Journal of Genetic Engineering and Biotechnology (2011) 9, 15–20

Academy of Scientific Research & Technology and
National Research Center, Egypt

Journal of Genetic Engineering and Biotechnology

www.elsevier.com/locate/jgeb

ARTICLE

Genetic polymorphism of two genes associated with carcass trait in Egyptian buffaloes

Othman E. Othman [a,*], Fawzia A. Zayed [b], Ali A. El Gawead [b], Medhat R.A. El-Rahman [a]

[a] *Cell Biology Department, National Research Centre, Dokki, Egypt*
[b] *Zoology Department, Faculty of Science, Zagazig University, Egypt*

Received 10 May 2011; accepted 10 May 2011
Available online 22 June 2011

KEYWORDS

Buffalo;
Leptin;
μ-Calpain;
PCR;
RFLP

Abstract Leptin and μ-calpain have been considered as two candidate genes for carcass performance and meat quality traits in the farm animals. The micromolar calcium-activated neutral protease (*CAPN1*) gene encodes μ-calpain that degrades myofibril proteins under the postmortem conditions which appears to be the primary enzyme in the postmortem tenderization process. Leptin is the hormone product of the obese (*LEP*) gene. The role of leptin as a lipostatic signal regulating whole-body energy metabolism makes it one of the best physiological markers of body weight, food intake, reproduction and immune system functions.

Genomic DNA extracted from 100 healthy buffaloes was amplified using primers that were designed from the cattle *CAPN1* and *LEP* gene sequences. The amplified fragments of *CAPN1* obtained from all tested buffalo DNA at 670-bp were digested with *Fok*I endonuclease. The result showed that all tested buffaloes are genotyped as CC for *CAPN1*. For *LEP* gene, the amplified fragments obtained from all tested buffalo DNA at 400-bp were digested with *Sau*3AI endonuclease. All buffalo animals investigated in the present study are genotyped as AA for *LEP* gene.

1. Introduction

There is a considerable interest in the application of molecular genetic technologies in the form of specific DNA markers that are associated with various QTL to promote more efficient and relatively easy selection and breeding of farm animals with an advantage for the inheritable traits of growth rate, body weight, carcass merit, feed intake as well as milk yield and composition [48]. Leptin and μ-calpain have been considered as two candidate genes for carcass performance and meat quality traits in the farm animals.

* Corresponding author.
E-mail address: othmanmah@yahoo.com (O.E. Othman).

1687-157X © 2011 Academy of Scientific Research and Technology. Production and hosting by Elsevier B.V. All rights reserved.

Peer review under National Research Center, Egypt.
doi:10.1016/j.jgeb.2011.05.003

Production and hosting by Elsevier

Calpain is a ubiquitous cytoplasmic cysteine protease and its activity is dependent on the concentration of calcium [47]. In cattle there are two genes of calpain that have been identified: *CAPN1* (μ-calpain) and *CAPN2* (m-calpain) [50]. Bovine *CAPN1* has been mapped to the telomeric end of BTA29 [46]. Recently, a QTL for meat tenderness was found to be segregating in this region of BTA29 [6,35]. The evaluation of *CAPN1* as a candidate gene for meat tenderness was supported by Meat Animal Research Center, MARC [1].

The *CAPN1* gene encodes a μ-calpain that degrades myofibril proteins under postmortem conditions and appears to be the primary enzyme in the postmortem tenderization process [26–28,3]. Regulation of μ-calpain activity has been correlated with variation in meat tenderness in cattle [20].

In *CAPN1* gene, more than 100 single nucleotide polymorphisms (SNPs) have been identified in cattle [46,41,24]. Among them, four polymorphisms, two non-synonymous SNPs (G316A and V530I) and two intronic SNPs (C4685T and C4751T) have been found to have significant effects on meat tenderness [41,42,52,36,44,51].

Leptin is the hormone product of the obese (leptin) gene that has been mapped to bovine chromosome (BTA) 4 [49,43]. It is a 16-kDa protein that is synthesized by white adipose tissue [55,22]. The role of leptin as a lipostatic signal in the regulation of whole-body energy metabolism makes it one of the best physiological markers of the body weight, food intake, energy expenditure [21,53,2], reproduction [11,18] and certain immune system functions [33].

Leptin has been considered as a candidate gene for performance, carcass and meat quality traits in beef cattle [17,4,29].

Genetic polymorphisms in the coding regions of the leptin gene in cattle have been associated with serum leptin concentration [31], feed intake [30,39], milk yield [32,5] and body fat [4,37].

With the aim of assessing the presence of sequence polymorphisms in the buffalo leptin gene, Orrù et al. [40] sequenced the entire coding region and part of the introns on a panel of Italian river buffaloes. They identified a new set of single nucleotide polymorphism (SNP) that is useful for the association studies between the sequence polymorphisms and the traits like the milk yield, feed intake, fat content and carcass and meat quality. To the best of our knowledge, this is the first published data on the genetic polymorphism of *CAPN1* gene in river buffalo.

In the present study, the PCR–RFLP technique was used to detect the genetic polymorphism within intron 14 of *CAPN*1 and intron 2 of *LEP* genes. These two introns exhibited many polymorphisms in cattle. Due to gene mapping conservation and nucleotide sequence homology between cattle and buffalo, we focused on the detection of genetic polymorphism within these two regions in Egyptian buffaloes for the first time.

2. Materials and methods

2.1. Animals

Water buffalo belongs to genus *Bubalus*, species *bubalis*. Water buffalo includes both river buffalo (*Bubalus bubalis bubalis*, $2n = 50$) and swamp buffalo (*Bubalus bubalis carabanesis*, $2n = 48$), the Egyptian buffaloes are of the river type. Egyptian river buffaloes have been classified according to minor phenotypic differences and their geographical locations, such as Beheiri, Menoufi and Balady which are found mainly in Northern-Egypt and Saiedy found in Southern-Egypt [12]. These differences are not well defined to be relied on as taxonomic classification. Few studies [15,14] have investigated the genetic variations in Egyptian buffaloes and concluded that all Egyptian river buffaloes belong to one population (*B. bubalis bubalis*).

2.2. Genomic DNA extraction

Genomic DNA was extracted from the whole blood of 100 unrelated healthy female buffaloes according to the method described by John et al. [23] with minor modifications. Briefly, 10 ml of blood taken on EDTA were mixed with 25 ml of cold sucrose-triton and double distilled water. The tubes were placed on ice for 10 min and mixed by inversion several times. After centrifugation, the pellet was re-suspended by nucleic lysis buffer using plastic pipette. The content was mixed with 108 μl of 20% SDS and 150 μl of proteinase. The tubes were placed in a water bath at 37 °C overnight.

After 24 h of incubation, the tube contents were transferred to a 15-ml polypropylene tube and 1 ml of saturated NaCl was added and shaken vigorously for 15 s. After centrifuging at 3500 rpm for 15 min at 4 °C, the supernatant was transferred to a clean 15-ml polypropylene tube and mixed with absolute ethanol. The tubes were agitated gently to mix the liquids and a fluffy white ball of DNA was formed. The precipitated DNA was picked up using the heat sealed pasture pipette, then washed twice in 70% ethanol and exposed to air to dry completely.

The DNA was dissolved in 200 μl TE buffer in 1.5-ml Microfuge tube and kept overnight in an incubator at 37 °C. DNA concentration was determined and diluted to the working concentration of 50 ng/μl, which is suitable for polymerase chain reaction.

2.3. Polymerase chain reaction (PCR)

The primers used in this study were designed from cattle *CAPN1* and *LEP* gene sequences. The 670-bp DNA fragment (exons 14–18) of *CAPN1* gene [24] and the 400-bp DNA fragment (intron 2) of *LEP* gene [30] were amplified using buffalo DNA.

CAPNI:
Primer forward: TTC AGG CCA ATC TCC CCG ACG
Primer reverse: GAT GTT GAA CTC CAC CAG GCC CAG

LEP:
Primer forward: TGG AGT GGC TTG TTA TTT TCT TCT
Primer reverse: GTC CCC GCT TCT GGC TAC CTA ACT

A PCR cocktail consists of 1.0 μM forward and reverse primers and 0.2 mM dNTPs, 10 mM Tris (pH 9), 50 mM KCl, 1.5 mM $MgCl_2$ and 1.25 U of Taq polymerase. The PCR cocktail (~23 μl) was added to 100 ng of buffalo DNA.