRNA_5415.4	16:1072072–1,076,246	764	1.93E-09	1.79E-07	8.12	ENSBTAT00000086621	*BTG2*, *CHI3L1*
lncRNA_9130.4	19:60774940–60,930,256	1149	3.50E-09	3.05E-07	3.36	ENSBTAT00000070427	-
lncRNA_18429.11	6:42982543–43,153,637	2562	2.42E-06	1.17E-04	2.97	ENSBTAT00000077875	*ENSBTAG00000033327*
lncRNA_16618.6	4:55292087–55,307,803	5601	2.90E-04	8.09E-03	2.61	ENSBTAT00000070495	*GPR85*
lncRNA_6086.4	16:79121509–79,142,756	3624	2.06E-09	1.89E-07	-8.23	ENSBTAT00000069212	*ZNF281*, *KIF14*
lncRNA_6442.5	17:53500021–53,509,696	6591	2.95E-09	2.63E-07	-4.31	ENSBTAT00000074572	*ENSBTAG00000052582*, *SETD1B*, *CFAP251*, *PSMD9*, *TMEM120B*
lncRNA_5876.4	16:55234389–55,238,149	615	4.57E-09	3.86E-07	-5.38	ENSBTAT00000081813	*KLHL20*, *DARS2*, *ZBTB37*, *CENPL*, *RC3H1*, *SERPINC1*
lncRNA_12348.9	23:50961431–50,974,711	5505	5.18E-09	4.33E-07	-7.90	ENSBTAT00000076672	*MYLK4*, *WRNIP1*
lncRNA_20062.7	8:22841637–22,873,487	2286	6.91E-07	3.79E-05	-11.23	ENSBTAT00000070622	*ENSBTAG00000048891*, *ENSBTAG00000054099*, *ENSBTAG00000055152*, *ENSBTAG00000050194*, *ENSBTAG00000052859*, *IFN-TAU*, *KLHL9*, *IFNAG*
lncRNA_6037.8	16:72109237–72,128,769	2734	8.69E-07	4.65E-05	-9.89	ENSBTAT00000067279	*RCOR3*, *TRAF5*

Continued

Feature ID	Positon	Length [a]	*p*-Value	FDR [b]	FC (log2)[c]	lncRNA annotated [d]	Gene Interaction [e]
lncRNA_3567.2	13:37839904–37,856,732	8398	1.01E-04	3.36E-03	-3.17	ENSBTAT00000085977	*ENSBTAG00000049520*, *ENSBTAG00000006043*, *DSTN*, *BFSP*, *PCSK2*
lncRNA_11324.5	22:36444667–36,922,968	1306	1.55E-04	4.84E-03	-11.06	ENSBTAT00000083930	*ENSBTAG00000019048*
lncRNA_843.3	1:148950230–148,972,709	1213	3.15E-04	8.64E-03	-2.59	ENSBTAT00000074316	*SIM2*

Table 3. Novel transcript lengths associated with annotated long genic noncoding RNAs that are differentially expressed in the *Longissimus thoracis* muscle of Nellore cattle with distinct fatty acid profiles. [a] Transcript Length in basis pair, [b] False Discovery Rate, [c] FC (log2) = Fold change (log2), [d] transcript association = lncRNA annotated in the bovine reference annotation (ARS.UCD1.2), [e] Gene Interaction = Genes associated with annotated lncRNAs within the 200 kb window, with 100 kb upstream and 100 kb downstream, relative to the start and end positions of the gene, respectively.

were identified (p-value < 0.05), which included 1 molecular function (MF), 47 biological processes (BP), and 13 cellular components (CC) terms (Table 6).

Discussion

The beef fatty acid profile contributes to its sensory properties and plays a significant role in determining the quality of the final product from a healthy perspective. Therefore, it is essential to select animals with genetic potential to produce healthier beef by increasing the levels of CLA and maintaining the optimal ratio of PUFA to SFA and ω6 to ω3. Aboujaoude et al.[11] reported that it is feasible to select beef FA profile in Nellore cattle given there is additive genetic variation for most beef FA profiles (h2 varying from 0.01 to 0.35). This study revealed substantial variations (P < 0.05) across animals within the population, which was observed in the clustering analysis (Table 10). The C1 cluster exhibited higher levels of polyunsaturated fatty acids (PUFAs) (16.9 ± 2.03 g/100 g), including ω-6, ω-3, linoleic, and α-linolenic acids, compared with C2 (10.80 ± 1.74 g/100 g) and C3 (7.78 ± 1.68 g/100 g) (Table 10). Given the beneficial roles of PUFAs in human metabolism, C1 represents a promising group for selection in animal breeding programs. The C2 cluster exhibited an intermediate fatty acid profile, while C3 showed a lower PUFA/SFA ratio and higher concentrations of saturated fatty acids (SFA, 46.13 ± 2.00 g/100 g), monounsaturated fatty acids (MUFA, 39.19 ± 2.95 g/100 g), and conjugated linoleic acid (CLA, 0.34 ± 0.12 g/100 g) compared to the other groups - C1 (SFA: 42.91 ± 1.75 g/100 g; CLA: 0.17 ± 0.10 g/100 g) and C2 (SFA: 43.29 ± 1.46 g/100 g; CLA: 0.27 ± 0.09 g/100 g) (Table 10).The simultaneous presence of elevated SFA, MUFA and CLA content in cluster C3 may be associated with the activity of the Δ9 desaturase enzyme, which catalyzes the conversion of saturated fatty acids (SFA) into monounsaturated fatty acids (MUFA), and trans-vaccenic acid (MUFA) into conjugated linoleic acid (CLA)[37]. This enzymatic activity suggests a metabolic interconnection and mutual dependence among these fatty acid classes. Furthermore, this cluster-based analysis reveals patterns of gene regulation across phenotypes, emphasizing coordinated lncRNA–gene networks rather than isolated gene effects.

The genome produces numerous lncRNAs, which are transcripts with more than 200 nucleotides and do not encode protein-coding genes. Studies have indicated that the structure of lncRNAs is one of the most critical factors regulating various biological processes at the epigenetic, transcriptional, and translational levels[38,39]. When involved in transcriptional regulation, lncRNAs often function as ligands that interact with transcription factors, forming complexes that modulate gene transcription[40].

LncRNAs can be classified into several categories based on their genomic location, structure, and function. In this study, we categorized them according to their genomic location as genic lncRNAs (Table 2 and 3) and long intergenic non-coding RNAs (lincRNAs) (Tables 7, 8 and 9). Genic lncRNAs are those that overlap with protein-coding genes; they may intersect with exons, introns, or an entire gene, and can affect the functionality of their associated mRNA[38]. In contrast, lincRNAs are located in intergenic regions and do not overlap with protein-coding genes. These transcripts exhibit distinct features that differentiate them from mRNAs and are known to participate in chromatin remodeling, genome architecture modification, transcriptional regulation, and RNA stabilization[41].

In the present study, we identified 25 differentially expressed (DE) genic lncRNAs that are annotated in the bovine reference genome (ARS-UCD1.2), along with 46 DE novel transcripts associated with annotated lncRNAs, and 194 DE novel lincRNAs linked to fatty acid (FA) content in beef cattle. These lncRNAs are likely to exert regulatory influence over genes involved in fatty acid biosynthesis and lipid metabolism, thereby contributing to variations in fatty acid deposition in beef. Nonetheless, further functional analyses are needed to elucidate the specific biological roles and mechanisms of action of these lncRNAs.

Differentially expressed genic long non-coding RNA

Cluster 1 was characterized by a favorable lipid profile, enriched in polyunsaturated fatty acids (PUFA) - including ω6 to ω3 ratio and reduced levels of monounsaturated (MUFAs) and saturated fatty acids (SFAs), such as palmitic, stearic, and miristic acids. Several upregulated lncRNAs associated with this phenotype, including *lncRNA_15786.3* (targeting *CCN1*), *lncRNA_13894.1* (targeting *BNIP3*), and *lncRNA_16456.3* (targeting *IL6*), may contribute to this metabolically advantageous fatty acid composition.

In particular, the upregulation of *lncRNA_15786.3* in Cluster 1 compared to Cluster 2, and its association with the *CCN1* gene (Cellular Communication Network Factor 1) (Table 3), suggests a potential regulatory

GO: ID	GOTerm	*p*-value	Nr. Genes *	Genes	lncRNA ID
Biological process					
GO:0022411	cellular component disassembly	0.00	5	*ARID2*, *DSTN*, *RUVBL1*, *SMN2*, *TOMM7*	*lincRNA_17358.2*, *lncRNA_3567.2*, *lincRNA_11659.21*, *lincRNA_11659.1*, *lincRNA_11659.14*, *lincRNA_11659.17*, *lincRNA_11659.3*, *lincRNA_10184.3*, *lincRNA_10184.1*, *lncRNA_16456.3*
GO:0048545	response to steroid hormone	0.01	4	*CNOT2*, *EIF4E*, *IL6*, *NR2F1*	*lincRNA_17393.3*, *lincRNA_18394.1*, *lncRNA_16456.3*, *lncRNA_19922.3*
GO:0008284	positive regulation of cell proliferation	0.01	7	*CD81*, *CCN1*, *HIPK1*, *IL6*, *KIF14*, *OSMR*, *SLC25A33*	*lincRNA_15009.1*, *lncRNA_15786.3*, *lincRNA_15543.1*, *lncRNA_16456.3*, *lncRNA_6086.4*, *lincRNA_10320.5*, *lincRNA_10320.8*, *lncRNA_10320.7*, *lincRNA_5714.2*
GO:0014070	response to organic cyclic compound	0.01	6	*CNOT2*, *DDX1*, *EIF4E*, *IL6*, *NR2F1*, *SMAD9*	*lincRNA_17393.3*, *lincRNA_2526.3*, *lincRNA_18394.1*, *lncRNA_16456.3*, *lncRNA_19922.3*, *lincRNA_3024.7*, *lincRNA_3024.5*
GO:0050731	positive regulation of peptidyl-tyrosine phosphorylation	0.01	3	*CD81*, *CSPG4*, *IL6*	*lincRNA_15009.1*, *lncRNA_10726.5*, *lncRNA_16456.3*
GO:0006403	RNA localization	0.02	3	*RUVBL1*, *SRSF1*, *ZNHIT6*	*lincRNA_11659.21*, *lincRNA_11659.1*, *lincRNA_11659.14*, *lincRNA_11659.17*, *lincRNA_11659.3*, *lncRNA_7981.4*, *lncRNA_15786.3*
GO:0071383	cellular response to steroid hormone stimulus	0.03	3	*CNOT2*, *EIF4E*, *NR2F1*	*lincRNA_17393.3*, *lincRNA_18394.1*, *lncRNA_19922.3*
GO:0009725	response to hormone	0.03	5	*CNOT2*, *EIF4E*, *IL6*, *NR2F1*, *SLC25A33*	*lincRNA_17393.3*, *lincRNA_18394.1*, *lncRNA_16456.3*, *lncRNA_19922.3*, *lincRNA_5714.2*
GO:0032147	activation of protein kinase activity	0.04	3	*CD81*, *CSPG4*, *KIF14*	*lincRNA_15009.1*, *lncRNA_10726.5*, *lncRNA_6086.4*
GO:0045860	positive regulation of protein kinase activity	0.04	4	*CD81*, *CSPG4*, *CCN1*, *KIF14*	*lincRNA_15009.1*, *lncRNA_10726.5*, *lncRNA_15786.3*, *lncRNA_6086.4*
GO:0043410	positive regulation of MAPK cascade	0.04	4	*CD81*, *CSPG4*, *IL6*, *UNC5CL*	*lincRNA_15009.1*, *lncRNA_10726.5*, *lncRNA_16456.3*, *lincRNA_11848.4*
GO:0071396	cellular response to lipid	0.04	4	*CNOT2*, *EIF4E*, *IL6*, *NR2F1*	*lincRNA_17393.3*, *lincRNA_18394.1*, *lncRNA_16456.3*, *lncRNA_19922.3*
GO:0044255	cellular lipid metabolic process	0.04	4	*AUH*, *CD81*, *ENSBTAT00000079956*	*lincRNA_20515.12*, *lincRNA_20515.13*, *lincRNA_20515.10*, *lincRNA_15009.1*, *lincRNA_20557.5*
GO:0071407	cellular response to organic cyclic compound	0.05	4	*CNOT2*, *EIF4E*, *NR2F1*, *SMAD9*	*lincRNA_17393.3*, *lincRNA_18394.1*, *lncRNA_19922.3*, *lincRNA_3024.7*, *lincRNA_3024.5*
GO:0001558	regulation of cell growth	0.05	3	*CCN1*, *KIF14*, *SLC25A33*	*lncRNA_15786.3*, *lncRNA_6086.4*, *lincRNA_5714.2*
GO:0006281	DNA repair	0.05	4	*DDX1*, *MMS22L*, *RUVBL1*, *WRNIP1*	*lincRNA_2526.3*, *lincRNA_20981.1*, *lincRNA_11659.21*, *lincRNA_11659.1*, *lincRNA_11659.14*, *lincRNA_11659.17*, *lincRNA_11659.3*, *lncRNA_12348.9*, *lncRNA_12348.11*
GO:0006839	mitochondrial transport	0.05	3	*RUVBL1*, *SLC25A33*, *TOMM7*	*lincRNA_11659.21*, *lincRNA_11659.1*, *lincRNA_11659.14*, *lincRNA_11659.17*, *lincRNA_11659.3*, *lincRNA_5714.2*, *lncRNA_16456.3*
GO:0050864	regulation of B cell activation	0.05	2	*CD81*, *IL6*	*lincRNA_15009.1*, *lncRNA_16456.3*
GO:0008380	RNA splicing	0.05	3	*DDX1*, *SMN2*, *SRSF1*	*lincRNA_2526.3*, *lincRNA_10184.3*, *lincRNA_10184.1*, *lncRNA_7981.4*
GO:0033674	positive regulation of kinase activity	0.05	4	*CD81*, *CSPG4*, *CCN1*, *KIF14*	*lincRNA_15009.1*, *lncRNA_10726.5*, *lncRNA_15786.3*, *lncRNA_6086.4*
Cellular Component					
GO:0036464	cytoplasmic ribonucleoprotein granule	0.00	4	*CNOT2*, *DDX1*, *EIF4E*, *SMN2*	*lincRNA_17393.3*, *lincRNA_2526.3*, *lincRNA_18394.1*, *lincRNA_10184.3*, *lincRNA_10184.1*
GO:0016604	nuclear body	0.02	6	*DDX1*, *HIPK1*, *KLHL20*, *NOC3L*, *SMN2*, *SRSF1*	*lincRNA_2526.3*, *lincRNA_15543.1*, *lncRNA_5876.5*, *lncRNA_5876.4*, *lincRNA_13547.2*, *lincRNA_10184.3*, *lincRNA_10184.1*, *lncRNA_7981.4*
Molecular function					
GO:0008134	transcription factor binding	0.01	6	*ATOH8*, *CNOT2*, *EIF4E*, *RUVBL1*, *TLE4*, *ZNHIT6*	*lincRNA_2305.1*, *lincRNA_17393.3*, *lincRNA_18394.1*, *lincRNA_11659.21*, *lincRNA_11659.1*, *lincRNA_11659.14*, *lincRNA_11659.17*, *lincRNA_11659.3*, *lincRNA_20224.2*, *lncRNA_15786.3*
GO:0004386	helicase activity	0.01	3	*DDX1*, *RUVBL1*, *WRNIP1*	*lincRNA_2526.3*, *lincRNA_11659.21*, *lincRNA_11659.1*, *lincRNA_11659.14*, *lincRNA_11659.17*, *lincRNA_11659.3*, *lncRNA_12348.9*, *lncRNA_12348.11*
GO:0016887	ATPase activity	0.01	5	*DDX1*, *DYNLL2*, *KIF14*, *RUVBL1*, *WRNIP1*	*lincRNA_2526.3*, *lncRNA_7981.4*, *lncRNA_6086.4*, *lincRNA_11659.21*, *lincRNA_11659.1*, *lincRNA_11659.14*, *lincRNA_11659.17*, *lincRNA_11659.3*, *lncRNA_12348.9*, *lncRNA_12348.11*

Table 4. Gene ontology (GO) - Biological process, cellular component, and molecular Function - associated with genes interacting with differentially expressed LncRNAs and LincRNAs identified in the comparison between C1 and C2 fatty acid clusters. *Nr. Genes: Number of genes.

mechanism promoting PUFA deposition through modulation of lipogenic pathways. This regulation may involve downstream effects on key enzymes such as *FASN* (Fatty Acid Synthase), crucial for fatty acid synthesis, contributing to lipid formation and fat accumulation in cells[42]. Previous studies identified *FASN* gene variants associated with increased PUFA concentrations in milk[43] and decreased SFA and MUFA levels in meat[44]aligning with the lipid profile observed in Cluster 1.

GO: ID	GOTerm	*p*-value	Nr. Genes*	Genes	lncRNAs
Biological process					
GO:0006366	transcription from RNA polymerase II promoter	0.00	18	*BRD3*, *CTCFL*, *E2F6*, *ELF2*, *GTF2A1*, *HOXC10*, *IER2*, *MNX1*, *NKX3-1*, *NR2F1*, *RCOR3*, *RUVBL1*, *SIM2*, *SMAD9*, *SOX4*, *TLE4*, *TRIM27*, *VEZF1*	*lincRNA_2832.1*, *lincRNA_3863.4*, *lncRNA_2540.2*, *lincRNA_2540.4*, *lincRNA_6258.4*, *lincRNA_6258.1*, *lincRNA_1840.2*, *lincRNA_17194.2*, *lincRNA_19092.2*, *lncRNA_17096.3*, *lncRNA_20382.10*, *lncRNA_19922.3*, *lncRNA_6037.8*, *lincRNA_11659.21*, *lincRNA_11659.1*, *lincRNA_11659.14*, *lincRNA_11659.17*, *lincRNA_11659.3*, *lncRNA_843.3*, *lincRNA_3024.7*, *lincRNA_3024.5*, *lncRNA_12504*, *lincRNA_20224.2*, *lincRNA_12128.6*, *lincRNA_12128.7*, *lncRNA_7981.4*
GO:0021953	central nervous system neuron differentiation	0.00	5	*ADARB1*, *BTG2*, *HOXC10*, *MNX1*, *SOX4*	*lincRNA_791.1*, *lncRNA_5415.4*, *lncRNA_5415.2*, *lincRNA_17194.2*, *lncRNA_17096.3*, *lncRNA_12504*
GO:0032774	RNA biosynthetic process	0.00	27	*BRD3*, *CTCFL*, *DDX1*, *E2F6*, *ELF2*, *GTF2A1*, *HOXC10*, *IER2*, *ENSBTAT00000053465*, *LOXL2*, *MLLT11*, *MNX1*, *MYT1L*, *NAB2*, *NKX3-1*, *NR2F1*, *RCOR3*, *RUVBL1*, *SIM2*, *SLC25A33*, *SMAD9*, *SMN2*, *SOX4*, *TLE4*, *TRAF5*, *TRIM27*, *VEZF1*	*lincRNA_2832.1*, *lincRNA_3863.4*, *lincRNA_2526.3*, *lincRNA_2540.2*, *lincRNA_2540.4*, *lincRNA_6258.4*, *lincRNA_6258.1*, *lincRNA_1840.2*, *lincRNA_17194.2*, *lincRNA_19092.2*, *lincRNA_7736.5*, *lincRNA_7736.1*, *lncRNA_20382.10*, *lincRNA_15403.1*, *lncRNA_17096.3*, *lincRNA_20692.2*, *lincRNA_17511.1*, *lincRNA_17511.6*, *lncRNA_19922.3*, *lncRNA_6037.8*, *lincRNA_11659.21*, *lincRNA_11659.1*, *lincRNA_11659.14*, *lincRNA_11659.17*, *lincRNA_11659.3*, *lncRNA_843.3*, *lincRNA_5714.2*, *lincRNA_3024.7*, *lincRNA_3024.5*, *lincRNA_10184.3*, *lincRNA_10184.1*, *lncRNA_12504*, *lincRNA_20224.2*, *lincRNA_12128.6*, *lincRNA_12128.7*, *lncRNA_7981.4*
GO:0001501	skeletal system development	0.01	6	*HOXC10*, *ITGB8*, *LOXL2*, *NAB2*, *SMAD9*, *SOX4*	*lincRNA_17194.2*, *lincRNA_16442.1*, *lncRNA_20382.10*, *lincRNA_17511.1*, *lincRNA_17511.6*, *lincRNA_3024.7*, *lincRNA_3024.5*, *lncRNA_12504*
GO:0000959	mitochondrial RNA metabolic process	0.01	2	*DARS2*, *SLC25A33*	*lncRNA_5876.4*, *lincRNA_5714.2*
GO:0006839	mitochondrial transport	0.02	4	*BNIP3*, *RUVBL1*, *SLC25A33*, *SLC25A37*	*lncRNA_13894.1*, *lincRNA_11659.21*, *lincRNA_11659.1*, *lincRNA_11659.14*, *lincRNA_11659.17*, *lincRNA_11659.3*, *lincRNA_5714.2*, *lncRNA_20382.10*
GO:2,000,113	negative regulation of cellular macromolecule biosynthetic process	0.02	11	*BTG2*, *E2F6*, *EIF4E*, *LOXL2*, *NAB2*, *NKX3-1*, *NR2F1*, *RCOR3*, *SIM2*, *TLE4*, *TRIM27*	*lncRNA_5415.4*, *lncRNA_5415.2*, *lincRNA_2540.2*, *lincRNA_2540.4*, *lincRNA_18394.1*, *lncRNA_20382.10*, *lincRNA_17511.1*, *lincRNA_17511.6*, *lncRNA_19922.3*, *lncRNA_6037.8*, *lncRNA_843.3*, *lincRNA_20224.2*, *lincRNA_12128.6*, *lincRNA_12128.7*
GO:0010822	positive regulation of mitochondrion organization	0.02	3	*BNIP3*, *MLLT11*, *RUVBL1*	*lncRNA_13894.1*, *lincRNA_15403.1*, *lincRNA_11659.21*, *lincRNA_11659.1*, *lincRNA_11659.14*, *lincRNA_11659.17*, *lincRNA_11659.3*
GO:0001666	response to hypoxia	0.03	3	*BNIP3*, *LOXL2*, *NKX3-1*	*lncRNA_13894.1*, *lncRNA_20382.10*
GO:0010638	positive regulation of organelle organization	0.03	6	*BNIP3*, *DCTN1*, *KIRREL1*, *MLLT11*, *RUVBL1*, *TRIM27*	*lncRNA_13894.1*, *lincRNA_2042.4*, *lncRNA_15244.2*, *lincRNA_15403.1*, *lincRNA_11659.21*, *lincRNA_11659.1*, *lincRNA_11659.14*, *lincRNA_11659.17*, *lincRNA_11659.3*, *lincRNA_12128.6*, *lincRNA_12128.7*
GO:0021954	central nervous system neuron development	0.03	2	*ADARB1*, *BTG2*	*lincRNA_791.1*, *lncRNA_5415.4*, *lncRNA_5415.2*
GO:0006869	lipid transport	0.04	3	*APOL3*, *NKX3-1*, *SPNS3*	*lincRNA_17681.1*, *lncRNA_20382.10*, *lincRNA_8294.10*, *lincRNA_8294.4*
GO:0051495	positive regulation of cytoskeleton organization	0.04	3	*DCTN1*, *KIRREL1*, *TRIM27*	*lincRNA_2042.4*, *lncRNA_15244.2*, *lincRNA_12128.6*, *lincRNA_12128.7*
GO:0070482	response to oxygen levels	0.04	3	*BNIP3*, *LOXL2*, *NKX3-1*	*lncRNA_13894.1*, *lncRNA_20382.10*
GO:0010821	regulation of mitochondrion organization	0.04	3	*BNIP3*, *MLLT11*, *RUVBL1*	*lncRNA_13894.1*, *lincRNA_15403.1*, *lincRNA_11659.21*, *lincRNA_11659.1*, *lincRNA_11659.14*, *lincRNA_11659.17*, *lincRNA_11659.3*
GO:0032271	regulation of protein polymerization	0.04	3	*DCTN1*, *KIRREL1*, *TRIM27*	*lincRNA_2042.4*, *lncRNA_15244.2*, *lincRNA_12128.6*, *lincRNA_12128.7*
GO:0032535	regulation of cellular component size	0.04	4	*KIRREL1*, *RAB22A*, *RAB5A*, *TRIM27*	*lncRNA_15244.2*, *lincRNA_3855.2*, *lincRNA_929.1*, *lincRNA_12128.6*, *lincRNA_12128.7*
GO:0060070	canonical Wnt signaling pathway	0.05	2	*RAB5A*, *SOX4*	*lincRNA_929.1*, *lncRNA_12504*
GO:0010876	lipid localization	0.05	3	*APOL3*, *NKX3-1*, *SPNS3*	*lincRNA_17681.1*, *lncRNA_20382.10*, *lincRNA_8294.10*, *lincRNA_8294.4*
GO:0070555	response to interleukin-1	0.05	2	*CHI3L1*, *NKX3-1*	*lncRNA_5415.4*, *lncRNA_5415.2*, *lncRNA_20382.10*
GO:0008380	RNA splicing	0.05	4	*DDX1*, *RBM38*, *SMN2*, *SRSF1*	*lincRNA_2526.3*, *lincRNA_3864.2*, *lincRNA_10184.3*, *lincRNA_10184.1*, *lncRNA_7981.4*
GO:0018205	peptidyl-lysine modification	0.05	4	*CTCFL*, *RUVBL1*, *SETD1B*, *SOX4*	*lincRNA_3863.4*, *lincRNA_11659.21*, *lincRNA_11659.1*, *lincRNA_11659.14*, *lincRNA_11659.17*, *lincRNA_11659.3*, *lncRNA_6442.5*, *lncRNA_12504*
GO:0045444	fat cell differentiation	0.05	3	*BNIP3*, *MEDAG*, *TMEM120B*	*lncRNA_13894.1*, *lincRNA_3050.2*, *lncRNA_6442.5*
GO:0071383	cellular response to steroid hormone stimulus	0.05	3	*EIF4E*, *NKX3-1*, *NR2F1*	*lincRNA_18394.1*, *lncRNA_20382.10*, *lncRNA_19922.3*
GO:0006631	fatty acid metabolic process	0.05	3	*AUH*	*lincRNA_20515.12*, *lincRNA_20515.13*, *lincRNA_20515.10*
Cellular component					
GO:0030904	retromer complex	0.00	2	*DCTN1*, *TRIM27*	*lincRNA_2042.4*, *lincRNA_12128.6*, *lincRNA_12128.7*
Continued					

GO: ID	GOTerm	*p*-value	Nr. Genes*	Genes	lncRNAs
GO:0010494	cytoplasmic stress granule	0.01	2	*DDX1*, *EIF4E*	*lincRNA_2526.3*, *lincRNA_18394.1*
GO:0034708	methyltransferase complex	0.01	3	*E2F6*, *RUVBL1*, *SETD1B*	*lincRNA_2540.2*, *lincRNA_2540.4*, *lincRNA_11659.21*, *lincRNA_11659.1*, *lincRNA_11659.14*, *lincRNA_11659.17*, *lincRNA_11659.3*, *lncRNA_6442.5*
GO:0045335	phagocytic vesicle	0.02	2	*RAB22A*, *RAB5A*	*lincRNA_3855.2*, *lincRNA_929.1*
GO:0015629	actin cytoskeleton	0.03	5	*DCTN1*, *DYNLL2*, *IVNS1ABP*, *RAB22A*, *RAB5A*	*lincRNA_2042.4*, *lncRNA_7981.4*, *lincRNA_5964.5*, *lincRNA_5964.1*, *lncRNA_5964.6*, *lincRNA_3855.2*, *lincRNA_929.1*
GO:1,990,234	transferase complex	0.04	7	*E2F6*, *GTF2A1*, *HOXC10*, *KLHL20*, *RUVBL1*, *SETD1B*, *VAC14*	*lincRNA_2540.2*, *lincRNA_2540.4*, *lincRNA_1840.2*, *lincRNA_17194.2*, *lncRNA_5876.5*, *lncRNA_5876.4*, *lincRNA_11659.21*, *lincRNA_11659.1*, *lincRNA_11659.14*, *lincRNA_11659.17*, *lincRNA_11659.3*, *lncRNA_6442.5*, *lincRNA_6802.5*
GO:0035770	ribonucleoprotein granule	0.04	3	*DDX1*, *EIF4E*, *SMN2*	*lincRNA_2526.3*, *lincRNA_18394.1*, *lincRNA_10184.3*, *lincRNA_10184.1*
Molecular function					
GO:0003677	DNA binding	0.00	20	*CTCFL*, *DDX1*, *E2F6*, *ELF2*, *GTF2A1*, *HOXC10*, *IER2*, *LBX2*, *LOC107132564*, *MNX1*, *NKX3-1*, *NR2F1*, *RCOR3*, *SIM2*, *SMAD9*, *SOX4*, *THAP9*, *TMPO*, *VEZF1*, *WRNIP1*	*lincRNA_3863.4*, *lincRNA_2526.3*, *lincRNA_2540.2*, *lincRNA_2540.4*, *lincRNA_6258.4*, *lincRNA_6258.1*, *lincRNA_1840.2*, *lincRNA_17194.2*, *lincRNA_19092.2*, *lincRNA_2036.1*, *lncRNA_17096.3*, *lncRNA_20382.10*, *lncRNA_19922.3*, *lncRNA_6037.8*, *lncRNA_843.3*, *lincRNA_3024.7*, *lincRNA_3024.5*, *lncRNA_12504*, *lincRNA_18674.6*, *lincRNA_18674.6*, *lincRNA_17577.2*, *lncRNA_7981.4*, *lncRNA_12348.9*, *lncRNA_12348.11*
GO:1,990,837	sequence-specific double-stranded DNA binding	0.01	8	*CTCFL*, *E2F6*, *HOXC10*, *IER2*, *NKX3-1*, *NR2F1*, *SOX4*, *VEZF1*	*lincRNA_3863.4*, *lincRNA_2540.2*, *lincRNA_2540.4*, *lincRNA_17194.2*, *lincRNA_19092.2*, *lncRNA_20382.10*, *lncRNA_19922.3*, *lncRNA_12504*, *lncRNA_7981.4*
GO:0001067	regulatory region nucleic acid binding	0.01	9	*CTCFL*, *E2F6*, *HOXC10*, *IER2*, *NKX3-1*, *NR2F1*, *RCOR3*, *SOX4*, *VEZF1*	*lincRNA_3863.4*, *lincRNA_2540.2*, *lincRNA_2540.4*, *lincRNA_17194.2*, *lincRNA_19092.2*, *lncRNA_20382.10*, *lncRNA_19922.3*, *lncRNA_6037.8*, *lncRNA_12504*, *lncRNA_7981.4*
GO:0003729	mRNA binding	0.05	3	*AUH*, *RBM38*, *SRSF1*	*lincRNA_20515.12*, *lincRNA_20515.13*, *lincRNA_20515.10*, *lincRNA_3864.2*, *lncRNA_7981.4*

Table 5. Gene ontology (GO) - Biological process, cellular component, and molecular Function - associated with genes interacting with differentially expressed LncRNAs and LincRNAs identified in the comparison between C1 and C3 fatty acid clusters. *Nr. Genes: Number of genes.

Cluster 2 exhibited an intermediate fatty acid profile, sharing characteristics of both C1 and C3. Although its molecular pattern was less pronounced, certain differentially expressed lncRNAs suggest a role in saturated fatty-acid (SFA) accumulation. For instance, *lncRNA_11324.5*, which is downregulated in Cluster 2 versus Cluster 3 (Table 3), targets the *ENSBTAG00000019048* (Mediator Complex Subunit 28 - *MED28*) gene in humans. *MED28* is ubiquitously expressed and has been shown to negatively regulate smooth muscle cell differentiation[48,46], but its involvement in lipid metabolism remains unexplored[47]. Notably, genome-wide association studies in cattle have linked *MED28* to intramuscular fat (IMF) content and carcass traits[48,49]. Therefore, the reduced expression of *lncRNA_11324.5* in C2 may reflect lower *MED28* activity and consequently somewhat reduced SFA levels compared to Cluster 3 — while still exceeding those in Cluster 1. Similarly, *lncRNA_19922.3*, associated with *NR2F1* (COUP transcription factor 1), was downregulated in both C1 vs. C2 and C1 vs. C3 comparisons (Table 3), suggesting a potential contribution to the lipid profile differences observed across clusters.

The *NR2F1* was enriched for GO biological function terms related to cellular lipid response (GO: 0071396) and to steroid hormone stimulus (GO:0071383) (Tables 4 and 5). *NR2F1* is a sterol-binding transcription factor involved in the regulation of triglyceride synthesis and transport in enterocytes and has been implicated in lipid, cholesterol, and carbohydrate metabolism[50–52]. It has been validated as a candidate gene for intramuscular fat (IMF) deposition using multi-omics data from pig muscle tissues[53,54]. Moreover, a genomic association study in pigs conducted by Pena et al.[55]indicated *NR2F1* within regions associated with palmitoleic acid, oleic acid, and MUFA. In the present study, C1 exhibited a lower total content of oleic acid and MUFA compared to C2 and C3 (Table 10), supporting the hypothesis that the reduced expression of *lncRNA_19922.3* in C1 reflects lower *NR2F1* activity and reduced MUFA levels.

Additionally, *lncRNA_20062.7* and *lncRNA_20062.10*, which interact with interferon-related genes such as *IFN-TAU* (Interferon Tau), *IFNAG* (Interferon-gamma), *IFNA5* (Interferon Alpha 5), and *IFNW1* (Interferon Omega 1) were downregulated in animals from C1 compared to C3, and in C2 compared to C3 (Table 3). These genes were enriched for GO biological processes related to immune activation, including cell activation involved in immune response (GO:0002263), leukocyte and lymphocyte activation (GO:0002366; GO:0002285) and adaptive immune response (GO:0002250) (Table 6). As members of the interferon family, these cytokines play central roles in immune regulation and inflammation[56,57]and they may also influence adipogenesis and fatty acid metabolism in muscle tissue[58]. In vitro studies have shown that oleic and palmitic acids can upregulate interferon-stimulated genes[59]while elevated levels of ω3 PUFA have been shown to suppress these pathways in mice[60]. The high concentrations of ω3 levels and reduced palmitic and oleic acid content observed in C1 and C2 may contribute to the reduced expression of interferon-associated lncRNAs in these groups.

Cluster 1 exhibited upregulation of *lncRNA_13894.1* relative to Cluster 2 and Cluster 3, which is associated with the *BNIP3* gene. *BNIP3* is implicated in mitochondrial β-oxidation and may influence the balance of ω6 and ω3 fatty acids[61,62].Gene Ontology analysis revealed enrichment for fat-cell differentiation (GO:0045444)

GO: ID	Term	*p*-value	Nr. Genes*	Genes	lncRNAs
Biological process					
GO:0042100	B cell proliferation	0.00	4	*CD180*, *CD81*, *IFN-TAU*, *IFNAG*	*lincRNA_10210.6*, *lincRNA_15009.1*, *lncRNA_20062.10*, *lncRNA_20062.7*
GO:0006898	receptor-mediated endocytosis	0.01	4	*ATAD1*, *CD81*, *LOXL2*, *RAB5A*	*lincRNA_13486.1*, *lincRNA_15009.1*, *lncRNA_20382.10*, *lincRNA_929.1*
GO:0016197	endosomal transport	0.01	4	*DCTN1*, *KLHL20*, *RAB5A*, *TRIM27*	*lincRNA_2042.4*, *lncRNA_5876.5*, *lncRNA_5876.4*, *lincRNA_929.1*, *lincRNA_12128.6*, *lincRNA_12128.7*
GO:0032147	activation of protein kinase activity	0.01	4	*CD81*, *CHI3L1*, *KIF14*, *NKX3-1*	*lincRNA_15009.1*, *lncRNA_5415.4*, *lncRNA_5415.2*, *lncRNA_6086.4*, *lncRNA_20382.10*
GO:0042113	B cell activation	0.01	4	*CD180*, *CD81*, *IFN-TAU*, *IFNAG*	*lincRNA_10210.6*, *lincRNA_15009.1*, *lncRNA_20062.10*, *lncRNA_20062.7*
GO:0046651	lymphocyte proliferation	0.01	4	*CD180*, *CD81*, *IFN-TAU*, *IFNAG*	*lincRNA_10210.6*, *lincRNA_15009.1*, *lncRNA_20062.10*, *lncRNA_20062.7*
GO:0043549	regulation of kinase activity	0.01	7	*ADARB1*, *CD81*, *CHI3L1*, *KIF14*, *NKX3-1*, *TRIM27*, *VAC14*	*lincRNA_791.1*, *lincRNA_15009.1*, *lncRNA_5415.4*, *lncRNA_5415.2*, *lncRNA_6086.4*, *lncRNA_20382.10*, *lincRNA_12128.6*, *lincRNA_12128.7*, *lincRNA_6802.5*
GO:0043112	receptor metabolic process	0.01	3	*ATAD1*, *CD81*, *RAB5A*	*lincRNA_13486.1*, *lincRNA_15009.1*, *lincRNA_929.1*
GO:0070661	leukocyte proliferation	0.02	4	*CD180*, *CD81*, *IFN-TAU*, *IFNAG*	*lincRNA_10210.6*, *lincRNA_15009.1*, *lncRNA_20062.10*, *lncRNA_20062.7*
GO:0021953	central nervous system neuron differentiation	0.02	3	*ADARB1*, *BTG2*, *HOXC10*	*lincRNA_791.1*, *lncRNA_5415.4*, *lncRNA_5415.2*, *lincRNA_17194.2*
GO:0032535	regulation of cellular component size	0.02	4	*DSTN*, *RAB22A*, *RAB5A*, *TRIM27*	*lncRNA_3567.2*, *lincRNA_3855.2*, *lincRNA_929.1*, *lincRNA_12128.6*, *lincRNA_12128.7*
GO:0002285	lymphocyte activation involved in immune response	0.02	3	*CD180*, *IFN-TAU*, *IFNAG*	*lincRNA_10210.6*, *lncRNA_20062.10*, *lncRNA_20062.7*
GO:0051495	positive regulation of cytoskeleton organization	0.03	3	*DCTN1*, *DSTN*, *TRIM27*	*lincRNA_2042.4*, *lncRNA_3567.2*, *lincRNA_12128.6*, *lincRNA_12128.7*
GO:0008285	negative regulation of cell proliferation	0.04	5	*ADARB1*, *ARID2*, *ATOH8*, *BTG2*, *NKX3-1*	*lincRNA_791.1*, *lincRNA_17358.2*, *lincRNA_2305.1*, *lncRNA_5415.4*, *lncRNA_5415.2*, *lncRNA_20382.10*
GO:0006869	lipid transport	0.04	3	*APOL3*, *NKX3-1*, *SPNS3*	*lincRNA_17681.1*, *lncRNA_20382.10*, *lincRNA_8294.10*, *lincRNA_8294.4*
GO:0002366	leukocyte activation involved in immune response	0.04	3	*CD180*, *IFN-TAU*, *IFNAG*	*lincRNA_10210.6*, *lncRNA_20062.10*, *lncRNA_20062.7*
GO:0002263	cell activation involved in immune response	0.04	3	*CD180*, *IFN-TAU*, *IFNAG*	*lincRNA_10210.6*, *lncRNA_20062.10*, *lncRNA_20062.7*
GO:0010638	positive regulation of organelle organization	0.05	5	*CNOT2*, *DCTN1*, *DSTN*, *RUVBL1*, *TRIM27*	*lincRNA_17393.3*, *lincRNA_2042.4*, *lncRNA_3567.2*, *lincRNA_11659.21*, *lincRNA_11659.1*, *lincRNA_11659.14*, *lincRNA_11659.17*, *lincRNA_11659.3*, *lincRNA_12128.6*, *lincRNA_12128.7*
GO:0033674	positive regulation of kinase activity	0.05	4	*CD81*, *CHI3L1*, *KIF14*, *NKX3-1*	*lincRNA_15009.1*, *lncRNA_5415.4*, *lncRNA_5415.2*, *lncRNA_6086.4*, *lncRNA_20382.10*
GO:0010876	lipid localization	0.05	3	*APOL3*, *NKX3-1*, *SPNS3*	*lincRNA_17681.1*, *lncRNA_20382.10*, *lincRNA_8294.10*, *lincRNA_8294.4*
GO:0014070	response to organic cyclic compound	0.05	5	*CNOT2*, *IFN-TAU*, *IFNAG*, *NKX3-1*, *SMAD9*	*lincRNA_17393.3*, *lncRNA_20062.10*, *lncRNA_20062.7*, *lncRNA_20382.10*, *lincRNA_3024.7*, *lincRNA_3024.5*
GO:0002250	adaptive immune response	0.05	3	*IFN-TAU*, *IFNAG*, *TRIM27*	*lncRNA_20062.10*, *lncRNA_20062.7*, *lincRNA_12128.6*, *lincRNA_12128.7*
GO:0034440	lipid oxidation	0.05	1	*AUH*	*lincRNA_20515.12*, *lincRNA_20515.13*, *lincRNA_20515.10*
Cellular component					
GO:1,990,234	transferase complex	0.01	8	*E2F6*, *GTF2A1*, *HOXC10*, *KLHL20*, *KLHL9*, *RUVBL1*, *SETD1B*, *VAC14*	*lincRNA_2540.2*, *lincRNA_2540.4*, *lincRNA_1840.2*, *lincRNA_17194.2*, *lncRNA_5876.5*, *lncRNA_5876.4*, *lncRNA_20062.10*, *lncRNA_20062.7*, *lincRNA_11659.21*, *lincRNA_11659.1*, *lincRNA_11659.14*, *lincRNA_11659.17*, *lincRNA_11659.3*, *lncRNA_6442.5*, *lincRNA_6802.5*
GO:0015629	actin cytoskeleton	0.02	5	*DCTN1*, *DSTN*, *IVNS1ABP*, *RAB22A*, *RAB5A*	*lincRNA_2042.4*, *lncRNA_3567.2*, *lincRNA_5964.5*, *lincRNA_5964.1*, *lncRNA_5964.6*, *lincRNA_3855.2*, *lincRNA_929.1*
GO:0035770	ribonucleoprotein granule	0.03	3	*CNOT2*, *PCBP1*, *SMN2*	*lincRNA_17393.3*, *lincRNA_2417.4*, *lincRNA_10184.3*, *lincRNA_10184.1*
GO:0005938	cell cortex	0.04	3	*BFSP1*, *DCTN1*, *DSTN*	*lncRNA_3567.2*, *lincRNA_2042.4*
Molecular function					
GO:0008134	transcription factor binding	0.00	7	*ATOH8*, *CNOT2*, *GTF2A1*, *NAB2*, *NKX3-1*, *RCOR3*, *RUVBL1*	*lincRNA_2305.1*, *lincRNA_17393.3*, *lincRNA_1840.2*, *lincRNA_17511.1*, *lincRNA_17511.6*, *lncRNA_20382.10*, *lncRNA_6037.8*, *lincRNA_11659.21*, *lincRNA_11659.1*, *lincRNA_11659.14*, *lincRNA_11659.17*, *lincRNA_11659.3*

Table 6. Gene ontology (GO) - Biological process, cellular component, and molecular Function - associated with genes interacting with differentially expressed LncRNAs and LincRNAs identified in the comparison between C2 and C3 fatty acid clusters. *Nr. Genes: Number of genes.

lncRNAs located in sense direction [a]								
Feature ID	**Positon**	**Length[b]**	***p*-Value**	**FDR [c]**	**FC(log2) [d]**	**mRNA Interaction [e]**	**Distance [f]**	**Interaction location [g]**
lincRNA_20557.5	8:91054949–91,086,297	791	6.50E-13	1.26E-10	6.76	ENSBTAT00000079956	14,797	upstream
lincRNA_7736.5	18:59685331–59,696,073	1112	2.60E-09	2.95E-07	6.23	ENSBTAT00000053465	78,735	upstream
lincRNA_2305.1	11:49143024–49,147,255	855	4.03E-08	3.76E-06	3.13	ATOH8-201	16,527	upstream
lincRNA_5255.1	15:65502793–65,506,317	2386	1.01E-04	4.25E-03	2.49	PDHX-201	170	downstream
lincRNA_9933.3	2:126223556–126,230,654	1190	1.69E-04	6.54E-03	2.04	TRNP1-201	927	downstream
lincRNA_10558.2	21:16690280–16,704,571	7132	3.04E-04	1.06E-02	3.26	ENSBTAT00000069947	17,948	downstream
lincRNA_9517.5	2:52904578–52,913,931	3169	2.69E-21	1.44E-18	-9.83	ARHGAP15-201	51,209	downstream
lincRNA_5265.1	15:65520195–65,531,625	1885	1.47E-19	6.62E-17	-5.83	PDHX-201	17,572	downstream
lincRNA_11848.4	23:14861716–14,866,193	1681	1.02E-17	3.58E-15	-7.26	UNC5CL-201	85,367	downstream
lincRNA_9517.4	2:52904578–52,913,931	3157	7.74E-17	2.50E-14	-5.91	ARHGAP15-201	51,221	downstream
lincRNA_20515.12	8:86704251–86,724,738	3110	3.62E-10	4.74E-08	-6.88	AUH-203	18,194	downstream
lincRNA_19092.2	7:12412390–12,426,648	2649	2.09E-09	2.40E-07	-3.82	IER2-201	7211	downstream
lincRNA_2526.3	11:82614309–82,639,458	4315	2.48E-08	2.38E-06	-8.31	DDX1-201	38,762	downstream
lincRNA_7736.1	18:59685331–59,696,073	1111	4.73E-06	2.79E-04	-2.86	ENSBTAT00000053465	78,735	upstream
lincRNA_10320.5	20:35647442–35,665,372	2876	5.10E-05	2.35E-03	-4.52	OSMR-201	75,932	upstream
lincRNA_4171.3	13:78409884–78,447,044	2212	1.67E-04	6.49E-03	-11.44	ENSBTAT00000079695	70,437	downstream
lincRNA_13086.2	25:25769133–25,823,226	880	1.70E-04	6.58E-03	12.97	SBK1-201	27,121	upstream
lincRNA_5964.5	16:66395474–66,405,633	1887	1.81E-04	6.90E-03	-4.48	IVNS1ABP-206	96,159	upstream
lincRNA_2540.2	11:86418716–86,464,641	1033	4.45E-04	1.42E-02	-12.02	E2F6-201	16,736	downstream
lincRNA_17103.9	5:5588820–5,627,943	3571	5.33E-04	1.65E-02	-11.12	PHLDA1-201	12,947	upstream
lincRNA_10900.4	21:58589214–58,593,395	651	6.15E-04	1.86E-02	-2.42	CCDC197-201	10,826	upstream
lincRNA_9841.2	2:120677247–120,682,616	3616	1.36E-03	3.55E-02	-2.02	AK2-201	55	downstream
lincRNA_5964.1	16:66395474–66,405,633	1811	1.52E-03	3.87E-02	-4.90	IVNS1ABP-206	96,159	upstream
lincRNAs located in antisense direction [a]								
Feature ID	**Positon**	**Length [b]**	**p-Value**	**FDR [c]**	**FC (log2) [d]**	**mRNA Interaction [e]**	**Distance [f]**	**Interaction location [g]**
lincRNA_5578.3	16:29120343–29,123,464	2479	7.94E-21	4.11E-18	6.63	H3-3 A-201	32,484	downstream
lincRNA_8796.3	19:44436479–44,443,655	1713	2.29E-08	2.21E-06	6.77	CCDC43-201	85	upstream
lincRNA_17393.3	5:43266505–43,268,113	357	4.92E-08	4.51E-06	14.53	CNOT2-201	110	upstream
lincRNA_5714.2	16:43978464–43,985,353	3333	6.05E-07	4.40E-05	2.89	SLC25A33-201	31,765	upstream
lincRNA_8294.10	19:24908019–24,914,528	4663	1.52E-04	6.01E-03	2.23	SPNS3-201	22,427	upstream
lincRNA_3119.2	12:34735808–34,750,812	3111	3.20E-04	1.11E-02	2.19	SGCG-201	29,925	upstream
lincRNA_18383.11	6:24210504–24,526,275	3655	3.57E-04	1.20E-02	2.08	DDIT4L-201	360	upstream
lincRNA_8294.4	19:24908019–24,914,528	4783	5.06E-04	1.58E-02	3.78	SPNS3-201	22,307	upstream
lincRNA_15543.1	3:29450215–29,462,055	2414	1.20E-03	3.20E-02	3.32	HIPK1-201	269	upstream
lincRNA_10184.3	20:10097610–10,105,934	1436	1.49E-03	3.82E-02	3.20	SMN2-201	44	upstream
lincRNA_13547.2	26:15877179–15,880,904	1156	1.85E-03	4.55E-02	2.65	NOC3L-202	84	upstream
lincRNA_15009.1	29:49217825–49,218,972	606	6.03E-19	2.58E-16	-5.78	CD81-201	1014	upstream
lincRNA_20981.1	9:52492142–52,495,493	706	7.48E-19	3.15E-16	-9.70	MMS22L-202	1636	upstream
lincRNA_17358.2	5:34560404–34,576,263	1840	2.78E-12	4.89E-10	-10.03	ARID2-202	41,974	upstream
lincRNA_15447.1	3:20935933–20,944,419	2180	2.31E-09	2.64E-07	-10.74	MGC134040-201	6782	upstream
lincRNA_12128.6	1:7104210–7,114,117	918	1.70E-07	1.41E-05	-6.87	TRIM27-201	88	upstream
lincRNA_20224.2	8:55767546–55,825,069	1610	2.56E-07	2.05E-05	-10.80	TLE4-204	37,635	downstream
lincRNA_280.3	1:69127181–69,127,765	425	2.91E-07	2.28E-05	-3.26	UMPS-201	102	upstream
lincRNA_10919.2	21:60379945–60,383,584	1353	1.99E-06	1.29E-04	-3.19	SYNE3-201	92	upstream
lincRNA_18394.1	6:25791826–25,795,075	1514	7.32E-05	3.21E-03	-4.56	EIF4E-202	86,079	downstream
lincRNA_8476.5	19:33209590–33,211,733	801	1.74E-04	6.68E-03	-13.53	LRRC75A-202	367	downstream
lincRNA_12128.6	23:30046878–30,066,974	1750	2.54E-04	9.14E-03	-12.48	TRIM27-201	1344	downstream
lincRNA_14743.1	29:37152089–37,169,227	896	3.20E-04	1.11E-02	-11.32	CCDC86-201	515	upstream
lincRNA_3024.7	12:24728114–24,731,701	742	3.72E-04	1.24E-02	-12.26	SMAD9-201	180	upstream
lincRNA_6783.6	17:72362986–72,382,214	4197	7.54E-04	2.19E-02	-6.53	SLC7A4-201	605	upstream
Continued								

lincRNAs located in antisense direction [a]								
Feature ID	Positon	Length [b]	p-Value	FDR [c]	FC (log2) [d]	mRNA Interaction [e]	Distance [f]	Interaction location [g]
lincRNA_6371.2	17:51604344–51,756,900	1413	7.54E-04	2.19E-02	-2.42	CCDC92-201	756	upstream
lincRNA_15447.4	3:20935933–20,944,419	1346	8.08E-04	2.31E-02	-2.26	MGC134040-201	6796	upstream
lincRNA_18711.6	6:102631399–102,685,256	2552	1.00E-03	2.77E-02	-10.93	ENSBTAT00000008532	565	downstream
lincRNA_11659.21	22:59504587–59,508,832	2979	1.78E-03	4.40E-02	-5.11	RUVBL1-201	221	upstream
lincRNA_7507.1	18:52318710–52,407,580	2625	1.97E-03	4.78E-02	-2.97	ZNF180-203	3074	upstream

Table 7. Novel long intergenic noncoding RNAs differentially expressed in *Longissimus thoracis* muscle of Nellore cattle in the C1-fatty acid cluster compared to the C2-fatty acid cluster. [a] Direction of transcription of proximal RNA transcripts; [b] Transcript Length in basis pair; [c] FC (log2) = Fold change (log2); [d] False Discovery Rate; [e] transcript interaction = lincRNA gene overlaps with a transcript gene from the bovine reference annotation (ARS.UCD1.2). ; [f] Distance = distance between lincRNA and closest RNA transcript in the bovine reference annotation (ARS.UCD1.2); [g] Interaction location = are defined according to the type of interactions (genic or intergenic), assuming that intergenic lncRNA are not overlapping any RNA transcript (RNA/gene), then it can be further classified in: upstream (the lincRNA is upstream transcribed in head-to-head or tail-to-tail orientation with RNA partner or yet both same orientation) or downstream (the lincRNA is downstream transcribed in head-to-head or tail-to-tail orientation with RNA partner or yet both in same orientation).

(Table 5). The *BNIP3* acts in different metabolic pathways, such as lipid metabolism[61]. Functionally, *BNIP3* interacts with acetyl-CoA acyltransferase 2 (*ACAA2*), a mitochondrial enzyme catalyzing the terminal step of fatty acid β-oxidation[61,63]. In sheep, single-nucleotide polymorphisms in *ACAA2* have been linked to variations in the milk ω6/ω3 ratio[64] and studies in fish have shown that dietary ω3 supplementation increases *BNIP3* expression[66]. Together, these data suggest that *lncRNA_13894.1* may drive *BNIP3* upregulation, supporting the ω6- and ω3-enriched lipid profile observed in Cluster 1 (Table 3).

Conversely, *lncRNA_16456.3*, which targets the *IL6* (Interleukin-6) gene, was downregulated in Cluster 1 versus Cluster 2 (Table 3). *IL6* encodes a pro-inflammatory adipokine that modulates lipid homeostasis, fatty acid oxidation, and lipolysis in adipocytes and skeletal muscle[65,66] and is enriched for cellular lipid response (GO:0071396) (Table 4). Interestingly, palmitic acid - a predominant saturated fatty acid - induces *IL6* expression via inflammatory signaling pathways[67–69]. The reduced expression of *lncRNA_16456.3* in C1 corresponds to diminished *IL6* activity, consistent with the lower SFA levels in this group. However, *IL6* has also been mapped near quantitative trait loci affecting PUFA, CLA, and SFA content in pigs, suggesting a pleiotropic role in fatty-acid composition[70–74].

Differentially expressed intergenic long non-coding RNA

In Cluster 1, several lincRNAs were significantly downregulated in relation to C2 and C3, respectively, potentially reflecting attenuated stress and inflammatory signaling. Notably, *lincRNA_18394.1* and *lincRNA_2526.3* were both downregulated in C1 compared to C2 and C3 (Tables 7 and 8). These lincRNAs target the *EIF4E* (Eukaryotic Translation Initiation Factor 4E) and *DDX1* (DEAD-Box Helicase 1) genes, respectively. The *EIF4E* was enriched to the "cellular response to lipid" (GO:0071396) GO term (Table 4). This gene is a key regulator of lipid homeostasis and energy metabolism in adipose tissue, coordinating fatty acid synthesis and oxidation[75] and it is known to play a role in milk fatty acid synthesis in bovine mammary epithelial cells[76]. By contrast, *DDX1* participates in DNA repair and mRNA transport[77]. Li et al.[78] observed that the DDX1 protein binds to the untranslated region of insulin mRNA, inhibiting its translation, and that saturated fatty acids such as palmitic acid diminish insulin secretion in pancreatic β-cells, contributing to insulin resistance and diabetes[79–83]. Indeed, chronic exposure to SFAs is recognized as a driver of insulin resistance. Thus, the downregulation of *lincRNA_18394.1* and *lincRNA_2526.3* in Cluster 1 may indicate reduced activation of stress and insulin-resistance pathways, consistent with the lower total SFA levels observed in this group.

In the comparison between Cluster 1 (C1) and Cluster 2 (C2), the differentially expressed lincRNA, *lincRNA_17393.3* was significantly upregulated in C1 and is associated with the *CNOT2* gene (Table 7). *CNOT2* (CCR4-NOT Transcription Complex Subunit 2) is enriched for GO biological process terms related to hormonal response (GO:0009725) and cellular response to lipid (GO:0071396) (Table 4). This gene encodes a key component of the CCR4-NOT complex, which plays a pivotal role in post-transcriptional and transcriptional regulation, including mRNA degradation and translational control[84,85]. Functionally, *CNOT2* has been implicated in adipocyte differentiation and fatty acids synthesis. Sohn et al.[86] demonstrated that overexpression of *CNOT2* in 3T3-L1 cells promotes adipogenesis and lipogenesis by upregulating transcription factors *PPARγ* and *CEBPα*, both of which are master regulators of lipid metabolism. Interestingly, several studies have reported that *PPARγ* and *CEBPα* expression is positively influenced by ω3 polyunsaturated fatty acids (PUFAs)[87,88]. Given the observed upregulation of *lincRNA_17393.3* in C1 and the corresponding increase in total PUFA (including ω3) levels compared to C2, this lincRNA may contribute to the enhanced PUFA profile in C1 through regulation of *CNOT2*-mediated lipogenic pathways.

In the C1 vs. C3 comparison, *lincRNA_17194.2* was downregulated in C1 and was associated with the *HOXC10* (Homeobox C10) gene (Table 8). *HOXC10* has previously been implicated in key biological processes such as angiogenesis[89]fat metabolism[90,91] and sexual regulation[92]. Kang et al.[93] reported *HOXC10* as a candidate gene

lncRNAs located in sense direction [a]								
Feature ID	**Positon**	**Length (pb)** [b]	***p*-Value**	**FDR** [c]	**FC (log2)** [d]	**mRNA Interaction** [e]	**Distance** [f]	**Interaction location** [g]
lincRNA_13486.1	26:9373528–9,377,974	2490	1.41E-20	1.01E-17	8.10	ATAD1-201	1300	downstream
lincRNA_3864.2	13:58736919–58,750,531	1453	1.77E-14	4.32E-12	7.13	RBM38-201	7936	downstream
lincRNA_20557.5	8:91054949–91,086,297	791	3.91E-13	7.73E-11	5.58	ENSBTAT00000079956	14,797	upstream
lincRNA_10320.8	20:35647442–35,665,372	2557	4.37E-09	3.98E-07	9.41	OSMR-201	75,932	upstream
lincRNA_5255.1	15:65502793–65,506,317	2386	3.61E-07	2.26E-05	3.91	PDHX-201	170	downstream
lincRNA_10900.4	21:58589214–58,593,395	651	3.66E-07	2.28E-05	5.83	CCDC197-201	10,826	upstream
lincRNA_10720.6	21:33142026–33,155,354	5446	3.95E-07	2.44E-05	8.44	ODF3L1-201	12,461	downstream
lincRNA_2540.4	11:86418716–86,464,641	856	1.18E-06	6.39E-05	8.12	E2F6-201	16,909	downstream
lincRNA_20515.13	8:86704251–86,724,738	2977	3.80E-06	1.83E-04	6.22	AUH-203	18,207	downstream
lincRNA_3050.2	12:30041465–30,044,471	2760	1.04E-05	4.54E-04	3.70	MEDAG-201	26,845	upstream
lincRNA_10558.1	21:16690280–16,704,571	7144	6.68E-05	2.49E-03	3.84	ENSBTAT00000069947	17,935	downstream
lincRNA_10558.14	21:16690280–16,704,571	6381	3.34E-04	9.72E-03	12.04	ENSBTAT00000069947	18,323	downstream
lincRNA_10558.9	21:16690280–16,704,571	6833	4.50E-04	1.23E-02	10.39	ENSBTAT00000069947	18,000	downstream
lncRNA_10320.7	20:35647442–35,665,372	2557	6.73E-04	1.70E-02	3.85	OSMR-201	75,932	upstream
lincRNA_16508.10	4:43223448–43,279,119	4572	1.14E-03	2.63E-02	11.50	PHTF2-201	41,821	downstream
lincRNA_20515.10	8:86704251–86,724,738	3120	1.78E-03	3.81E-02	4.97	AUH-203	18,184	downstream
lincRNA_9517.4	2:52904578–52,913,931	3157	1.66E-22	1.76E-19	-7.92	ARHGAP15-201	51,221	downstream
lincRNA_5265.1	15:65520195–65,531,625	1885	1,55E-20	1,08E-17	-6,58	PDHX-201	17,572	downstream
lincRNA_1878.2	10:102749910–102,772,451	4686	2.82E-17	1.09E-14	-6.47	U6-201	8839	downstream
lincRNA_17681.1	5:74669803–74,711,373	1010	1.74E-11	2.49E-09	-5.55	APOL3-201	11,739	upstream
lincRNA_20515.12	8:86704251–86,724,738	3110	3.79E-10	4.27E-08	-6.90	AUH-203	18,194	downstream
lincRNA_19092.2	7:12412390–12,426,648	2649	1.69E-09	1.69E-07	-4.10	IER2-201	7211	downstream
lincRNA_2526.3	11:82614309–82,639,458	4315	1.42E-06	7.54E-05	-8.61	DDX1-201	38,762	downstream
lincRNA_5964.1	16:66395474–66,405,633	1811	7.94E-05	2.91E-03	-6.48	IVNS1ABP-206	96,159	upstream
lincRNA_5964.6	16:66395474–66,405,633	1890	2.63E-04	8.01E-03	-4.09	IVNS1ABP-206	96,159	upstream
lincRNA_16442.1	4:29193479–29,195,379	688	3.29E-04	9.59E-03	-2.59	ITGB8-201	77,720	downstream
lincRNA_10720.7	21:33142026–33,155,354	2129	1.69E-03	3.63E-02	-3.79	ODF3L1-201	12,368	downstream
lincRNA_7736.1	18:59685331–59,696,073	1111	1.82E-03	3.87E-02	-2.09	ENSBTAT00000053465	78,735	upstream
lincRNA_9841.2	2:120677247–120,682,616	3616	2.36E-03	4.84E-02	-2.64	AK2-201	55	downstream
lincRNAs located in antisense direction [a]								
Feature ID	**Positon**	**Length (pb)** [b]	**p-Value**	**FDR** [c]	**FC (log2)** [d]	**mRNA Interaction** [e]	**Distance** [f]	**Interaction location** [g]
lincRNA_18738.3	6:108831172–108,833,800	2254	1.62E-21	1.43E-18	8.41	BOD1L1-201	165	upstream
lincRNA_17511.1	5:56346812–56,351,225	553	8.71E-18	3.72E-15	9.25	NAB2-201	286	upstream
lincRNA_16747.1	4:76603706–76,606,680	2756	2.08E-16	7.21E-14	7.95	CCM2-203	239	upstream
lincRNA_18738.2	6:108831172–108,833,800	2257	6.65E-15	1.71E-12	6.13	BOD1L1-201	162	upstream
lincRNA_18001.6	5:108915063–108,924,973	5266	2.19E-12	3.76E-10	8.62	CECR2-201	12,869	upstream
lincRNA_10184.1	20:10097610–10,105,934	1477	2.29E-11	3.18E-09	7.74	SMN2-201	62	upstream
lincRNA_10586.5	21:21519283–21,537,669	2678	8.21E-11	1.03E-08	11.66	ENSBTAT00000031184	2866	downstream
lincRNA_6258.4	17:18453124–18,474,698	914	3.88E-10	4.36E-08	8.57	ELF2-201	147	upstream
lincRNA_17577.2	5:62725526–62,731,661	1158	3.38E-09	3.16E-07	4.51	TMPO-201	12,982	downstream
lincRNA_8796.1	19:44436479–44,443,655	1717	7.06E-09	6.25E-07	9.87	CCDC43-201	76	upstream
lincRNA_15455.1	3:20557127–20,560,106	1103	7.88E-09	6.89E-07	10.62	VPS45-201	139	upstream
lincRNA_2042.4	11:10233550–10,238,310	936	1.14E-08	9.67E-07	8.13	DCTN1-201	2229	upstream
lincRNA_5578.3	16:29120343–29,123,464	2479	2.08E-07	1.37E-05	6.95	H3-3 A-201	32,484	downstream
lincRNA_11659.1	22:59504587–59,508,832	3008	7.97E-07	4.54E-05	10.73	RUVBL1-201	189	upstream
lincRNA_12128.7	23:30046878–30,066,974	1748	1.93E-06	9.93E-05	10.44	TRIM27-201	1344	downstream
lincRNA_11659.14	22:59504587–59,508,832	3229	2.97E-06	1.47E-04	7.29	RUVBL1-201	187	upstream
lincRNA_18674.6	6:97716507–97,750,095	1377	3.65E-06	1.77E-04	6.65	THAP9-201	2602	upstream
lincRNA_3855.2	13:58045731–58,049,015	1933	7.19E-06	3.23E-04	3.17	RAB22A-201	101	upstream
lincRNA_791.1	1:144934585–144,937,622	2739	7.97E-06	3.55E-04	4.48	ADARB1-201	204	upstream
lincRNA_8476.6	19:33209590–33,211,733	1021	2.85E-05	1.14E-03	7.20	LRRC75A-202	367	downstream
lincRNA_929.1	1:157196721–157,198,350	1337	5.73E-05	2.17E-03	4.94	RAB5A-201	151	upstream
lincRNA_11659.17	22:59504587–59,508,832	3069	9.52E-05	3.42E-03	6.37	RUVBL1-201	188	upstream
lincRNA_2036.1	11:10142822–10,146,171	2000	1.22E-04	4.23E-03	3.14	LBX2-201	2603	downstream
lincRNA_8294.4	19:24908019–24,914,528	4783	1.70E-04	5.61E-03	12.95	SPNS3-201	22,307	upstream

Continued

lincRNAs located in antisense direction [a]								
Feature ID	Positon	Length (pb) [b]	p-Value	FDR [c]	FC (log2) [d]	mRNA Interaction [e]	Distance [f]	Interaction location [g]
lincRNA_3433.4	13:17510294–17,518,504	1169	1.72E-04	5.65E-03	12.94	IL15RA-201	121	upstream
lincRNA_5714.2	16:43978464–43,985,353	3333	1.97E-04	6.32E-03	2.53	SLC25A33-201	31,765	upstream
lincRNA_3024.5	12:24728114–24,731,701	896	2.16E-04	6.82E-03	12.14	SMAD9-201	165	upstream
lincRNA_20692.2	8:111823798–111,834,775	3395	2.38E-04	7.38E-03	2.49	MYT1L-201	86,892	upstream
lincRNA_8476.12	19:33209590–33,211,733	1027	2.57E-04	7.88E-03	12.76	LRRC75A-202	367	downstream
lincRNA_17511.6	5:56346812–56,351,225	562	2.62E-04	7.99E-03	12.74	NAB2-201	1804	upstream
lincRNA_18001.15	5:108915063–108,924,973	4906	2.85E-04	8.57E-03	12.62	CECR2-201	12,876	upstream
lincRNA_6258.1	17:18453124–18,474,698	801	3.24E-04	9.48E-03	12.45	ELF2-201	1432	upstream
lincRNA_18001.17	5:108915063–108,924,973	5263	4.86E-04	1.31E-02	3.22	CECR2-201	12,878	upstream
lincRNA_1840.2	10:92511630–92,522,957	1195	7.11E-04	1.78E-02	3.83	GTF2A1-201	161	upstream
lincRNA_11659.3	22:59504587–59,508,832	3272	8.38E-04	2.03E-02	11.46	RUVBL1-201	188	upstream
lincRNA_15403.1	3:19689039–19,726,184	1166	1.28E-03	2.90E-02	11.14	MLLT11-201	410	downstream
lincRNA_18674.5	6:97716507–97,750,095	1465	1.77E-03	3.79E-02	9.31	THAP9-201	1096	upstream
lincRNA_17653.3	5:73720268–73,724,709	3062	2.17E-03	4.50E-02	2.30	RASD2-201	24,336	upstream
lincRNA_18001.14	5:108915063–108,924,973	5266	2.24E-03	4.62E-02	3.19	CECR2-201	12,869	upstream
lincRNA_20041.2	8:16267188–16,287,915	1056	1.77E-19	1.01E-16	-10.37	LINGO2-201	11,117	upstream
lincRNA_20981.1	9:52492142–52,495,493	706	2.65E-19	1.45E-16	-10.80	MMS22L-202	1636	upstream
lincRNA_17194.2	5:26047734–26,049,634	869	2.23E-12	3.81E-10	− 9.12	HOXC10-201	369	upstream
lincRNA_20224.2	8:55767546–55,825,069	1610	3.43E-08	2.67E-06	-13.89	TLE4-204	37,635	downstream
lincRNA_38.1	1:7104210–7,114,117	918	3.84E-07	2.38E-05	-8.67	CCT8-201	88	upstream
lincRNA_15447.4	3:20935933–20,944,419	1346	1.87E-05	7.75E-04	-3.37	MGC134040-201	6796	upstream
lincRNA_2832.1	11:104798248–104,801,001	2140	4.33E-05	1.68E-03	-2.47	BRD3-201	3413	downstream
lincRNA_12128.6	23:30046878–30,066,974	1750	7.19E-05	2.66E-03	-14.61	TRIM27-201	1344	downstream
lincRNA_14743.1	29:37152089–37,169,227	896	1.01E-04	3.58E-03	-13.40	CCDC86-201	515	upstream
lincRNA_3024.7	12:24728114–24,731,701	742	1.53E-04	5.12E-03	-14.73	SMAD9-201	180	upstream
lincRNA_6802.5	18:1343804–1,405,315	1285	1.41E-04	4.79E-03	12.72	VAC14-202	6224	upstream
lincRNA_6783.6	17:72362986–72,382,214	4197	6.04E-04	1.56E-02	-7.45	SLC7A4-201	605	upstream
lincRNA_18394.1	6:25791826–25,795,075	1514	8.06E-04	1.97E-02	-4.31	EIF4E-202	86,079	downstream
lincRNA_3863.4	13:58694491–58,705,765	984	1.12E-03	2.60E-02	-2.57	CTCFL-201	11,892	downstream
lincRNA_8796.4	19:44436479–44,443,655	1699	1.61E-03	3.50E-02	-2.61	CCDC43-201	98	upstream
lincRNA_18001.20	5:108915063–108,924,973	5271	1.87E-03	3.95E-02	-4.23	CECR2-201	12,869	upstream
lincRNA_10586.3	21:21519283–21,537,669	5164	2.28E-03	4.70E-02	-4.16	ENSBTAT00000031184	2866	downstream

Table 8. Novel long intergenic noncoding RNAs differentially expressed in *Longissimus thoracis* muscle of Nellore cattle in the C1-fatty acid cluster compared to C3-fatty acid cluster. [a] Direction of transcription of proximal RNA transcripts; [b] Transcript Length in basis pair; [c] FC (log2) = Fold change (log2); [d] False Discovery Rate; [e] transcript interaction = lincRNA gene overlaps with a transcript gene from the bovine reference annotation (ARS.UCD1.2). ; [f] Distance = distance between lincRNA and closest RNA transcript in the bovine reference annotation (ARS.UCD1.2); [g] Interaction location = are defined according to the type of interactions (genic or intergenic), assuming that intergenic lncRNA are not overlapping any RNA transcript (RNA/gene), then it can be further classified in: upstream (the lincRNA is upstream transcribed in head-to-head or tail-to-tail orientation with RNA partner or yet both same orientation) or downstream (the lincRNA is downstream transcribed in head-to-head or tail-to-tail orientation with RNA partner or yet both in same orientation).

influencing adipose deposition in fat-tailed sheep, while human study suggests its role in obesity and cholesterol regulation[94,95]. It is interesting to note that the presence of fatty acids can also modulate cellular cholesterol uptake and lipoprotein secretion. A study in humans showed that the presence of ω3 PUFAs reduced the expression of 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase), a key enzyme in cholesterol synthesis[96,97]. Taken together, these data suggest that reduced expression of *lincRNA_17194.2* in C1 may reflect lower *HOXC10* activity in line with the high PUFA levels observed in C1 compared to Cluster 3.

In both C1 vs. C3 and C2 vs. C3 comparison, the lincRNA, *lincRNA_17681.1*, was downregulated and was associated with the *APOL3* (APOL-I to VI) gene (Tables 8 and 9). The *APOL3* was enriched to GO-biological function terms related to lipid localization (GO: 0010876) and to lipid transport (GO:0006869) (Tables 5 and 6). *APOL3* is known to be involved in cholesterol and sphingolipids transport and recycling in skeletal muscle in human[98,99]and in intramuscular fat (IMF) deposition in cattle[100,101]. The lower expression of *lincRNA_17681.1* in C1 and C2 may reflect reduced activity of *APOL3*, consistent with the lower SFA levels (including myristic, palmitic, and stearic acids) observed in these clusters relative to C3. Together, these findings suggest that

lincRNAs located in sense direction [a]								
Feature ID	**Positon**	**Length [b]**	***p*-Value**	**FDR [c]**	**FC (log2) [d]**	**mRNA Interaction [e]**	**Distance [f]**	**Interaction location [g]**
lincRNA_7736.5	18:59685331–59,696,073	1112	8.56E-11	1.05E-08	6.16	ENSBTAT00000053465	78,735	upstream
lincRNA_9933.3	2:126223556–126,230,654	1190	5.22E-09	4.35E-07	3.61	TRNP1-201	927	downstream
lincRNA_10558.2	21:16690280–16,704,571	7132	2.29E-07	1.41E-05	4.98	ENSBTAT00000069947	17,948	downstream
lincRNA_2305.1	11:49143024–49,147,255	855	2.07E-06	1.01E-04	2.73	ATOH8-201	16,527	upstream
lincRNA_13486.1	26:9373528–9,377,974	2490	5.24E-22	4.27E-19	-8.31	ATAD1-201	1300	downstream
lincRNA_9517.5	2:52904578–52,913,931	3169	2.16E-19	1.17E-16	-9.76	ARHGAP15-201	51,209	downstream
lincRNA_3864.2	13:58736919–58,750,531	1453	5.77E-16	1.65E-13	-7.39	RBM38-201	7936	downstream
lincRNA_10900.4	21:58589214–58,593,395	651	7.42E-11	9.24E-09	-8.25	CCDC197-201	10,826	upstream
lincRNA_17681.1	5:74669803–74,711,373	1010	6.50E-10	6.72E-08	-4.77	APOL3-201	11,739	upstream
lincRNA_11848.4	23:14861716–14,866,193	1681	3.04E-09	2.70E-07	-6.31	UNC5CL-201	85,367	downstream
lincRNA_10320.8	20:35647442–35,665,372	2557	5.36E-09	4.46E-07	-9.86	OSMR-201	75,932	upstream
lincRNA_20515.13	8:86704251–86,724,738	2977	8.68E-08	5.83E-06	-7.23	AUH-203	18,207	downstream
lincRNA_10720.6	21:33142026–33,155,354	5446	9.51E-08	6.34E-06	-8.51	ODF3L1-201	12,461	downstream
lincRNA_5964.5	16:66395474–66,405,633	1887	4.68E-07	2.70E-05	-12.51	IVNS1ABP-206	96,159	upstream
lincRNA_2540.4	11:86418716–86,464,641	856	4.46E-06	2.01E-04	-7.85	E2F6-201	16,909	downstream
lincRNA_3470.1	13:23312186–23,369,059	4082	1.02E-04	3.38E-03	-2.32	ENSBTAT00000067334	45,318	downstream
lincRNA_20515.10	8:86704251–86,724,738	3120	1.89E-04	5.67E-03	-5.74	AUH-203	18,184	downstream
lincRNA_10558.14	21:16690280–16,704,571	6381	3.30E-04	8.99E-03	-11.93	ENSBTAT00000069947	18,323	downstream
lincRNA located in antisense direction [a]								
Feature ID	**Positon**	**Length [b]**	**p-Value**	**FDR [c]**	**FC (log2) [d]**	**mRNA Interaction [e]**	**Distance [f]**	**Interaction location [g]**
lincRNA_20041.2	8:16267188–16,287,915	1056	2.05E-09	1.89E-07	4.57	LINGO2-201	11,117	upstream
lincRNA_17393.3	5:43266505–43,268,113	357	4.00E-08	2.85E-06	14.89	CNOT2-201	110	upstream
lincRNA_5578.2	16:29120343–29,123,464	2479	3.23E-06	1.51E-04	2.96	H3-3 A-201	32,484	downstream
lincRNA_2417.4	11:68352230–68,367,299	1440	8.63E-06	3.68E-04	4.14	PCBP1-201	44,166	upstream
lincRNA_8294.10	19:24908019–24,914,528	4663	3.12E-04	8.59E-03	2.87	SPNS3-201	22,427	upstream
lincRNA_7978.2	19:8080110–8,091,884	3121	3.53E-04	9.51E-03	13.23	MSI2-205	451	upstream
lincRNA_18738.2	6:108831172–108,833,800	2257	1.33E-20	8.89E-18	-7.61	BOD1L1-201	162	upstream
lincRNA_17511.1	5:56346812–56,351,225	553	1.86E-19	1.03E-16	-9.49	NAB2-201	286	upstream
lincRNA_16747.1	4:76603706–76,606,680	2756	1.13E-17	4.34E-15	-8.66	CCM2-203	239	upstream
lincRNA_15009.1	29:49217825–49,218,972	606	2.22E-13	4.30E-11	-6.76	CD81-201	1014	upstream
lincRNA_17194.2	5:26047734–26,049,634	869	3.11E-13	5.92E-11	-9.73	HOXC10-201	369	upstream
lincRNA_10184.1	20:10097610–10,105,934	1477	1.88E-11	2.67E-09	-7.02	SMN2-201	62	upstream
lincRNA_2042.4	11:10233550–10,238,310	936	6.55E-11	8.23E-09	-8.15	DCTN1-201	2229	upstream
lincRNA_10586.5	21:21519283–21,537,669	2678	7.73E-10	7.86E-08	-9.83	ENSBTAT00000031184	2866	downstream
lincRNA_15455.1	3:20557127–20,560,106	1103	4.17E-09	3.56E-07	-9.59	VPS45-201	139	upstream
lincRNA_3433.2	13:17510294–17,518,504	1039	1.80E-08	1.36E-06	-3.38	IL15RA-201	109	upstream
lincRNA_8796.1	19:44436479–44,443,655	1717	1.16E-07	7.59E-06	-8.42	CCDC43-201	76	upstream
lincRNA_17577.2	5:62725526–62,731,661	1158	3.57E-07	2.12E-05	-3.59	TMPO-201	12,982	downstream
lincRNA_3855.2	13:58045731–58,049,015	1933	9.01E-07	4.80E-05	-3.24	RAB22A-201	101	upstream
lincRNA_17358.2	5:34560404–34,576,263	1840	1.26E-06	6.49E-05	-9.96	ARID2-202	41,974	upstream
lincRNA_11659.14	22:59504587–59,508,832	3229	1.62E-06	8.15E-05	-7.35	RUVBL1-201	187	upstream
lincRNA_11659.1	22:59504587–59,508,832	3008	2.17E-06	1.05E-04	-10.27	RUVBL1-201	189	upstream
lincRNA_12128.7	23:30046878–30,066,974	1748	3.26E-06	1.53E-04	-9.98	TRIM27-201	1344	downstream
lincRNA_18674.6	6:97716507–97,750,095	1377	2.35E-05	9.10E-04	-5.76	THAP9-201	2602	upstream
lincRNA_10919.2	21:60379945–60,383,584	1353	3.69E-05	1.38E-03	-6.04	SYNE3-201	92	upstream
lincRNA_791.1	1:144934585–144,937,622	2739	4.16E-05	1.53E-03	-3.66	ADARB1-201	204	upstream
lincRNA_11659.17	22:59504587–59,508,832	3069	4.95E-05	1.79E-03	-8.28	RUVBL1-201	188	upstream
lincRNA_2036.1	11:10142822–10,146,171	2000	7.48E-05	2.56E-03	-3.11	LBX2-201	2603	downstream
lincRNA_3024.5	12:24728114–24,731,701	896	1.02E-04	3.37E-03	-11.63	SMAD9-201	165	upstream
lincRNA_929.1	1:157196721–157,198,350	1337	1.03E-04	3.39E-03	-4.11	RAB5A-201	151	upstream
lincRNA_3433.4	13:17510294–17,518,504	1169	1.13E-04	3.68E-03	-14.02	IL15RA-201	121	upstream
lincRNA_8476.14	19:33209590–33,211,733	514	1.24E-04	4.00E-03	-4.73	LRRC75A-202	367	downstream
lincRNA_6802.5	18:1343804–1,405,315	1285	1.70E-04	5.23E-03	-12.54	VAC14-202	6224	upstream
lincRNA_1840.2	10:92511630–92,522,957	1195	1.85E-04	5.58E-03	-3.74	GTF2A1-201	161	upstream
lincRNA_18001.17	5:108915063–108,924,973	5263	2.73E-04	7.70E-03	-2.95	CECR2-201	12,878	upstream

Continued

lincRNA located in antisense direction [a]								
Feature ID	Positon	Length [b]	p-Value	FDR [c]	FC (log2) [d]	mRNA Interaction [e]	Distance [f]	Interaction location [g]
lincRNA_6258.1	17:18453124–18,474,698	801	2.95E-04	8.20E-03	-11.43	ELF2-201	1432	upstream
lincRNA_10210.6	20:12278005–12,356,729	2124	3.10E-04	8.53E-03	-12.26	CD180-201	61,290	upstream
lincRNA_280.3	1:69127181–69,127,765	425	3.50E-04	9.45E-03	-3.12	UMPS-201	102	upstream

Table 9. Novel long intergenic noncoding RNAs differentially expressed in *Longissimus thoracis* muscle of Nellore cattle in the C2-fatty acid cluster compared to C3-fatty acid cluster. [a] Direction of transcription of proximal RNA transcripts; [b] Transcript Length in basis pair; [c] FC (log2) = Fold change (log2); [d] False Discovery Rate; [e] transcript interaction = lincRNA gene overlaps with a transcript gene from the bovine reference annotation (ARS.UCD1.2). ; [f] Distance = distance between lincRNA and closest RNA transcript in the bovine reference annotation (ARS.UCD1.2); [g] Interaction location = are defined according to the type of interactions (genic or intergenic), assuming that intergenic lncRNA are not overlapping any RNA transcript (RNA/gene), then it can be further classified in: upstream (the lincRNA is upstream transcribed in head-to-head or tail-to-tail orientation with RNA partner or yet both same orientation) or downstream (the lincRNA is downstream transcribed in head-to-head or tail-to-tail orientation with RNA partner or yet both in same orientation).

Fatty acids	Cluster 1 ($n = 14$)	Cluster 2 ($n = 24$)	Cluster 3 ($n = 10$)	*p*-value
PUFA/SFA	$0.37^{a} \pm 0.05$	$0.24^{b} \pm 0.04$	$0.16^{c} \pm 0.03$	5.97E-15
PUFA	$16.19^{a} \pm 2.03$	$10.80^{b} \pm 1.74$	$7.78^{c} \pm 1.68$	6.97E-15
ω6	$10.19^{a} \pm 1.40$	$6.88^{b} \pm 1.33$	$5.07^{c} \pm 1.18$	2.09E-12
ω3	$5.72^{a} \pm 0.87$	$3.58^{b} \pm 0.58$	$2.33^{c} \pm 0.57$	6.22E-16
Linolenic	$9.37^{a} \pm 1.25$	$6.34^{b} \pm 1.22$	$4.69^{c} \pm 1.12$	1.98E-12
α-Linolenic	$0.94^{a} \pm 0.13$	$0.62^{b} \pm 0.11$	$0.46^{c} \pm 0.12$	1.42E-12
ω6/ω3	$1.79^{b} \pm 0.19$	$1.92^{b} \pm 0.25$	$2.18^{a} \pm 0.18$	1.93E-04
CLA	$0.17^{b} \pm 0.10$	$0.27^{a} \pm 0.09$	$0.34^{a} \pm 0.12$	5.70E-04
Oleic	$28.71^{b} \pm 2.03$	$33.18^{a} \pm 2.26$	$33.18^{a} \pm 2.26$	3.04E-07
MUFA	$33.88^{b} \pm 2.30$	$39.06^{a} \pm 2.32$	$39.19^{a} \pm 2.95$	1.27E-07
SFA	$42.91^{b} \pm 1.75$	$43.29^{b} \pm 1.46$	$46.13^{a} \pm 2.00$	2.48E-05
Stearic	$13.34^{b} \pm 1.64$	$13.96^{b} \pm 1.43$	$15.84^{a} \pm 1.68$	7.06E-04
Miristic	$1.54^{c} \pm 0.30$	$2.19^{b} \pm 0.26$	$2.71^{a} \pm 0.48$	1.23E-10
Palmitic	$19.69^{c} \pm 1.56$	$21.98^{b} \pm 0.99$	$23.59^{a} \pm 0.97$	1.36E-09

Table 10. Least square means of fatty acids (g/100 g of total fatty acids) in the *Longissimus thoracis* muscle of three clusters of Nellore bulls, grouped based on similarities in their fatty acid profiles. Data presented as mean and standard deviation. The concentration of fatty acids was expressed as a percentage of total fatty acid methyl esters. Means sharing different letters between columns were significantly different ($p < 0.05$) from one another according to Tukey's test.

lincRNA_17681.1 may serve as regulatory marker linked to SFA-rich phenotypes, with decreased expression supporting the leaner lipid profiles in C1 and C2.

Overall, the enrichment analysis for the C2 vs. C3 comparison revealed gene ontology (GO) terms related to immune response, including cell activation involved in immune response (GO:0002263), adaptive immune response (GO:0002250), leukocyte activation involved in immune response (GO:0002366), lymphocyte activation involved in immune response (GO:0002285), and B cell activation (GO:0042113) (Table 6). These findings align with the higher saturated fatty acid (SFA) content observed in both C2 and C3 clusters, which promote inflammation in adipose tissue through various of these mechanisms. SFAs activate pattern recognition receptors (PRRs) and toll-like receptor 4 (*TLR4*), leading to the production of pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (*IL6*)[102–104]. Moreover, SFAs induce endoplasmic reticulum stress and activate inflammatory signaling pathways, such as nuclear factor-kappa B (NF-κB), which further amplifies adipose tissue inflammation[105]. These biological processes are consistent with the immune-related GO enrichments detected in C2 and C3, suggesting a link between SFA accumulation and inflammatory activation.

In this study, we identified several differentially expressed genic and intergenic lncRNAs associated with fatty acid composition in beef. While our findings provide new insights into the potential regulatory roles of lncRNAs in lipid metabolism and inflammatory processes, further functional studies are required to elucidate the specific mechanisms of action by which these lncRNAs interact with coding genes and modulate fatty acid metabolism in beef cattle. Understanding these interactions will be crucial for advancing genetic selection strategies aimed at improving the nutritional quality and health value of Nellore beef.

Conclusion

This study identified 38 novels genic lncRNAs and a total of 194 intergenic lncRNAs, highlighted *lncRNA_15786.3*, *lncRNA_11324.5*, *lncRNA_13894.1*, *lncRNA_19922.3*, and *lincRNA_17393.3* that were interacting with genes essential to fatty acid metabolism. These interactions likely contribute to enhanced polyunsaturated (PUFA), ω3, ω6 and monounsaturated (MUFA) fatty acid profiles, consequently a healthier meat fat content. Specifically, these lncRNAs and lincRNAs target genes involved in adipogenesis (*CCN1)*, muscle cell differentiation *(MED28)*, β-oxidation (*BNIP3)*, lipogenesis (*CNOT2*), and transport of triglycerides *(NR2F1*). Together, these findings provide valuable information about the complex regulatory network of lncRNAs, lincRNAs, and their gene partners affecting fatty acid composition in beef cattle, expanding our knowledge of noncoding regulatory elements associated with meat quality traits. This study lays the groundwork for future in-deep functional investigations aimed at unraveling the precise molecular mechanisms controlling FA deposition in bovine muscle.

Materials and methods

Ethics approval and consent to participate

This study was approved to carry out procedures involving animals by ethics committee of the Faculty of Agrarian Sciences and Veterinary, Sao Paulo State University (UNESP) (certificate number 18340/16). Furthermore, all the data sampling was performed following the CEUA/ FCAV-UNESP guidelines and regulations according to Regulations for the Administration of Affairs Concerning Experimental Animals (Ministry of Science and Technology, Brazil). In addition, we confirmed the statement that the study was conducted following the ARRIVE guidelines.

Animals, samples collection and fatty acids composition

A total of forty-eight young Nellore bulls belonging to the Capivara farm in São Paulo State, Brazil, were used. These animals were included in the Nellore Qualitas (QLT) commercial breeding program. During the growth phase, animals were raised under a grazing production system (*Brachiaria sp.* and *Panicum sp.*) and received mineral supplementation. The animals were finished in feedlots for 90 days, receiving a mixed diet based on corn silage and supplemented with concentrates based on sorghum grain and soybean meal in the proportion of concentrate/silage ratio (50/50 to 70/30). All animals belonged to the same contemporary group and were finished with the same nutritional management. The animals were slaughtered with an average age of 24 months and body weight of 550 kg in commercial slaughterhouses, following the Brazilian Federal Inspection Service procedures.

RNA was extracted from *Longissimus thoracis* (LT) muscle tissue collected immediately after slaughter from the left half-carcass of each animal, between the 12th and 13th ribs. The samples were frozen in liquid nitrogen and stored at − 80 °C until further RNA-Seq analysis. After 48 h *postmortem* at 0–2 °C, the samples were collected from the *Longissimus thoracis* muscle (12–13th ribs of left half carcass) from each animal and stored at − 80 °C for posterior fatty acid (FA) assessment analyses.

Beef FAs were extracted from the LT muscle samples (~ 100 g) according to the method described by Folch et al.[107]. The muscle samples were ground, and the lipids were extracted by homogenizing the sample with a solution of chloroform and methanol in the ratio of 2:1. Then NaCl was added at a concentration of 1.5% to isolate the lipids. The isolated lipids were subjected to methylation, and the resulting methyl esters were formed according to Kramer et al.[108]. The fatty acid composition was quantified using gas chromatography (GC-2010 Plus - Shimadzu AOC 20i auto-injector) equipped with an SP-2560 capillary column (100 m x 0.25 mm diameter with 0.02 mm thickness, Supelco, Bellefonte, PA), as described in Berton et al.[18]. The FA profile was quantified by normalizing the area under the curve of methyl esters using the GS Solution 2.42 software and then expressed as g/100 g of total FA of meat. From the identified FA profile, fourteen FAs were selected based on their health importance (seven individuals and seven groups of FAs) as described in Table 1.

Animals clustering by amount of fatty acids

The K-means method was used to classify 48 animals into three groups by their similarities in beef FA profiles (myristic, palmitic, stearic, oleic, linoleic, conjugated linoleic (CLA), α-linolenic and the groups of saturated fatty acids (SFA), monounsaturated (MUFA), polyunsaturated (PUFA), ω3, ω6, PUFA/SFA ratio and ω6/ω3). These three groups were determined using the gap statistics to compare the total intracluster variation for different values of k clusters with their expected values under the null reference distribution of the data. Normality for the FA profile was tested using Shapiro–Wilk's normality test[109]and followed a normal distribution. The differences between the three groups on the FA profile were compared using the Least-Squares Means package in R[110] and Tukey's test ($P < 0.05$).

RNA-sequencing

Total RNA was isolated from 48 muscle tissue samples (~ 50 mg) using the RNeasy Lipid Tissue Mini Kit (Qiagen, Valencia, CA, USA) in accordance with the manufacturer's instructions. The extracted RNA's purity was assessed by measuring its absorbance in a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific). The quality of the total RNA extraction was evaluated using an Agilent 2100 Bioanalyzer, where only samples with RIN > 8 were used. The concentration and the presence of genomic DNA contamination were quantified using Qubit® 2.

The mRNA paired-end libraries were created from each RNA sample using the Illumina TruSeq mRNA library preparation kit. Sequencing was conducted on the Illumina HiSeq 2500 platform to generate paired-end reads with 2 × 100 bp.

Quality control and reads alignment

The FastQC program (Babraham Bioinformatics; Andrews S 2010. FastQC High Throughput Sequence QC Report)[111] was used for quality control of the reads considering the following parameters: (1) quality scores, (2) GC-content, (3) N-content, (4) length distributions, (5) duplication, (6) overrepresented sequences, and (7) K-mer content.

The raw reads were processed in two steps using Atropos (v1.1.19)[112]starting with the insert match algorithm, followed by the adapter match algorithm for the unprocessed reads that passed by the first step. Thereafter, the low-quality regions were also trimmed using "PRINSEQ-lite" software (v.0.20.3.;[113]). After filtering, HISAT2 (v.2.0.5)[114] was used to map trimmed paired-end reads to the bovine genome reference (ARS-UCD1.2 Bos Taurus; http://ftp.ensembl.org/pub/current_fasta/bos_taurus/).

Mapped reads were assembled using Cufflinks program (v2.2.1). Then, a matrix with transformed expression values was calculated by logarithm transformation (log2) and normalized using the cuffilinks packages (Cuffmerge/Cuffquant/Cuffnorm pipeline; http://cole-trapnell-lab.github.io/cufflinks/manual/) with default settings. To ensure validity and reliability of any downstream analysis like DE, isoforms with no detectable expression (FPKM value "0" in all samples), or low expression (mean FPKM less than 0.01 in all samples) and transcripts that were shorter than 200 bp were filtered out[115].

Differential expression LncRNA analyses

The LncDIFF is a powerful differential analysis tool for low abundance non-coding RNA expression data and was used to perform differential expression analysis[115]. The package "lncDIFF" was used with its parameter settings: link.function = "log," simulated.pvalue = FALSE, permutation = 300 (https://CRAN.R-project.org/package=lncDIFF). This package adopts the generalized linear model with zero-inflated exponential quasi-likelihood to estimate group effect on normalized counts and employs the likelihood ratio test in the differentially expressed genes. A pairwise group comparison (C1 vs. C2, C1 vs. C3 and C2 vs. C3) was used. Additionally, a Fold Change (FC) > | 2 |, p-value < 0.01 and FDR < 0.05 were used to filter DE transcripts and then identify and classify the DE lncRNAs.

Identification of LncRNA

Feelnc filter pipeline[116] was used to identify potential long non-coding RNAs (lncRNAs) from 1,206,35 transcript models. Transcripts shorter than 200 bp, biotype-coding protein, single-exon transcripts, and biexonic transcripts with one exon size shorter than 25 bp were discarded[117,118]. After filtering, the FEELnc coding potential module (FEELnc codpot) was used to separate putative long noncoding RNAs (lncRNAs) from protein-coding RNAs by first computing a coding potential core (CPS, ranging from 0 to 1) for each transcript and then computing a CPS cut-off that maximizes both the lncRNA sensitivity and specificity using a tenfold cross-validation according to the input training files. This process helped classify the transcripts as putative lncRNAs or protein-coding RNAs[27]. The FEELnc classifier pipeline was also leveraged to classify each lncRNA with respect to its location and orientation compared to its closest annotated protein-coding genes.

The FEELnc classifier module was used for possible function prediction of differentially expressed lncRNAs based on their nearest-neighbor protein-coding genes. The transcripts in this module are categorized in relation to the nearest RNA: Genic or Intergenic (lincRNA), with three subtypes that are the divergent, convergent and same-strand sub-classes, as detailed on the FEELnc website (https://github.com/tderrien/FEELnc). Additional information can be found in the FEElnc GitHub database (https://github.com/tderrien/FEELnc).

This classification was applied to independently analyze the differentially expressed (DE) lncRNAs within each category. For novel transcript lengths associated with annotated lncRNAs, nearby genes located within a 200 kb window — defined as 100 kb upstream of the lncRNA start site and 100 kb downstream of its stop site — were annotated using the Ensembl Genome Browser (ARS-UCD1.2 assembly; https://useast.ensembl.org/Bos_taurus/Location/).

The ClueGO plug-in of the Cytoscape software was used to perform functional annotations of genes associated with DE lncRNAs, applying a p-value threshold of smaller than 5% for each group contrast. The ClueGO network was constructed using a kappa score < 0.05, to determine the strength of the association between genes and biological pathways.

Data availability

44 RNAseq data samples are available in the publicly accessible Sequence Read Archive (SRA) database under the corresponding accession number PRJNA780472.

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Author contributions

L.G.A. conceived and led the coordination of the study. B.M.S, M.M.M.M, L.F.S.F and L.G.A performed the study design. B.M.S, C.S.T and L.F.M.M contributed to the statistical analysis. B.M.S led the functional analysis, data analysis and manuscript preparation. B.M.S, M.M.M.M, L.F.S.F, L.F.M.M, D.B.S.S, F.B, G.B.F, A.S.C.P and M.S-G contributed to data preparation and analysis. All authors read and approved the final manuscript version.

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Declarations

Competing interests
The authors declare no competing interests.

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