Single nucleotide polymorphisms in the promoter region of the paraoxonase 1 gene in *Bos indicus* cows

Polimorfismos de nucleotídeo único na região promotora do gene da paraoxonase 1 em vacas *Bos indicus*

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Natália Ávila de Castro
Doutora em Veterinária
Universidade Federal de Pelotas
Rua Gomes Carneiro, 01 – Centro, Pelotas – RS
E-mail: nataliavetufpel@gmail.com

Pedro Augusto Silva Silveira
Doutor em Veterinária
Instituto Sul Riograndense, Campus CAVG, Av. Ildefonso Simões Lopes, 2791 – Pelotas – RS
E-mail: pedrosilveira3@hotmail.com

Luiz Francisco Machado Pfeifer
Doutor em Ciências, Pesquisador A - Embrapa Rondônia
Empresa Brasileira de Pesquisa Agropecuária
BR-364, s/n – Porto Velho – RS
E-mail: luiz.pfeifer@embrapa.br

Carlos Castilho de Barros
Doutor em Biotecnologia, Professor – Departamento de Nutrição, Faculdade de Nutrição
Universidade Federal de Pelotas
Rua Gomes Carneiro, 01 – Centro, Pelotas – RS
E-mail: barros_cc@yahoo.com.br

Augusto Schneider
Doutor em Biotecnologia, Professor – Departamento de Nutrição, Faculdade de Nutrição
Universidade Federal de Pelotas
Rua Gomes Carneiro, 01 – Centro, Pelotas – RS
E-mail: augustoschneider@gmail.com

ABSTRACT

Paraoxonase 1 is an enzyme whose activity serves as a biomarker of uterine health and has been linked to reproductive parameters in cattle, as well as in other species. The aims of the present study were to characterize single nucleotide polymorphisms (SNPs) in the promoter region of the paraoxonase 1 (PON1) gene and to determine the relationship between SNPs and PON1 serum activity in *Bos indicus* beef cows. Samples from Nelore cows were used for genetic sequencing (n=17) and for genotyping of the SNP-221 and

PON1 activity analysis (n=52). Eight SNPs were identified in the promoter region of PON1, from these three SNPs were previously observed in *Bos taurus* dairy cows. There was no association between any SNP position and PON1 activity. Our results suggest that SNPs in the PON1 promoter does not affect serum PON1 activity in *B. indicus* beef cows.

Keywords: Cattle, paraoxonase 1, polymorphism

RESUMO
A paraoxonase 1 é uma enzima cuja atividade serve de biomarcador de saúde uterina e tem sido relacionada com parâmetros reprodutivos em bovinos, bem como em outras espécies. Os objetivos do presente estudo foram caracterizar polimorfismos de nucleotídeo único (SNPs) na região promotora do gene da paraoxonase 1 (PON1) e determinar a relação entre os SNPs e a atividade sérica de PON1 em vacas de corte *Bos indicus*. Amostras de vacas da raça Nelore foram usadas para sequenciamento genético (n=17) e para genotipagem do SNP loclizado na posição -221 e análise da atividade de PON1 (n=52). Oito SNPs foram identificados na região promotora do gene da PON1, sendo que três destes foram previamente observados em vacas leiteiras *Bos taurus*. Nenhuma associação entre SNP e atividade de PON1 foi detectada. Os nossos resultados sugerem que os SNPs presentes na região promotora de PON1 não afetam a atividade sérica de PON1 em vacas *B. indicus* de corte.

Palavras-chave: Bovinos, paraoxonase 1, polimorfismo

1 INTRODUCTION

The paraoxonase 1 (PON1) is an enzyme synthetized in the liver and associated with high-density lipoproteins (HDL; CERON et al., 2014). PON1 protects against the oxidation of low density lipoprotein (LDL) and cell membranes (AVIRAM, 1999). PON1 reduces its activity during inflammatory processes (BIONAZ et al., 2007), and can serve as a biomarker of uterine health in postpartum dairy cows (SCHNEIDER et al., 2013). In addition, dairy cows with increased PON1 activity are more likely to resume postpartum ovarian activity (KRAUSE et al., 2014). Moreover, during bovine in vitro fertilization, addition of recombinant PON1 increased D7 blastocyst rates (RINCÓN et al., 2016).

Recently, seven single nucleotide polymorphisms (SNPs) were described in the promoter region of the bovine PON1 gene, and the SNP present at the -221 position was strongly associated with serum PON1 activity (SILVEIRA et al., 2015) and calving conception interval (SILVEIRA et al., 2019). The position -221 was identified as a site for transcription factors regulating the acute phase response (WEDEL & ZIEGLER-HEITCROCK, 1995). However, to date there are no studies evaluating the occurrence and location of SNPs in the promoter region of the PON1 gene in *Bos indicus* cattle.

Based on these considerations, the aims of this study were to identify SNPs in the promoter region of the bovine PON1 gene and to evaluate the genotype distribution of the PON1 -221 SNP and its association to PON1 activity in Nelore cows.

2 MATERIALS AND METHODS

This study was conducted in a commercial farm in the state of Rondônia, Brazil. All animals were kept in *Brachiaria brizantha* pasture, with free access to water and mineral supplement. Blood samples from 52 suckled Nelore cows subjected to a fixed-timed artificial insemination (FTAI) protocol were used. Cows were between 3 and 6 years old, 69.8 ± 11.4 d postpartum and body condition score (BCS) between 2.5 and 3.5 (1 = cachectic, 5 = obese).

On the day of the insemination, cows were evaluated by transrectal ultrasound (SIUI CTS-900, probe linear with 5MHZ, Gangdong, China) to confirm that none had uterine abnormalities. At this moment blood samples were collected for PON1 activity analysis and DNA extraction. Determination of serum PON1 arylesterase activity (U/mL) was performed according to the method described by BROWNE et al. (2007).

The promoter region of the bovine PON1 gene (828 bp) was amplified by the PCR technique using specific primers (Forward: 5′-CGGTAATCCCTGAAGAATGC-3′ and Reverse: 5′-GCACTTCCTACCCTGCTTTG-3), as described by SILVEIRA et al. (2015). The PCR products from 17 samples were purified and submitted to sequencing (HELIXXA, São Paulo, Brazil) to identify the occurrence and location of SNPs. The obtained sequences were aligned using the BioEdit software (Ibis Bioscienses), using the bovine (*B. taurus*) PON1 sequence published in NCBI (number: AC_000161.1) as reference. The positions of the SNPs were numbered relative to the first nucleotide of the published mRNA sequence (NM_001046269.2; Silveira et al., 2015).

Additionally, genotyping of the SNP-221 was performed in all samples (n=52) according to the described by SILVEIRA et al. (2019). The PCR product was incubated with the *BseL*I enzyme at 37 °C for 12 hours and genotype was determined after 2h of electrophoresis of the DNA fragments in a 2.5% agarose gel. Control samples from each genotype were included in every run. Statistical analysis was performed using GraphPad software and means were compared by the *t* test. Differences were considered significant when $P \leq 0.05$. Data is presented as least square means. The independent variable was the genotype and the dependent variable was the serum PON1 activity.

3 RESULTS AND DISCUSSION

Sequencing indicated the presence of eight SNPs, located at positions -105, -111, -130, -221, -267, -392, -440 and -455 in the promoter region of the PON1 gene of Nelore cows (Table 1). There was no association between any of the identified SNPs and PON1 activity (Table 1). Interestingly, 70% of cows presented the combination G-C-A-G-A-A-T-T as homozygous at all SNP positions identified. The PON1 serum activity of cows that had this profile was 98.9 ± 8.4 U/mL and 79.5 ± 13.9 U/mL for cows that were not homozygous in these positions (P=0.24). The prevalence of genotypes AA, AG and GG for the -221 position in the population was 5.8% (3/52), 28.8% (15/52) and 65.4% (34/52), respectively. The SNP at -221 position was not associated to serum PON1 activity (Table 1; P>0.05).

Table 1. Single nucleotide polymorphisms (SNPs) identified in the promoter region of the bovine paraoxonase 1 (PON1) gene in Nelore postpartum cows.

SNP position	Genotypes / PON1 activity*			*P*-Value
-105	AA	AG	GG	AA+AG vs GG
	5.9% (1/17)	5.9% (1/17)	88% (15/17)	
	43.9	116.7	95 ± 7.2	0.54
-111	CC	CT	TT	CC vs TT
	70.6% (12/17)	0% (0/17)	71.4% (5/17)	
	99 ± 8.1	--	79.5 ± 12.5	0.24
-130	AA	AG	GG	AA vs GG
	70.6% (12/17)	0% (0/17)	29.4% (5/17)	
	99 ± 8.1	--	79.5 ± 12.5	0.24
-221	AA	AG	GG	AA+AG vs GG
	5.9% (1/17)	5.9% (1/17)	88% (15/17)	
	43.9	116.7	95 ± 7,2	0.54
-267	AA	AG	GG	AA vs GG
	70.6% (12/17)	0% (0/17)	29.4% (5/17)	
	99 ± 8.1	--	79.5 ± 12.5	0.24
-392	AA	AC	CC	AA vs AC
	88% (15/17)	11,8% (2/17)	0% (0/17)	
	95 ± 7,2	80.3 ± 25.7	--	0.54
-440	CC	CT	TT	CC+CT vs TT
	23.5% (4/17)	5.9% (1/17)	70.6% (12/17)	
	72.8 ± 13.7	106.43	99 ± 8.1	0.24
-455	CC	CT	TT	CC vs TT
	29.4% (5/17)	0% (0/17)	70.6% (12/17)	
	79.5 ± 12.5	--	99 ± 8.1	0.24

*PON1 activity was evaluated on day of timed artificial insemination and is expressed in mean of U/mL ± Standard error.

From the eight SNPs characterized in the promoter region of the PON1 gene in *B. indicus* beef cows, three were previously described in *B. taurus* Holstein cows (-105, -221 and -392; SILVEIRA et al., 2015; 2019). However, different from what was observed in Holstein cows (SILVEIRA et al., 2015; 2019), none of the polymorphism was

associated with serum PON1 activity in Nelore cows from our study. Previously, we observed that PON1 activity was not associated with ovulation and pregnancy in *B. indicus* beef cows (CASTRO et al., 2018), in contrast to observations in *B. taurus* dairy cows (SCHNEIDER et al., 2013, SILVEIRA et al., 2019). These results may be associated to the metabolic and endocrine differences between *B. taurus* dairy and *B. indicus* beef breeds, as described elsewhere (for review see SARTORI et al., 2016). It is important to mention that cows from this study were all more than 45 days postpartum, healthy and had a body condition score > 3.0. This condition may have affected PON1 activity, which was relatively high for all cows. Furthermore, we recognized the limitations regarding the number of cows used in this study and only one time point per cow was used for measuring PON1 activity. However, this was an initial screening study to determine the existence of these SNPs in the PON1 gene of *B. indicus* cows and larger population and/or more samples per animal should be collected in order to confirm the effect of these SNPs on PON1 activity and other productive parameters.

The genotyping of the SNP location -221 was performed because this SNP has a strong association with PON1 activity and with fertility rates in Holstein cows (SILVEIRA et al., 2015; 2019). More specifically, the allele A was associated with higher PON1 activity and shorter calving conception interval in Holstein cows (SILVEIRA et al., 2019). In our study, the A allele was present in 35% of *B. indicus* beef cows, with 65% of the cows having the GG genotype. In contrast, the A allele was the most prevalent (~ 80%) among *B. taurus* dairy cows in previous studies (SILVEIRA et al., 2015; 2019). The same difference in prevalence was observed for the SNP positions -105 and -392, which were also previously identified in *B. taurus* dairy cows (Silveira et al., 2015). In our study with *B. indicus* beef cows the highest prevalence was for the -105 GG and -392 AA genotypes (88% for both). However, in *B. taurus* dairy cows from previous studies the proportion was the opposite, with 4% of cows having -105 GG genotype and 35% having the -392 AA genotype (SILVEIRA et al., 2015, 2019). Our preliminary study suggest therefore differences between the prevalence of PON1 SNPs between *B. indicus* beef cattle in comparison to the *B. taurus* dairy cattle.

4 CONCLUSION

In summary, eight SNPs were identified in the promoter region of the bovine PON1 gene in *B. indicus* beef cows. However, no association among SNPs and PON1 activity was detected, suggesting that PON1 does not appear to be a significant genetic

marker for use in *B. indicus* beef cows. Important differences in the prevalence of specific alleles were observed in comparison to previous studies with *B. taurus* dairy cows and need to be confirmed in large scale studies in different *B. indicus* populations.

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