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Preliminary analysis of Stearoyl Co-A Desaturase gene transcripts in River buffalo

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ABSTRACT: Stearoyl-CoA desaturase (SCD) is a key enzyme in the biosynthesis of monounsaturated fatty acids (MUFAs). In cattle, SCD gene extends over a DNA segment of ~17.0 Kb, and it is organized in 6 exons and 5 introns. The SCD gene has been indicated as the candidate gene to change the saturated/unsaturated FAs ratio and hence it has been suggested as the gene influencing the fat quality. In cattle, eight SNPs have been identified and one of them, (T→C) at 231st nt of 5th exon, is responsible for the Val→Ala amino acid change. The C allele has been associated with higher content of MUFAs in carcasses, and it is positively related to a higher index of desaturation (C18:0/C18:1 and C16:0/C16:1) in the milk. In this study, we report on preliminary results of analysis of transcripts of the SCD encoding gene in river buffalo. The electrophoretic analysis of the RT-PCR products and the subsequent sequencing showed at least five different populations of mRNA. The most represented population is correctly assembled (~1300 bp), followed by the one which is deleted of ~750bp, corresponding to the 3rd, 4th and 5th exon and partially to the 2nd and 6th exon.

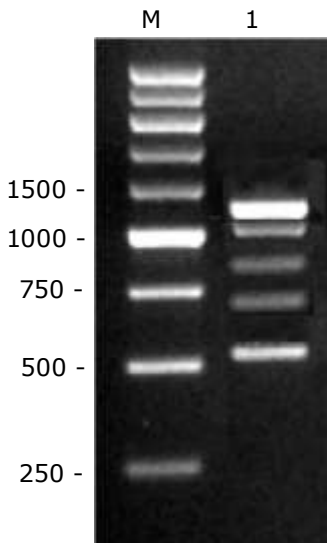
Key words: River buffalo, Stearoyl Co-A Desaturase, SCD gene, Transcripts.

INTRODUCTION - Stearoyl-CoA desaturase is a key enzyme in the biosynthesis of monounsaturated fatty acids (MUFAs) and it plays a central role in regulation of FA metabolism (Heinemann and Ozols, 2003), catalyzing the insertion of a double bond between carbon atoms 9 and 10 in a spectrum of saturated fatty acids (Palmquist *et al.*, 1993). SCD is responsible for saturated/monounsaturated FAs ratio in the composition of the triacylglycerol and in the phospholipids membrane. The alteration of this ratio has been implicated in many diseases, such as diabetes, obesity and cardiovascular diseases (Heinemann and Ozols, 2003). In cattle, the SCD gene extends over a DNA segment of ~17.0 Kb and it is organized in 6 exons and 5 introns. The complete bovine SCD mRNA spans 5.3 Kb and it codes for a protein of 359 amino acids. The SCD gene has been indicated as the candidate gene to change the saturated/unsaturated FAs ratio and therefore, to influence the fat quality (Taniguchi *et al.*, 2004). So far, eight SNPs have been identified in cattle, forming two different haplotypes: A and B. Three SNPs are located on the 5th exon and one of them, (T→C) in position 231st, is responsible for the Val→Ala amino acid change (Taniguchi *et al.*, 2004). The C allele has been associated with higher content of MUFAs in carcasses, and it

is positively related to a higher index of desaturation measured by the ratios (C18:0/C18:1 and C16:0/C16:1) in the milk (Medrano, 2002). In river buffalo very little information on SCD gene is available. Nowadays, only the sequences of the 4th, 5th and 6th exon of the SCD gene are known (DQ088625; DQ646700; DQ646701). In the present study we report on a preliminary characterization of the river buffalo SCD gene transcripts.

MATERIAL AND METHODS - Total RNA was extracted from milk somatic cells of six lactating Mediterranean river buffaloes of comparable age, type of feed, diet, feeding level and lactation stage. Primers for amplification and sequencing were designed by DNA-sis software (Hitachi) using as template the sequence of the bovine *SCD* gene (EMBL: AY241932). The reverse transcription of total RNA was performed using an oligo dT(18). RT mix (20- μ l) was performed using Improm-II Reverse Transcriptase (Promega). PCR was carried out using the following primers: *forward* 5'-CAGCGGAAGGTCCCGA-3' and *reverse* 5'-TGGGTAAACGTCATCTTTAGCA-3', corresponding respectively to nt 3-18 of the 1st exon and complementary to nt 290-311 of the 6th exon of the bovine SCD gene. The 100- μ l PCR reaction mix comprised: the 20- μ l of RT product, 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 2 mM MgCl₂, 10 pmol of each primer, 0.2 mM each dNTPs, 5 U of Taq DNA Polymerase (Promega). Amplification consisted of initial denaturation at 95°C for 4 min followed by 37 cycles carried out at 95°C for 45 sec, 62°C for 45 sec, 72°C for 90 sec, and 10 min of final extension in the last cycle. All the amplicons were analysed by electrophoresis on 1.5 % agarose gel in TBE 1X buffer. Densitometric analysis of RT-PCR products was displayed using QuantityOne software (BioRad). PCR products were purified from gel with QIAquick kit (QIAGEN) and sequenced, twice in both directions, according to Sanger *et al.* (1977) method.

Figure 1. RT-PCR (1st – 6th exon) of mRNA transcribed from river buffalo SCD gene. M: Marker 1000 bp (Promega); lane 1: RT-PCR product.



RESULTS AND CONCLUSIONS - The electrophoresis analysis of the RT-PCR products of six lactating Mediterranean river buffaloes showed patterns characterized by at least five bands of sizes ~1300 bp, ~1100 bp, ~900 bp, ~700 bp and ~550 bp (Figure 1).

The most represented mRNA population is ~1300 bp long (~52%), followed by the one of about 550 bp (~30%). These two RT-PCR products have been sequenced. The first sequenced transcript is 1250 bp long (EMBL acc. no. AM600640) and it shares a total similarity with the corresponding bovine cDNA of ~97.8%. The subsequent comparison with the bovine SCD gene allowed the subdivision in 6 exons (from the 53rd nt of the exon 1 to the 278th nt of the exon 6), confirming that such transcript is correctly assembled and it

Figure 2. Homology between the river buffalo SCD cDNA sequences of the correctly assembled transcript (upper line) and the incorrectly assembled. Dashes represent missing nucleotides, capital and small letters are translated and untranslated exons respectively, bold letters are amino acids.

	10	20	30	40	50	60	70	80	90	
1	tcaggaacta	gtctacactc	agtttggact	gccccgaact	ccgctccgca	gtctcagccc	cgagaaaagt	atccccagtg	cctagaggcc	90
1	tcaggaacta	gtctacactc	agtttggact	gccccgaact	ccgctccgca	gtctcagccc	cgagaaaagt	atccccagtg	cctagaggcc	90
	100	110	120	130	140	150	160	170	180	
	M P A N L L Q E E I S S S Y T T T I T A P F S R V L Q									
91	agATGCCGGC	CCACTTGGTG	CAAGGGAGA	TCTCTAGCTC	CTACACAACC	ACCACCACCA	TCACAGACAC	TCCTCCAGG	GTCTCCGAGA	180
91	agATGCCGGC	CCACTTGGTG	CAAGGGAGA	TCTCTAGCTC	CTACACAACC	ACCACCACCA	TCACAGACAC	TCCTCCAGG	GTCTCCGAGA	180
	190	200	210	220	230	240	250	260	270	
	N G G G K L E K T P L Y L E E D I R P E T R D D I Y D P T Y									
141	ATGGAGGGGG	CAAAITGGAA	AAGACTCCCC	TATACTTGGG	AGAAGACATC	CGCCCTGAAA	CGAGAGATGA	CATCTATGAC	CCAACCTACC	270
141	ATGGAGGGGG	CAAAITGGAA	AAGACTCCCC	TATACTTGGG	AGAAGACATC	CGCCCTGAAA	CGAGAGATGA	CATCTATGAC	CCAACCTACC	270
	280	290	300	310	320	330	340	350	360	
	Q D K E G P K F P K L E Y V W R N I I L M F L L H L G A L Y G									
271	AGGATAAGGA	GGCCCCAAG	CCCAAGCTTG	AGTATGTTTG	GAGAACAATC	ATCCTCATGT	TTCTGTACA	CTTGGAGGC	CTATATGGGA	360
271	AGGATAAGGA	GGCCCCAAG	CCCAAGCTTG	AGTATGTTTG	GAGAACAATC	ATCCTCATGT	TTCTGTACA	CTTGGAGGC	CTATATGGGA	360
	370	380	390	400	410	420	430	440	450	
	I T L I P T C K I Y T Y L W V L F Y Y L M G A L G I T A G A									
361	TACATGAT	CCCCACTG	AAGATATAGA	CCTATCTCTG	GGTGTATTC	TACTATCTGA	TGGGTGCCCT	GGGCATCACA	GCAGGGGCC	450
361	TACATGAT	CCCCACTG	AAGATATAGA	CCTATCTCTG	GGTGTATTC	TACTATCTGA	TGGGTGCCCT	GGGCATCACA	GCAGGGGCC	450
	460	470	480	490	500	510	520	530	540	
	H R L W S H R T Y K A R L P L R V F L I I G N T M A P Q N D									
451	ATCGGCTGTG	GAGTCACCGA	ACCTACAAAG	CTCGCTGCC	CCCGCGGTC	TTCTGTATCA	TCGGCACAC	CATGGCGTTC	CAGAATGAGC	540
451	ATCGGCTGTG	GAGTCACCGA	ACCTACAAAG	CTCGCTGCC	CCCGCGGTC	TTCTGTATCA	TCGGCACAC	CATGGCGTTC	CAGAATGAGC	540
	550	560	570	580	590	600	610	620	630	
	V F F W S R D H R A H H K F S E T D A D P H N S R R G F F F									
541	TTTTTGAATG	GTCCCGAGAT	CACCGTGCC	ACCACAAGTT	TTCAGAAACG	GATGCTGATC	CCACAAATTC	CCGACGTGGC	TTTTTCTTCT	630
541	TTTTTGAATG	GTCCCGAGAT	CACCGTGCC	ACCACAAGTT	TTCAGAAACG	GATGCTGATC	CCACAAATTC	CCGACGTGGC	TTTTTCTTCT	630
	640	650	660	670	680	690	700	710	720	
	S H V G W L L V R K H P A V I E K G S T L N L S D L K A E K									
631	CTCAGCTGGG	TTGGCTGCTT	GTCCGCARAC	ACCCAGCTGT	CATAGAAAAG	GGTCTACGC	TAAATTTATC	CGACCTAAA	CCCGAGAAGC	720
631	CTCAGCTGGG	TTGGCTGCTT	GTCCGCARAC	ACCCAGCTGT	CATAGAAAAG	GGTCTACGC	TAAATTTATC	CGACCTAAA	CCCGAGAAGC	720
	730	740	750	760	770	780	790	800	810	
	L V M F Q R R Y Y K P G V L L L C F I L P T L V P W Y L M G									
721	TGGTAATGTT	CCAGAGGAGG	TACTACARAC	CTGGTGTCTT	GTGTTGTGTC	TTCATCTGTC	CCACACTGCT	GCCGTGGTAT	CTGTGGGGTG	810
721	TGGTAATGTT	CCAGAGGAGG	TACTACARAC	CTGGTGTCTT	GTGTTGTGTC	TTCATCTGTC	CCACACTGCT	GCCGTGGTAT	CTGTGGGGTG	810
	820	830	840	850	860	870	880	890	900	
	E T F Q N S L F F A T L F R Y A L G L N I T W L V N S A A N									
771	AAACGTTTCA	AAACGCTCTG	TTTTTTGCCA	CCTTATTCGG	TTATGCCCTT	GGGCTCAACA	TCACCTGGCT	GUTGAATAGT	CGTGGCCATA	900
771	AAACGTTTCA	AAACGCTCTG	TTTTTTGCCA	CCTTATTCGG	TTATGCCCTT	GGGCTCAACA	TCACCTGGCT	GUTGAATAGT	CGTGGCCATA	900
	910	920	930	940	950	960	970	980	990	
	M Y G Y R P Y D K T I S P R E N I L V S L G A V G E G F H N									
901	TGTATGGATA	TCCGCTTAT	GACAAGACCA	TCAGCCCCG	AGAGAAATAT	CTGGTTTTC	TGGGAGCTGT	GGGTGAGGC	TTCCACAAC	990
901	TGTATGGATA	TCCGCTTAT	GACAAGACCA	TCAGCCCCG	AGAGAAATAT	CTGGTTTTC	TGGGAGCTGT	GGGTGAGGC	TTCCACAAC	990
	1000	1010	1020	1030	1040	1050	1060	1070	1080	
	Y N H T F P Y D Y S A S E Y R W H I N F T T F T I D C M A A									
991	ACCACCACAC	CTTTCCTTAT	GACTACTCAG	CCAGTGAGTA	CCGCTGGCAC	ATCAAACTTA	CCACGTTCTT	CATTGATTGC	ATGGCTGCCA	1080
991	ACCACCACAC	CTTTCCTTAT	GACTACTCAG	CCAGTGAGTA	CCGCTGGCAC	ATCAAACTTA	CCACGTTCTT	CATTGATTGC	ATGGCTGCCA	1080
	1090	1100	1110	1120	1130	1140	1150	1160	1170	
	I G L A Y D R K K V S K A I L A R M K R T G E S Y K S G *									
1081	TCGGCTGCG	TTATGACCGG	AGAAAGTAT	CCAAGCTGC	CATCTGGCC	AGGAATGAAA	GAACTGGAGA	GGAAAGCTAC	AAGAGTGCCT	1120
1081	TCGGCTGCG	TTATGACCGG	AGAAAGTAT	CCAAGCTGC	CATCTGGCC	AGGAATGAAA	GAACTGGAGA	GGAAAGCTAC	AAGAGTGCCT	1120
	1180	1190	1200	1210	1220	1230	1240	1250		
	**									
1121	GAatttggg	tcctttgggt	tccttttcca	aaagccatct	gggcagaggt	ttaaatgttct	gtttattaac	tactgaataa	
1121	-----	-----	tccttttcca	aaagccatct	gggcagaggt	ttaaatgttct	gtttattaac	tactgaataa	
			S F S K S H L G R G L M F C L L T T E ***							

codifies for 359 amino acids of the protein. The translation stop codon TGA is realized between 198-200 nucleotides of exon 6. A comparison of the sequenced cDNA with the partial published sequences of the buffalo SCD gene (DQ088625; DQ646700; DQ646701) showed 3 polymorphic sites, one of which, realized at the 231st nucleotide of exon 5, is responsible for an amino acid change ($GCG^{Ala} \rightarrow GTG^{Val}$). Similar to what has already been observed in cattle (Taniguchi *et al.*, 2004; Medrano, 2002), such transition could be associated to a different content of MUFAs in buffalo carcasses and milk. The remaining two mutations are conservative transversions realized in position 220 of exon 5 ($TCC^{Ser} \rightarrow TCA$) and 107

of exon 6 (GCA^{Ala}→GCC), respectively. The second sequenced fragment is 565 bp long and it is a uncorrectly assembled transcript. In fact, the amplicon is deleted of 765 bp, from the 228th nucleotide of exon 2 to the 217th nucleotide of exon 6, probably as a result of an alternative splicing. As a consequence, the corresponding protein is not 359 amino acids long, but probably 104. In fact, as shown in figure 2, the transcript uncorrectly assembled is characterized by the lack of the canonical stop codon (TGA), and the identification of a new stop codon (TAA) realized between the 275-277 nucleotides of the exon 6. Such event would lead to the translation of a part of the 6th exon (last 19 amino acids), normally only transcribed. These findings probably suggest that the skipping of a great part of the SCD exons brings up the accumulation of shortened but stable spliced mRNAs, which enable to encode for a functional protein, but this needs to be further investigated. The preliminary analysis of SCD gene transcripts further increases the interest towards the study of the fatty acid composition in the milk and its elaborated systems of gene regulation.

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