Use of PCR-RFLP (Polymerase Chain Reaction - Restricted Fragment Length Polymorphism) in the gene of the enzyme Stearoyl-CoA-Desaturase in *Bubalus bubalis*

G.M.F. de Camargo, R.B. Thomazine, L. Boarini,
F.G. Camargo, A.L.F. Lima, H. Tonhati

São Paulo State University F.C.A.V / Unesp - Jaboticabal - Brasil

Corresponding author: H. Tonhati. Via de Acesso - Paulo Donato Castellane s.nº, F.C.A.V / Unesp / Jaboticabal, Estado de São Paulo, Brasil, CEP - 14884-900 - Tel. +55 16 3209-2678 - Email: tonhati@fcav.unesp.br

ABSTRACT: The milk is an important food because it contents Conjugated Linoleic Acids (CLA). These fatty acids are synthesized in mammary gland under action of the enzyme Stearoyl CoA-Desaturase (SCD) and have showed some positive effects in human disease prevention and treatments. A variation of CLA in milk fat exists and can be partially explained by the different levels of expression of SCD. The aim was to study part of the encoding regions of SCD´s gene using PCR-RFLP (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism). Genomic DNA was extracted from lactating Murrah females. After this, PCR reactions were made by using primers Z ▶◀ D1 that encloses exon I, II and intron I. The fragments amplified are composed by 938 pb. Then, RFLP techniques were applied in the fragments using the restriction enzymes Pst I and Sma I. The enzyme Pst I has generated fragments of 788pb and 150bp and the Sma I has generated fragments of 693pb and 245pb. All the animals showed the same migration standard for both enzymes, characterizing a genetic monomorphism for this region of SCD gene. The analysis determined that there aren't genetic differences between these animals in the studied regions by using Pst I and Sma I enzymes.

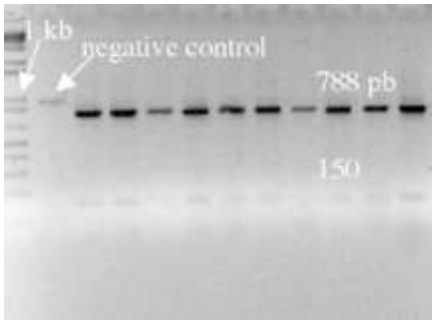
Key words*:* CLA, PCR, RFLP, Stearoyl-CoA-Desaturase.

INTRODUCTION - The conjugated linoleic acid (CLA) is an important lipid for human health. There are many studies in human medicine showing that this lipid can be used in cancer prevention (Park *et al*., 2000; Miller *et al*., 2001) in diabetic, high blood pressure treatments (Agnieska and Ntambi, 2005) and also in obesity treatments (Pariza *et al*., 1996) This fatty acid is composed by isomers with 18 carbons and two double bonds (Bauman *et al*.,2000) and the cis-9 trans-11CLA and trans-10 cis-12CLA have the biggest biological activity (Pariza, 2000). The main sources of this fatty acid are the dairy products.(Parodi *et al*., 1997) This research has as an objective to study a region of the gene of Stearoyl-CoA-Desaturase (SCD), the enzyme which is responsible by converting the Vacenic acid which is circulating in blood into CLA in the mammary gland (Griinari *et al*., 2000). This reaction integrates the endogenous syntheses, the most important one, considering the production

of cis-9 trans-11CLA (Griinari *et al*., 2000; Benjamin *et al*., 2001) Studies showed that buffaloes at the same dietary have different levels of CLA in its milk. (Fernandes, 2004) One of the reasons of this fact could be the different level of SCD in the mammary gland. A possible explanation is that could exist polymorphisms in the gene of SCD, changing the structure of the enzyme and modifying its function. The objective of this work was to study and try to identify possible polymorphisms in the gene of SCD using the molecular biology technique of PCR-RFLP and try to correlate them with CLA quantity in the milk, and after this, make a selection of the animals that have a greater quantity of this fatty acid in its milk. This brings benefits to the breeder and the society.

MATERIAL AND METHODS - A sample of hair's tails was collected from 55 lactating bubaline females which begin to the breed Murrah. Then the genomic DNA was extracted. The technique used to do this was the method phenol-chloroform-isoamilic alcohol (PCL) that consists in inserting in the samples TE-Tween e Proteinase-K during incubation, followed by inserting a volume of PCL, precipitation with sodium acetate (0,3M) e absolute ethanol and make a DNA solution using TE (10 mM Tris HCl pH = 7.6 e 1mM EDTA pH = 8.0) in proportion de 10:1, and after keep them stocked at 4 ºC. 10 µL from each DNA solution sample have been mixed with 5 µL of bromophenol blue 0.05% and were put in a agarose gel (0,8%) stained with Ethidium Bromide to electrophoresis procedure be done with the intention to detect the quantity and purity of DNA. It was visualized under UV light in a Gel Logic system (Kodak). After the extractions, PCR reactions were made by using 100 ng de DNA, 0.5µM from each primer, 1X PCR buffer [10 mM Tris-HCl (pH 9.0), 1.5 mM MgCl2 e 50 mM KCl], 100 µM de dNTPs e 0.5 U de Taq polimerase in final volume that contents 25 µl. The primers Z ▶◀ D1 encloses the first and second exons and the first intron. The fragments amplified are composed by 938 pb. After the reactions in termociclator, the material was mixed with 5µl of bromophenol blue 0.05% Then, they were put in agarose gel (1.5%) stained with Ethidium Bromide to electrophoresis procedure be done and confirm the amplification. It was seen using under UV light in a Gel Logic system (Kodak) .After that, RFLP techniques were applied in the fragments using the restriction enzymes Pst I and Sma I. RFLP reactions were done using 8µl of PCR, 2µl of buffer solution and 0.5µl of the enzyme and has completed with water to the volume of 20µl. After the reactions, each sample was mixed with 5µl of bromophenol blue 0.05%. Then they were put in agarose gel (3.0%) with Ethidium Bromide to electrophoresis procedure be done and confirm were the enzymes have cut the fragments. The standard of fragments were visualized. under UV light in a Gel Logic system (Kodak).

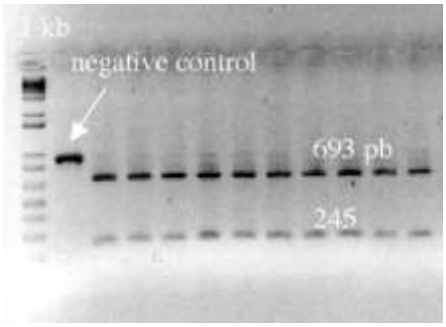
Figure 1. RFLP technique using the enzyme Pst I and the fragments of 788 pb and 150 pb.



RESULTS AND CONCLUSION
The enzyme Pst I has generated fragments of 788pb and 150pb and the

Sma I has generated fragments of 693pb and 245pb in all animals (as we can see in Figure 1 and 2). We can conclude that there are no polymorphisms in the region of the gene of SCD analyzed and no correlation with CLA production could be done.

Figure 2. RFLP technique using the enzyme Sma I and the fragments of 693 pb and 245 pb.



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