Maejo Int. J. Sci. Technol. **2016**, *10*(02), 154-165

Maejo International Journal of Science and Technology

ISSN 1905-7873

Available online at www.mijst.mju.ac.th

Full Paper

Characterisation of 5′ upstream and coding sequences of myostatin gene (*MSTN*) in Thai swamp buffalo (*Bubalus bubalis*)

Krailerk Taweechue [1, 2], Preeda Lertwatcharasarakul [3], Thawornchai Limjindaporn [4], Sornthep Tumwasorn [5] and Theera Rukkwamsuk [6,*]

[1] Centre for Agricultural Biotechnology, Kasetsart University, Kamphaeng Sean Campus, Nakhon Pathom 73140, Thailand

[2] Centre of Excellence on Agricultural Biotechnology (AG-BIO/PERDO-CHE), Bangkok 10900, Thailand

[3] Department of Pathology, Faculty of Veterinary Medicine, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand

[4] Department of Anatomy, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand

[5] Department of Animal Husbandry, Faculty of Agriculture, Kasetsart University, Bangkok 10930, Thailand

[6] Department of Large Animal and Wildlife Clinical Sciences, Faculty of Veterinary Medicine, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand

* Corresponding author, e-mail: fvettrr@ku.ac.th; tel. +66 34 351901; fax +66 34 351405

Received: 4 April 2015 / Accepted: 9 June 2016 / Published: 20 June 2016

Abstract: To characterise the 5′ upstream and coding sequences of the myostatin gene (*MSTN*) in Thai swamp buffalo (*Bubalus bubalis*), polymerase chain reaction and direct DNA sequencing were performed. The sequences obtained were compared with those of other livestock animals. The study successfully characterised the 5′ upstream and coding sequences of the Thai swamp buffalo *MSTN* gene. Both the common and muscle-specific transcription factor binding sites were observed in the 5′ upstream region and the coding sequences in the Thai swamp buffalo were similar to those in cattle (*Bos taurus*). From the multiple alignment method, the coding sequence identity of the tested buffalo was 98, 94 and 81% as compared to cattle, pig and chicken respectively. Two variants (g.264C>T and g. 414G>A) located in exon 1 were also identified. Based on the obtained DNA sequences, the amino acid sequence identity of the tested buffalo was 98, 94 and 86% as compared to cattle, pig and chicken respectively. Thai swamp buffalo have 100% sequence identity of Arg-Ser-Arg-Arg (RSRR) aligned between the positions 263 and 266 of the amino acid sequence.

Maejo Int. J. Sci. Technol. **2016**, *10*(02), 154-165

These results indicate that the 5′ upstream and the coding sequences of Thai swamp buffalo *MSTN* gene display a high similarity with other livestock animals, especially cattle.

Keywords: myostatin gene, coding sequence, Thai swamp buffalo, *Bubalus bubalis*, transcription factor binding site

INTRODUCTION

In Thailand most buffalo (*Bubalus bubalis*) are of the swamp buffalo [1]. Traditionally, they have been raised as draught animals but more recently are increasingly being raised for meat production. At present, there is a decrease in the number of Thai swamp buffalo raised for draught purpose due to socio-economic changes, while buffalo are still used as a major meat source [2]. Buffalo meat is consumed by many Thais, especially in the north of the country, and the demand for this meat has been increasing due to an increasing population. To improve productivity, several biotechnologies have been developed, which include artificial insemination (a simple biotechnology commonly used in field work), embryo transfer and cloning.

Interestingly, there is evidence that genetic factors are associated with the muscle phenotype in livestock animals and several studies have shown that either mutation or variation in several genes is associated with meat quality [3]. The myostatin gene (*MSTN*) is an example of an economically important gene. Fluorescence *in situ* hybridisation (FISH) mapping has revealed that the buffalo *MSTN* gene is located on chromosome 2 [4]. This gene plays a critical role in regulating skeletal muscle mass. Mutations of this gene result in the double muscling phenotype in cattle [5-7]. In addition, mutation or variation identified in other livestock animals is also associated with either the growth traits or the double muscling phenotype [8-12]. However, the *MSTN* gene in swamp buffalo, particularly raised in Thailand, has not been elucidated.

The objective of this study is therefore to characterise the 5′ upstream and coding sequences of Thai swamp buffalo *MSTN* gene. The characterised sequences can be used as basic data for providing molecular insights in terms of the gene's variation and mutation, including its influence on a number of biological activities.

MATERIALS AND METHODS

DNA Samples

Three millilitres each of jugular blood from eight Thai swamp buffalo kept by the Livestock Research and Breeding Centre in Surin and Lopburi provinces were collected into blood collecting tubes with ethylene diamine tetra-acetic acid as the blood coagulant [Vacuette® K3E K3EDTA, Greiner Bio-One Ltd., Thailand]. Genomic DNA was extracted from white blood cells by the salting-out method as described by Miller et al. [13]. DNA concentrations were measured using a spectrophotometer (NanoDrop 2000, Thermo Fisher Scientific, USA).

Primer Design, Polymerase Chain Reaction (PCR) and Direct DNA Sequencing

All three exons of the *MSTN* gene were amplified using overlapping primer pairs designed by primer3 software [14]. The primer design and coding sequence positions were based on DNA sequence data of the river buffalo *MSTN* gene (GenBank accession number DQ091762) [15]. Primers used for amplification of the 5′ upstream sequence were designed based on cattle

Maejo Int. J. Sci. Technol. **2016**, *10*(02), 154-165

MSTN gene (GenBank accession number AF348479) [16]. The regions amplified and sizes of the amplicons are shown in Table 1. The total volume of PCR reactions was 15 µL containing 50 ng of buffalo genomic DNA, 0.4 µM of each primer (Bio Basic Inc., Canada), 0.2 mM of each deoxynucleotide triphosphate (dNTPs) (Promega, UK), 4% DMSO, 2 mM $MgCl_2$, 1.5 µL of 10X ImmoBuffer and 0.45 unit of DNA polymerase (IMMOLASE^{TM} DNA polymerase, Bioline USA Inc, USA). The PCR profile consisted of initial denaturation at 95°C for 10 min., 35 cycles of denaturation at 95°C for 30 sec., annealing at optimal temperature as listed in Table 1 for 30 sec. and extension at 72°C for 45 sec., and final extension at 72°C for 10 min.

The purification of PCR products was carried out using exonuclease I/shrimp alkaline phosphatase (ExoSAP-IT®, USB Corporation, USA) according to the manufacturer's instructions. Direct DNA sequencing was performed by the DNA sequencing services (First BASE DNA Sequencing Services, First BASE Laboratories, Selangor, Malaysia).

Sequence Analysis and Amino Acid Alignments

The obtained sequences from each fragment were joined together. Putative transcription binding sites in the 5′ upstream region were predicted using MatInspector software [17]. Full-length coding sequences obtained from the joined fragments and generated amino acids were analysed using the Biology WorkBench 3.2 online program [18].

Table 1. List of primers used for 5′ upstream and coding sequence characterisation. Fragment 1-6 primers were designed based on river buffalo *MSTN* gene (GenBank accession number DQ091762). The 5′Ups 1 and 5′Ups 2 primers were designed based on the cattle *MSTN* gene (GenBank accession number AF348479). Both GenBank accession numbers were retrieved from the NCBI database.

Name	Forward primer (5′→3′)	Reverse primer (5′→3′)	Product size (bp)	Annealing temperature (°C)
5′Ups 1	CTTCAAATGCTTACT TAAATAG	AATATAAACAGAGA TTTGCAGTT	575	58
5′Ups 2	AGTAATTCCATGAGC AATTTA	CTCTTGCTCCACAAT GAAT	525	58
Fragment 1 (Exon 1)	GGAAGAAGTAAGAA CAAGGGA	CTCTCTGGACATCGA ACTGA	340	60
Fragment 2 (Exon 1)	TCCTCAAGACTAGA AGCCATAA	CTCCTTACATACAAG CCAGCA	341	60
Fragment 3 (Exon 2)	GATATGGAGGTGTTC GTTCG	TGATTTCAATGCCTA AGTTGG	378	57
Fragment 4 (Exon 2)	TGAGACCTGTCAAG ACTCCT	TAAGCACAGGAAAC TGGTAG	370	60
Fragment 5 (Exon 3)	GGCATTCAGATACTC AAACC	TGTCTGTTACCTTGA CTTCTA	293	60
Fragment 6 (Exon 3)	GGATGGGATTGGAT TATTG	AGACCTTCCATGTTT GAGG	291	60

Maejo Int. J. Sci. Technol. **2016**, *10*(02), 154-165

RESULTS AND DISCUSSION

Characterisation of 5′ Upstream Sequence of Buffalo *MSTN* Gene

Approximately 700 base pairs of this region were amplified using two overlapping primer pairs with annealing temperature at 58°C. By using the cattle *MSTN* gene as a reference sequence for primer designing, the 5′ upstream sequence in Thai swamp buffalo is amplified and characterised (Scheme 1). Multiple alignments with those of cattle (GenBank accession number AF348479), goat (AY827576), sheep (AY918121), horse (GQ183900) and pig (AF093798) show that this region shares 99, 97, 97, 88 and 88 % sequence identity respectively. The bioinformatics analysis using MatInspector software exhibits three TATA boxes (TATA-1, TATA-2 and TATA-3). Apart from the common transcription factor binding sites, muscle-specific transcription factor binding sites (E-boxes, MEF2, SMAD and FoxO) are observed as shown in Scheme 1. In addition, we find that each transcription factor binding site shows a high conservation among the compared animal sequences, especially for ruminants. The high conservation of these transcription factor binding sites among the animals compared indicates that it may be important for transcriptional regulation of the buffalo *MSTN* gene as well as other livestock. For example, Du et al. [19] studied the transcriptional regulation activities by detecting the fluorescence strength of enhanced green fluorescent protein in C2C12 myoblasts transfected with the vectors. The results indicate that some regions such as E3 and MEF2 have an influence on the transcription and expression of the sheep *MSTN* gene. Moreover, the mutagenesis of the highly conserved FoxO or SMAD binding sites significantly decreases the *MSTN* promoter activity [20]. Actually, several transcription factor binding sites located in the 5′ regulatory region of *MSTN* have been observed in other animals [21-23]. However, the 5′ upstream sequence and transcription factor binding sites located in this region of both the river and swamp buffalo have not been identified before. This study is the first report describing these issues.

Scheme 1. Results of analysis of 5′ upstream sequence of Thai swamp buffalo *MSTN* gene. Putative transcription factor binding sites are indicated by boxes. The atg indicates the start codon.

Maejo Int. J. Sci. Technol. **2016**, *10*(02), 154-165

Characterisation of Coding Sequences of Buffalo *MSTN* Gene

The coding sequences of the *MSTN* gene, which has been reported as a negative regulator of skeletal muscle growth [24], have previously been analysed in Brazilian Murrah buffalo [25] and Indian river buffalo [26]. In this study the characterisation of the *MSTN* coding sequences was performed in eight Thai swamp buffalo. The procedure consisted of amplification, sequencing and comparing the obtained sequences with those from cattle, pig and chicken *MSTN* genes. By using the river buffalo sequence as a reference for primer designing, the study succeeded in characterising the entire coding sequence of the *MSTN* gene (Scheme 2). The results show that the coding sequences in all 8 Thai swamp buffalo tested are identical to the river buffalo sequences (data not shown). A close relationship between the swamp and river buffalo *MSTN* genes has been previously reported. A molecular tree shows a cluster of their own and an apparent differentiation from 4 *Bos* species [27].

We also found that these sequences of the Thai buffalo *MSTN* gene are close to the *MSTN* cattle sequence rather than to the pig or chicken *MSTN* genes. In comparison to other livestock species, the entire coding sequence of Thai swamp buffalo *MSTN* gene has 98, 94 and 81% similarity to cattle, pig and chicken respectively (Scheme 3). These findings correlate well with their evolutionary relationships. Moreover, the *MSTN* gene variations are also firstly identified in Thai swamp buffalo. Two variants (g.264C>T and g.414G>A) located in exon 1 are found with the ratio of wild type to heterozygous variant samples at 5:3. The position g.264 (the number being based on river buffalo *MSTN* gene accession number DQ091762) is in codon 73 (AGC). Substitution of the third nucleotide (AGC>AGT) does not result in any change of the amino acid (serine). Likewise, the G>A transition at genomic DNA position g.414 (ACG>ACA) does not result in an alteration of threonine at codon position 123.

In their previous studies of river buffalo, Mota et al. [25] and Tantia et al. [26] did not find any variant in coding regions among the buffalo. It can thus be suggested that both the identified variants in our study may be specific for Thai swamp buffalo. We suggest that, as DNA variations in genomic DNA positions 264 and 414 are identified with high frequencies, these can be used as molecular markers for buffalo identification.

Based on the nucleic acid sequences obtained for the buffalo *MSTN* gene, amino acids were generated using the Biology WorkBench 3.2. It was found that the amino acid sequence of Thai swamp buffalo is 98, 94 and 86% identical to that of cattle, pig and chicken respectively (Scheme 4). To generate a biologically active myostatin ligand (a COOH-terminal fragment), cleavage by the furin-family enzyme at Arg-Ser-Arg-Arg (RSRR) aligned between positions 263 and 266 of amino acids is needed [31]. Although DNA sequences encoding for RSRR are not fully conserved among the species shown in Scheme 3, these sequences encode the same amino acid sequence. Analysis results in this study have revealed that this four-amino-acid region of Thai swamp buffalo is 100% identical to that of cattle, pig and chicken. In addition, a COOH-terminal fragment of Thai swamp buffalo shows 99, 97 and 96% amino acid similarity to myostatin of cattle, pig and chicken respectively. These results indicate that the myostatin of Thai swamp buffalo may have the same function as in other livestock animals. In this study although we did not find missense, nonsense and frameshift mutations (which can result in increased muscle mass) in any of the Thai swamp buffalo tested, further study on a larger number of animals should be done to clarify the possibility of mutation.

Maejo Int. J. Sci. Technol. **2016**, *10*(02), 154-165

```
     M  Q  K  L  Q  I  S  V  Y  I  Y  L  F  M  L  I  V  A  G  P
   1 atgcaaaaactgcaaatctctgtttatatttacctatttatgctgattgttgctggccca   60
     V  D  L  N  E  N  S  E  Q  K  E  N  V  E  K  E  G  L  C  N
  61 gtggatctgaatgagaacagcgagcagaaggaaaatgtggaaaaagaggggctgtgtaat  120
     A  C  L  W  R  E  N  T  T  S  S  R  L  E  A  I  K  I  Q  I
 121 gcatgtttgtggagggaaaacactacatcctcaagactagaagccataaaaatccaaatc  180
     L  S  K  L  R  L  E  T  A  P  N  I  S  K  D  A  I  R  Q  L
 181 ctcagtaaacttcgcctggaaacagctcctaacatcagcaaagatgctatcagacaactt  240
     L  P  K  A  P  P  L  L  E  L  I  D  Q  F  D  V  Q  R  D  A
 241 ttgcccaaggctcctccactcctggaactgattgatcagttcgatgtccagagagatgcc  300
     G  S  D  G  S  L  E  D  D  D  Y  H  A  R  T  D  A  V  I  T
 301 ggcagtgacggctccttggaagacgatgactaccacgccaggacggacgcggtcattacc  360
     M  P  T  E  S  D  L  L  T  Q  V  E  G  K  P  K  C  C  F  F
 361 atgcccacggagtctgatcttctaacgcaagtggaaggaaaacccaaatgttgcttcttt  420
     Q  F  S  S  K  I  Q  Y  N  K  L  V  K  A  Q  L  W  I  Y  L
 421 caatttagctctaagatacaatacaataaactagtaaaggcccaactgtggatatatctg  480
     R  P  V  K  T  P  A  T  V  F  V  Q  I  L  R  L  I  K  P  M
 481 agacccgtcaagactcctgcgacagtgtttgtgcagatcctgagactcatcaaacccatg  540
     K  D  G  T  R  Y  T  G  I  R  S  L  K  L  D  M  N  P  G  T
 541 aaagacggtacaaggtatactggaatccgatctctgaaacttgacatgaacccaggcact  600
     G  I  W  Q  S  I  D  V  K  T  V  L  Q  N  W  L  K  Q  P  E
 601 ggtatttggcagagcattgatgtgaagacagtgttgcaaaactggctcaaacaacctgaa  660
     S  N  L  G  I  E  I  K  A  L  D  E  N  G  H  D  L  A  V  T
 661 tccaacttaggcattgaaatcaaagctttagatgagaatggtcatgatcttgctgtaacc  720
     F  P  E  P  G  E  D  G  L  T  P  F  L  E  V  K  V  T  D  T
 721 ttcccagaaccaggagaagatggactgactccttttttagaagtcaaggtaacagacaca  780
     P  K  R  S  R  R  D  F  G  L  D  C  D  E  R  S  T  E  S  R
 781 ccaaaaagatctaggagagattttgggcttgattgtgatgagcgctccacagaatctcga  840
     C  C  R  Y  P  L  T  V  D  F  E  A  F  G  W  D  W  I  I  A
 841 tgctgtcgttaccctctaactgtggattttgaagctttggatgggattggattattgca  900
     P  K  R  Y  K  A  N  Y  C  S  G  E  C  E  F  V  F  L  Q  K
 901 cctaaaagatataaggccaattactgctctggagaatgtgaatttgtatttttgcaaaag  960
     Y  P  H  T  H  L  V  H  Q  A  N  P  R  G  S  A  G  P  C  C
 961 tatcctcatacccatcttgtgcaccaagcaaaccccagaggttcagctggcccctgctgc 1020
     T  P  T  K  M  S  P  I  N  M  L  Y  F  N  G  E  G  Q  I  I
1021 actcctacaaagatgtctccaattaatatgctatattttaatggcgaaggacaaataata 1080
     Y  G  K  I  P  A  M  V  V  D  R  C  G  C  S  *
1081 tatgggaagattccagccatggtagtagatcgctgtgggtgttcatga  1128
```

Scheme 2. Coding and amino acid sequences of Thai swamp buffalo *MSTN* gene. The first codon is underlined. Asterisk indicates stop codon.

Maejo Int. J. Sci. Technol. **2016**, *10*(02), 154-165

```
Swamp buffalo    1 ATGCAAAAACTGCAAATCTCTGCTTATATTTACCTATTTATGCTGATTGTTGCTGGCCCA
Cattle             ATGCAAAAACTGCAAATCTCTGTTTATATTTACCTATTTATGCTGATTGTTGCTGGCCCA
Pig                ATGCAAAAACTGCAAATCTATGTTTATATTTACCTGTTTATGCTGATTGTTGCTGGTCCC
Chicken            ATGCAAAAGCTAGCAGTCTATGTTTATATTTACCTGTTCATGCAGATCGCGGTTGATCCA
                   ******** **   * *** ** ************ ** **** *** *  * **  **

Swamp buffalo   61 GTGGATCTGAATGAGAACAGCGAGCAGAAGGAAAATGTGGAAAAAGAGGGGCTGTGTAAT
Cattle             GTGGATCTGAATGAGAACAGCGAGCAGAAGGAAAATGTGGAAAAAGAGGGGCTGTGTAAT
Pig                GTGGATCTGAATGAGAACAGCGAGCAAAAGGAAAATGTGGAAAAAGAGGGGCTGTGTAAT
Chicken            GTGGCTCTGGATGGCAGTAGTCAGCCCACAGAGAACGCTGAAAAAGACGGACTGTGCAAT
                   **** **** ***  *  **  ***  *  ** ** *  ******** ** ***** ***

Swamp buffalo  121 GCATGTTTGTGGAGGGAAAGCACTACATCCTCAAGACTAGAAGCCATAAAAATCCAAATC
Cattle             GCATGTTTGTGGAGGGAAAACACTACATCCTCAAGACTAGAAGCCATAAAAATCCAAATC
Pig                GCATGTATGTGGAGACAAAACACTAAATCTTCAAGACTAGAAGCCATAAAAATTCAAATC
Chicken            GCTTGTACGTGGAGACAGAATACAAAATCCTCCAGAATAGAAGCCATAAAAATTCAAATC
                   ** ***  ******  * *  ** * *** ** *** *************** ******

Swamp buffalo  181 CTCAGTAAACTTCGCCTGGAAACAGCTCCTAACATCAGCAAAGATGCTATCAGACAACTT
Cattle             CTCAGTAAACTTCGCCTGGAAACAGCTCCTAACATCAGCAAAGATGCTATCAGACAACTT
Pig                CTCAGTAAACTTCGCCTGGAAACAGCTCCTAACATTAGCAAAGATGCTATAAGACAACTT
Chicken            CTCAGCAAACTGCGCCTGGAACAAGCACCTAACATTAGCAGGGACGTTATTAAGCAGCTT
                   ***** ***** *********  *** ******** ****  ** * *** *  ** ***

Swamp buffalo  241 TTGCCCAAGGCTCCTCCACTCCTGGAACTGATTGATCAGTTCGATGTCCAGAGAGATGCC
Cattle             TTGCCCAAGGCTCCTCCACTCCTGGAACTGATTGATCAGTTCGATGTCCAGAGAGATGCC
Pig                TTGCCCAAAGCTCCTCCACTCCGGGAACTGATTGATCAGTACGATGTCCAGAGAGATGAC
Chicken            TTACCCAAAGCTCCTCCACTGCAGGAACTGATTGATCAGTATGATGTCCAGAGGGACGAC
                   ** ***** *********** * *****************  *********** ** * *


Swamp buffalo  301 GGCAGTGACGGCTCCTTGGAAGACGATGACTACCACGCCAGGACGGACGCGGTCATTACC
Cattle             AGCAGTGACGGCTCCTTGGAAGACGATGACTACCACGCCAGGACGGAAACGGTCATTACC
Pig                AGCAGTGATGGCTCCTTGGAAGATGATGATTATCACGCTACGACGGAAACGATCATTACC
Chicken            AGTAGCGATGGCTCTTTGGAAGACGATGACTATCATGCCACAACCGAGACGATTATCACA
                    * ** ** ***** ******** ***** ** ** ** *  ** **  ** * ** **

Swamp buffalo  361 ATGCCCACGGAGTCTGATCTTCTAACGCAAGTGGAAGGAAAACCCAAATGTTGCTTCTTT
Cattle             ATGCCCACGGAGTCTGATCTTCTAACGCAAGTGGAAGGAAAACCCAAATGTTGTTTCTTT
Pig                ATGCCTACAGAGTCTGATCTTCTAATGCAAGTGGAAGGAAAACCCAAATGCTGCTTCTTT
Chicken            ATGCCTACGGAGTCTGATTTTCTTGTACAAATGGAGGGAAAACCAAAATGTTGCTTCTTT
                   ***** ** ********* ****    *** **** ******** ***** ** ******

Swamp buffalo  421 CAATTTAGCTCTAAGATACAATACAATAAACTAGTAAAGGCCCAACTGTGGATATATCTG
Cattle             AAATTTAGCTCTAAGATACAATACAATAAACTAGTAAAGGCCCAACTGTGGATATATCTG
Pig                AAATTTAGCTCTAAAATACAATACAATAAAGTAGTAAAGGCCCAACTGTGGATATATCTG
Chicken            AAGTTTAGCTCTAAAATACAATATAACAAAGTAGTAAAGGCACAATTATGGATATACTTG
                    * *********** ******** ** *** ********** *** * ********  **

Swamp buffalo  481 AGACCCGTCAAGACTCCTGCGACAGTGTTTGTGCAGATCCTGAGACTCATCAAACCCATG
Cattle             AGGCCTGTCAAGACTCCTGCGACAGTGTTTGTGCAAATCCTGAGACTCATCAAACCCATG
Pig                AGACCCGTCAAGACTCCTACAACAGTGTTTGTGCAAATCCTGAGACTCATCAAACCCATG
Chicken            AGGCAAGTCCAAAAACCTACAACGGTGTTTGTGCAGATCCTGAGACTCATTAAGCCCATG
                   ** *  *** * *  *** * ** *********** ************** ** ******

Swamp buffalo  541 AAAGACGGTACAAGGTATACTGGAATCCGATCTCTGAAACTTGACATGAACCCAGGCACT
Cattle             AAAGACGGTACAAGGTATACTGGAATCCGATCTCTGAAACTTGACATGAACCCAGGCACT
Pig                AAAGACGGTACAAGGTATACTGGAATCCGATCTCTGAAACTTGACATGAACCCAGGCACT
Chicken            AAAGACGGTACAAGATATACTGGAATTCGATCTTTGAAACTTGACATGAACCCAGGCACT
                   ************** ************ ****** *************************
```

Scheme 3. Multiple alignments of *MSTN* coding sequences in four livestock animals: swamp buffalo, cattle (GenBank accession number AY160688) [28], pig (AY448008) [29] and chicken (AY448007) [30]. The first codon is underlined. The nucleotide positions 1126-1128 are stop codon. Asterisks indicate consensus sequence between four species. Box indicates variations of DNA sequence among species encoding amino acids for cleavage site (RSRR).

Maejo Int. J. Sci. Technol. **2016**, *10*(02), 154-165

```
Swamp buffalo  601 GGTATTTGGCAGAGCATTGATGTGAAGACAGTGTTGCAAAACTGGCTCAAACAACCTGAA
Cattle             GGTATTTGGCAGAGCATTGATGTGAAGACAGTGTTGCAGAACTGGCTCAAACAACCTGAA
Pig                GGTATTTGGCAGAGCATTGATGTGAAGACAGTGTTGCAAAATTGGCTCAAACAACCTGAA
Chicken            GGTATCTGGCAGAGTATTGATGTGAAGACAGTGCTGCAAAATTGGCTCAAACAGCCTGAA
                   ***** ******** ****************** **** ** *********** ******

Swamp buffalo  661 TCCAACTTAGGCATTGAAATCAAAGCTTTAGATGAGAATGGTCATGATCTTGCTGTAACC
Cattle             TCCAACTTAGGCATTGAAATCAAAGCTTTAGATGAGAATGGCCATGATCTTGCTGTAACC
Pig                TCCAACTTAGGCATTGAAATCAAAGCTTTAGATGAGAATGGTCATGATCTTGCTGTAACC
Chicken            TCCAATTTAGGCATCGAAATAAAAGCTTTTGATGAGACTGGACGAGATCTTGCTGTCACA
                   ***** ******** ***** ******** ******* *** *  *********** **

Swamp buffalo  721 TTCCCAGAACCAGGAGAAGATGGACTGACTCCTTTTTTAGAAGTCAAGGTAACAGACACA
Cattle             TTCCCAGAACCAGGAGAAGATGGACTGACTCCTTTTTTAGAAGTCAAGGTAACAGACACA
Pig                TTCCCAGGACCAGGAGAAGATGGGCTGAATCCCTTTTTAGAAGTCAAGGTAACAGACACA
Chicken            TTCCCAGGACCGGGTGAAGATGGATTGAACCCATTTTTAGAGGTCAGAGTTACAGACACA
                   ******* *** ** ********  ***  ** ******** ****  ** *********

Swamp buffalo  781 CCAAAAAGATCTAGGAGAGATTTTGGGCTTGATTGTGATGAGCGCTCCACAGAATCTCGA
Cattle             CCAAAAAGATCTAGGAGAGATTTTGGGCTTGATTGTGATGAACACTCCACAGAATCTCGA
Pig                CCAAAAAGATCCAGGAGAGATTTTGGACTCGACTGTGATGAGCACTCAACAGAATCTCGA
Chicken            CCGAAACGGTCCCGCAGAGATTTTGGCCTTGACTGTGATGAGCACTCAACGGAATCCCGA
                   ** *** * **  * ********* ** ** ******** * *** ** ***** ***

Swamp buffalo  841 TGCTGTCGTTACCCTCTAACTGTGGATTTTGAAGCTTTTGGATGGGATTGGATTATTGCA
Cattle             TGCTGTCGTTACCCTCTAACTGTGGATTTTGAAGCTTTTGGATGGGATTGGATTATTGCA
Pig                TGCTGTCGTTACCCTCTAACTGTGGATTTTGAAGCTTTTGGATGGGACTGGATTATTGCA
Chicken            TGTTGTCGCTACCCGCTGACAGTGGATTTCGAAGCTTTTGGATGGGACTGGATTATAGCA
                   ** ***** ***** ** ** ******** ***************** ******** ***

Swamp buffalo  901 CCTAAAAGATATAAGGCCAATTACTGCTCTGGAGAATGTGAATTTGTATTTTTGCAAAAG
Cattle             CCTAAAAGATATAAGGCCAATTACTGCTCTGGAGAATGTGAATTTGTATTTTTGCAAAAG
Pig                CCCAAAAGATATAAGGCCAGTTACTGCTCTGGAGAGTGTGAATTTGTATTTTTACAAAAA
Chicken            CCTAAAAGATACAAAGCCAATTACTGCTCCGGAGAATGTGAATTTGTGTTTCTACAGAAA
                   ** ******** ** **** ********* ***** *********** *** * ** **

Swamp buffalo  961 TATCCTCATACCCATCTTGTGCACCAAGCAAACCCCAGAGGTTCAGCTGGCCCCTGCTGC
Cattle             TATCCTCATACCCATCTTGTGCACCAAGCAAACCCCAGAGGTTCAGCCGGCCCCTGCTGT
Pig                TACCCTCACACTCATCTTGTGCACCAAGCAAACCCCAGAGGTTCAGCAGGCCCCTGCTGT
Chicken            TACCCGCACACTCACCTGGTACACCAAGCAAATCCCAGAGGCTCAGCAGGCCCTTGCTGC
                   ** ** ** ** ** ** ** ********** ******** ***** ***** *****

Swamp buffalo 1021 ACTCCTACAAAGATGTCTCCAATTAATATGCTATATTTTAATGGCGAAGGACAAATAATA
Cattle             ACTCCTACAAAGATGTCTCCAATTAATATGCTATATTTTAATGGCGAAGGACAAATAATA
Pig                ACTCCCACAAAGATGTCTCCAATCAATATGCTATATTTTAATGGCAAAGAACAAATAATA
Chicken            ACACCCACCAAGATGTCCCCTATAAACATGCTGTATTTCAATGGAAAAGAACAAATAATA
                   ** ** ** ******** ** ** ** ***** ***** *****  *** **********

Swamp buffalo 1081 TATGGGAAGATTCCAGCCATGGTAGTAGATCGCTGTGGGTGTTCATGA 1128
Cattle             TACGGGAAGATTCCAGCCATGGTAGTAGATCGCTGTGGGTGTTCATGA
Pig                TATGGGAAAATTCCAGCCATGGTAGTAGATCGCTGTGGGTGCTCATGA
Chicken            TATGGAAAGATACCAGCCATGGTTGTAGATCGTTGCGGGTGCTCATGA
                   ** ** ** ** *********** ******** ** ***** ******
```

Scheme 3 (Continued).

Maejo Int. J. Sci. Technol. **2016**, *10*(02), 154-165

```
Swamp buffalo    1 MQKLQISVYIYLFMLIVAGPVDLNENSEQKENVEKEGLCNACLWRENTTSSRLEAIKIQI  60
Cattle             MQKLQISVYIYLFMLIVAGPVDLNENSEQKENVEKEGLCNACLWRENTTSSRLEAIKIQI
Pig                MQKLQIYVYIYLFMLIVAGPVDLNENSEQKENVEKEGLCNACMWRQNTKSSRLEAIKIQI
Chicken            MQKLAVYVYIYLFMQIAVDPVALDGSSQPTENAEKDGLCNACTWRQNTKSSRIEAIKIQI
                   ****   ******* *   ** *   *   ** ** ****** ** ** *** *******

Swamp buffalo   61 LSKLRLETAPNISKDAIRQLLPKAPPLLELIDQFDVQRDAGSDGSLEDDDYHARTDAVIT 120
Cattle             LSKLRLETAPNISKDAIRQLLPKAPPLLELIDQFDVQRDASSDGSLEDDDYHARTETVIT
Pig                LSKLRLETAPNISKDAIRQLLPKAPPLRELIDQYDVQRDDSSDGSLEDDDYHATTETIIT
Chicken            LSKLRLEQAPNISRDVIKQLLPKAPPLQELIDQYDVQRDDSSDGSLEDDDYHATTETIIT
                   ******* ***** * * ********* ***** *****  ************ *   **

Swamp buffalo  121 MPTESDLLTQVEGKPKCCFFQFSSKIQYNKLVKAQLWIYLRPVKTPATVFVQILRLIKPM 180
Cattle             MPTESDLLTQVEGKPKCCFFKFSSKIQYNKLVKAQLWIYLRPVKTPATVFVQILRLIKPM
Pig                MPTESDLLMQVEGKPKCCFFKFSSKIQYNKVVKAQLWIYLRPVKTPTTVFVQILRLIKPM
Chicken            MPTESDFLVQMEGKPKCCFFKFSSKIQYNKVVKAQLWIYLRQVQKPTTVFVQILRLIKPM
                   ****** * * ********* ********* ********** *  * *************

Swamp buffalo  181 KDGTRYTGIRSLKLDMNPGTGIWQSIDVKTVLQNWLKQPESNLGIEIKALDENGHDLAVT 240
Cattle             KDGTRYTGIRSLKLDMNPGTGIWQSIDVKTVLQNWLKQPESNLGIEIKALDENGHDLAVT
Pig                KDGTRYTGIRSLKLDMNPGTGIWQSIDVKTVLQNWLKQPESNLGIEIKALDENGHDLAVT
Chicken            KDGTRYTGIRSLKLDMNPGTGIWQSIDVKTVLQNWLKQPESNLGIEIKAFDETGRDLAVT
                   ********************************************** ** * *****

Swamp buffalo  241 FPEPGEDGLTPFLEVKVTDTPKRSRRQFGLDCDERSTESRCCRYPLTVDFEAFGWDWIIA 300
Cattle             FPEPGEDGLTPFLEVKVTDTPKRSRRQFGLDCDEHSTESRCCRYPLTVDFEAFGWDWIIA
Pig                FPGPGEDGLNPFLEVKVTDTPKRSRRQFGLDCDEHSTESRCCRYPLTVDFEAFGWDWIIA
Chicken            FPGPGEDGLNPFLEVRVTDTPKRSRRQFGLDCDEHSTESRCCRYPLTVDFEAFGWDWIIA
                   ** ****** ***** ****************** ************************

Swamp buffalo  301 PKRYKANYCSGECEFVFLQKYPHTHLVHQANPRGSAGPCCTPTKMSPINMLYFNGEGQII 360
Cattle             PKRYKANYCSGECEFVFLQKYPHTHLVHQANPRGSAGPCCTPTKMSPINMLYFNGEGQII
Pig                PKRYKANYCSGECEFVFLQKYPHTHLVHQANPRGSAGPCCTPTKMSPINMLYFNGKEQII
Chicken            PKRYKANYCSGECEFVFLQKYPHTHLVHQANPRGPAGPCCTPTKMSPINMLYFNGKEQII
                   ********************************** ******************  ***

Swamp buffalo  361 YGKIPAMVVDRCGCS 375
Cattle             YGKIPAMVVDRCGCS
Pig                YGKIPAMVVDRCGCS
Chicken            YGKIPAMVVDRCGCS
                   ***************
```

Scheme 4. Multiple alignment of myostatin amino acid sequences in four livestock animals: swamp buffalo, cattle (GenBank accession number AF320998) [32], pig (EF490986) [33] and chicken (AF346599) [34]. The cleavage site (RSRR) is shown in the box. Asterisks indicate consensus amino acid residues between the four species.

CONCLUSIONS

Our results have demonstrated that the 5′ upstream, coding and amino acid sequences of the Thai swamp buffalo *MSTN* gene show high sequence identity with other livestock animals, especially cattle. To our knowledge, this is the first *MSTN* gene report for Thai swamp buffalo. The output provides basic information that can be used in further study such as buffalo breeding.

ACKNOWLEDGMENTS

We thank the staff at Surin and Lamphayaklang Livestock Research and Breeding Centre for allowing us to use the buffalo and for their assistance during blood sampling. We also thank Veterinary Research and Development Centre (Lower North-eastern Region) for allowing us to use

their facilities. We would like to give a special thank to all staff members at the biotechnology laboratory, Department of Pathology, Faculty of Veterinary Medicine, Kasetsart University, Kamphaeng Saen Campus for their support with the use of laboratory equipment.

This research was supported by the Centre of Excellence on Agricultural Biotechnology, Science and Technology Postgraduate Education and Research Development Office, Commission on Higher Education, Ministry of Education (AG-BIO/PERDO-CHE).

REFERENCES

1. K. Triwitayakorn, B. Moolmuang, S. Sraphet, S. Panyim, A. Na-Chiangmai and D. R. Smith, "Analysis of genetic diversity of the Thai swamp buffalo (*Bubalus bubalis*) using cattle microsatellite DNA markers", *Asian-Australas. J. Anim. Sci.,* **2006**, *19*, 617-621.
2. S. Uriyapongson, "Buffalo and buffalo meat in Thailand", *Buffalo Bull.*, **2013**, *32*, 329-332.
3. H. K. Koopaei and A. E. Koshkoiyeh, "Application of genomic technologies to the improvement of meat quality in farm animals", *Biotechnol. Mol. Biol. Rev.*, **2011**, *6*, 126-132.
4. M. Strazzullo, C. Rossetti, G. Fusco, C. Campanile, D. Vecchio, G. Campanile, A. Perucatti, G. P. Di Meo, F. Filippini, A. Eggen, L. Ferrara and M. D′Esposito, "Genomic characterization and chromosomal mapping of 5 river buffalo skeletal muscle differentiation master genes", *Cytogenet. Genome Res*., **2010**, *128*, 221-227.
5. C. Marchitelli, M. C. Savarese, A. Crisa, A. Nardone, P. A. Marsan and A. Valentini, "Double muscling in Marchigiana beef breed is caused by a stop codon in the third exon of myostatin gene", *Mamm. Genome.*, **2003**, *14*, 392-395.
6. J. L. Gill, S. C. Bishop, C. McCorquodale, J. L. Williams and P Wiener, "Associations between the 11-bp deletion in the myostatin gene and carcass quality in Angus-sired cattle", *Anim. Genet.*, **2009**, *40*, 97-100.
7. P. Wiener, J. A. Woolliams, A. Frank-Lawale, M. Ryan, R. I. Richardson, G. R. Nute, J. D. Wood, D. Homer and J. L. Williams, "The effects of a mutation in the myostatin gene on meat and carcass quality", *Meat Sci.*, **2009**, *83*, 127-134.
8. I. A. Boman, G. Klemetsdal, T. Blichfeldt, O. Nafstad and D. I. Vage, "A frameshift mutation in the coding region of the myostatin gene (*MSTN*) affects carcass conformation and fatness in Norwegian White Sheep (*Ovis aries*)", *Anim. Genet.*, **2009**, *40*, 418-422.
9. I. A. Boman, G. Klemetsdal, O. Nafstad, T. Blichfeldt and D. I. Vage, "Impact of two myostatin (*MSTN*) mutations on weight gain and lamb carcass classification in Norwegian White Sheep (*Ovis aries*)", *Genet. Sel. Evol.*, **2010**, *42*, doi:10.1186/1297-9686-42-4
10. T. Tozaki, F. Sato, E. W. Hill, T. Miyake, Y. Endo, H. Kakoi, H. Gawahara, K. Hirota, Y. Nakano, Y. Nambo and M. Kurosawa, "Sequence variants at the myostatin gene locus influence the body composition of Thoroughbred horses", *J. Vet. Med. Sci.*, **2011**, *73*, 1617-1624.
11. G. Zhang, L. Zhang, Y. Wei, J. Wang, F. Ding, G. Dai and K. Xie, "Polymorphisms of the myostatin gene and its relationship with reproduction traits in the Bian chicken", *Anim. Biotechnol.*, **2012**, *23*, 184-193.
12. Z. J. Zhang, Y. H. Ling, L. J. Wang, Y. F. Hang, X. F. Guo, Y. H. Zhang, J. P. Ding and X. R. Zhang, "Polymorphisms of the myostatin gene (*MSTN*) and its relationship with growth traits in goat breeds", *Genet. Mol. Res.*, **2013**, *12*, 965-971.
13. S. A. Miller, D. D. Dykes and H. F. Polesky, "A simple salting out procedure for extracting DNA from human nucleated cells", *Nucleic Acids Res.*, **1988**, *16*, 1215.

14. T. Koressaar and M. Remm, "Enhancement and modifications of primer design program primer3", *Bioinformatics*, **2007**, *23*, 1289-1291.
15. National Centre of Biotechnology Information (NCBI), "*Bubalus bubalis* myostatin gene, complete cds", http://www.ncbi.nlm.nih.gov/nuccore/DQ091762 (Accessed: January 2014).
16. National Centre of Biotechnology Information (NCBI), "*Bos taurus* myostatin gene", http://www.ncbi.nlm.nih.gov/nuccore/AF348479 (Accessed: January 2014).
17. Genomatix, "Genomatix software suite", http://www.genomatix.de, (Accessed: January 2014).
18. San Diego Supercomputer Centre (SDSC), "Biology WorkBench version 3.2", http://workbench.sdsc.edu, (Accessed: January 2014).
19. R. Du, X. R. An, Y. F. Chen and J. Qin, "Some motifs were important for myostatin transcriptional regulation in sheep (*Ovis aries*)", *J. Biochem. Mol. Biol.*, **2007**, *40*, 547-553.
20. D. L. Allen and T. G. Unterman, "Regulation of myostatin expression and myoblast differentiation by FoxO and SMAD transcription factors", *Am. J. Physiol. Cell Physiol.*, **2007**, *292*, 188-199.
21. D. L. Allen and M. Du, "Comparative functional analysis of the cow and mouse myostatin genes reveals novel regulatory elements in their upstream promoter regions", *Comp. Biochem. Physiol. B Biochem. Mol. Biol.,* **2008**, *150*, 432-439.
22. S. Dall'Olio, L. Fontanesi, L. N. Costa, M. Tassinari, L. Minieri and A. Falaschini, "Analysis of horse myostatin gene and identification of single nucleotide polymorphisms in breeds of different morphological types", *J. Biomed. Biotechnol.*, **2010**, *2010*, Art. ID 542945.
23. J. Li, J. Deng, S. Yu, J. Zhang, D. Cheng and H. Wang, "The virtual element in proximal promoter of porcine myostatin is regulated by myocyte enhancer factor 2C", *Biochem. Biophys. Res. Commun.*, **2012**, *419*, 175-181.
24. A. C. McPherron and S. J. Lee, "Double muscling in cattle due to mutations in the myostatin gene", *Proc. Natl. Acad. Sci. USA.*, **1997**, *94*, 12457-12461.
25. L. S. L. S. Mota, R. A. Curi, D. A. Palmieri, A. S. Borges, C. R. Lopes, J. D. Babosa and M. A. Gemines, "Sequence characterization of coding regions of the myostatin gene (*GDF8*) from Brazilian Murrah buffaloes (*Bubalus bubalis*) and comparison with the *Bos taurus* sequence", *Genet. Mol. Biol*., **2006**, *29*, 79-82.
26. M. S. Tantia, R. K. Vijh, B. Mishra and S. T. B. Kumar, "Sequence of *GDF8* (Myostatin) gene in *Bubalus bubalis*", *Anim. Biotechnol.*, **2007**, *18*, 177-181.
27. L. P. Wang, R. Q. Geng, D. J. Ji, H. Chang, C. F. Chang and Y. H. Li, "Molecular evolution character of complete coding sequence of bovine myostatin gene", *Acta Agric. Bor. Sin*., **2009**, *24*, 46-49.
28. National Centre of Biotechnology Information (NCBI), "*Bos taurus* myostatin (*GDF8*) mRNA, complete cds", http://www.ncbi.nlm.nih.gov/nuccore/AY160688 (Accessed: January 2014).
29. National Centre of Biotechnology Information (NCBI), "*Sus scrofa* myostatin (*MSTN*) mRNA, complete cds", http://www.ncbi.nlm.nih.gov/nuccore/AY448008 (Accessed: January 2014).
30. National Centre of Biotechnology Information (NCBI), "*Gallus gallus* myostatin (*MSTN*) mRNA, complete cds", http://www.ncbi.nlm.nih.gov/nuccore/AY448007 (Accessed: January 2014).
31. Z. Huang, X. Chen and D. Chen, "Myostatin: A novel insight into its role in metabolism, signal pathways, and expression regulation", *Cell. Signal.*, **2011**, *23*, 1441-1446.
32. National Centre of Biotechnology Information (NCBI), "*Bos taurus* myostatin (*GDF8*) gene, complete cds", http://www.ncbi.nlm.nih.gov/nuccore/AF320998 (Accessed: January 2014).

Maejo Int. J. Sci. Technol. **2016**, *10*(02), 154-165

33. National Centre of Biotechnology Information (NCBI), "*Sus scrofa* breed Large White myostatin (*MSTN*) gene, complete cds", http://www.ncbi.nlm.nih.gov/nuccore/EF490986 (Accessed: January 2014).
34. National Centre of Biotechnology Information (NCBI), "*Gallus gallus* myostatin (*MSTN*) gene, promoter region, exons 1, 2 and 3 and complete cds", http://www.ncbi.nlm.nih.gov/nuccore/AF346599 (Accessed: January 2014).