

SHORT COMMUNICATION

Effects of calpastatin gene polymorphism on hematology and selected serum biochemical parameters in Awassi lambs

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ABSTRACT

Objective: This study was conducted to investigate the effect of *MspI* polymorphism in the calpastatin (CAST) gene on hematology and selected serum biochemical parameters in Awassi lambs.

Materials and Methods: CAST genotypes of 31 Awassi lambs were determined using polymerase chain reaction—restricted fragment length polymorphism method. Hematology, serum biochemical analyses, serum levels of triiodothyronine, thyroxine, and cortisol were determined using routine laboratory procedures.

Results: Two CAST genotypes were detected with frequencies of 0.65 and 0.35 for MN (three major bands of 622, 336, and 268 bp) and NN (two major bands of 336 and 268 bp), respectively. Allele frequencies were 0.49 and 0.51 for M and N alleles, respectively. Animals with MN *MspI* CAST genotype had significantly ($p < 0.05$) higher neutrophil percentage and neutrophil to lymphocyte ratio but, significantly ($p < 0.05$) lower lymphocyte percentage and neutrophil to lymphocyte ratio than NN *MspI* CAST genotype. Serum T3 and cortisol concentrations were significantly ($p < 0.05$) higher in MN *MspI* CAST genotype than the NN *MspI* CAST genotype.

Conclusion: Results of this study indicate that CAST gene heterozygous individuals are healthier than homozygous individual, which may explain the superiority of the CAST gene heterozygous animals in growth performance.

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Introduction

Calpastatin (CAST) is a member of the calpain system which is believed to control skeletal muscle turnover and protein degradation [1]. Growth of skeletal muscles was found to require inhibition of calpain activity in order to achieve a significant reduction in muscle protein degradation [1]. This inhibition of calpain activity is associated with stimulation of CAST activity [1]. It was found that calpain modulation is affected by the indigenous inhibitor (CAST) that regulates the calpain activity [1]. CAST gene polymorphism has been investigated using several restriction enzymes such as *MspI* [2–5], *NcoI*, and *Hin6I* [6]. CAST gene was found to have a significant effect on pre-weaning growth rate and carcass quality [6].

Various animal production traits are greatly affected by the genetic potential of each individual animal [7]. High producing animals could be under chronic stress that adversely affects their productivity and welfare. Assessment of various hematology and serum biochemistry parameters, thyroid gland functions, and serum cortisol levels could be used to evaluate the general health status of production animals [8]. In this context, the effect of CAST gene polymorphism on various hematology and serum biochemistry parameters has not been investigated before in sheep. Therefore, the objectives of this study were to investigate the effect of CAST gene polymorphism on the hematology and selected serum biochemistry parameters in Awassi lambs.

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Material and Methods

This project was approved by the Animal Care and Use Committee at Jordan University of Science and Technology.

Animals

Animals involved in the study were subjected to a complete physical examination to determine their health status. Only apparently healthy animals were used in the study. A total of 31 ram lambs aged between 3 and 5 months were used.

PCR-RFLP procedure

Approximately, 5 ml of whole blood was collected via Jugular venipuncture using vacutainer needles and tubes containing EDTA (BD Vacutainer, UK). In the laboratory, E.Z.N.A. Blood DNA kit (BioTek, Winooski, VT) was used to isolate genomic DNA from whole blood samples within 3–4 h after collection. (polymerase chain reaction—restricted fragment length polymorphism) method was carried out for genotyping the region of the first encoding repetitive domain region (exon and intron 1) using previously published primer [9]. In each PCR reaction of 20 μ l, a mixture of 10 μ l of nuclease-free water, 100 ng of isolated DNA, 0.5 μ M of the primer, and 5 unit/ μ l of HOT FIRE Pol[®] DNA polymerase were added. The PCR cyclers were set to perform denaturation at 95°C for 3 min, 30 cycles of denaturation at 95°C for 30 sec, annealing at 63°C for 50 sec, and polymerization at 72°C for 60 sec, and final extension at 72°C for 10 min.

A PCR product of 622 bp was then digested using *MspI* restriction enzyme. Briefly, the reaction mixture contained 10 μ l PCR product, 20 units *MspI* enzyme, 2 μ l nuclease free water, 1 μ l bovine albumen, and 6 μ l buffers. The digestion took place at 37°C for 12 h. The digestion products were then viewed under UV light on ethidium bromide, 1.5% agarose stained gels.

Hematology and serum biochemistry analyses

A sample of whole blood was collected from each animal in the early morning via Jugular venipuncture and placed in plain and EDTA containing tubes. Samples were transferred to laboratory on ice where hematology analysis was performed within 3–4 h after collection using electronic cell counter (ABC Vet hematology analyzer, ABX Diagnostics, France). The following parameters were determined: total white blood cell count (WBC), red blood cell count (RBC), hemoglobin concentration (Hb), packed cell volume (PCV), platelet count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC).

Serum was obtained by centrifugation of clotted blood at 10,000 \times g for 10 min. The following serum biochemical parameters were determined: total protein using the Biuret method, albumin using the Bromocresol Green method,

urea nitrogen using the colorimetric method, creatinine using the kinetic method, serum glucose using glucose oxidase-phenol and 4 aminophenazone method, total triglyceride using glycerol phosphate oxidase method, and serum cholesterol using glucose oxidase peroxidase method. In addition, serum concentrations of triiodothyronine (T3), thyroxine (T4), and cortisol were determined using commercially available ELISA kits (Biocheck, San Francisco, CA) according to the manufacturer's instructions.

Data analysis

Genotype and allelic frequencies were calculated manually [10]. Mixed model of general linear model procedure (SAS, 2004) was used for analyzing the hematological and serum biochemistry values. The effect of CAST genotypes and the interaction between them on all studied traits were included in the statistical model.

Results

Figure 1 shows two different CAST genotypes with frequencies of 0.65 and 0.35 for MN (three major bands) and NN (two major bands), respectively. The allele frequencies of these genotypes were 0.49 and 0.51 for M and N alleles, respectively.

Various hematology parameters (means \pm SE) are shown in Table 1. No significant variation were detected in the mean values of WBC, RBC, Hb, PCV, platelets, MCV, MCH, and MCHC between the two CAST genotypes of Awassi lambs. However, the percentages of neutrophils, lymphocytes, and the neutrophil to lymphocytes ratio were significantly ($p < 0.05$) affected by genotype. Animals with MN CAST genotype had significantly ($p < 0.05$) higher neutrophil percentage and higher N/L but significantly ($p < 0.05$) lower lymphocyte percentage and lower N/L than NN genotype. No other significant

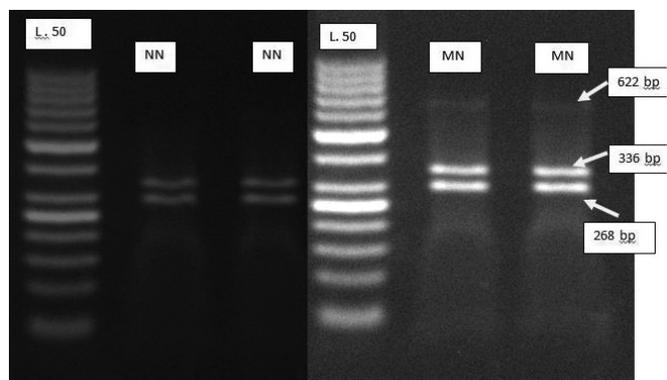


Figure 1. Image of 1.5% agarose gel electrophoresis showing MN and NN *MspI* CAST genotypes detected in Awassi lambs.

Table 1. Least square means \pm S.E. of various hematology values in Awassi sheep with different genotypes of the *MspI* CAST gene.

| Parameters | CAST gene genotypes | |
|--|------------------------------|-----------------------------|
| | NN (n = 16) | MN (n = 15) |
| White blood cell count ($\times 10^3/\mu\text{l}$) | 10 \pm 0.6 | 11 \pm 0.7 |
| Red blood cell count ($\times 10^6/\mu\text{l}$) | 11 \pm 0.3 | 11 \pm 0.4 |
| Hemoglobin (g/dl) | 11 \pm 0.2 | 11 \pm 0.3 |
| Packed cell volume (fl) | 35 \pm 1.1 | 33 \pm 1.4 |
| Mean corpuscular volume (pg) | 31 \pm 0.3 | 31 \pm 0.4 |
| Mean corpuscular hemoglobin (g/dl) | 10 \pm 0.2 | 10 \pm 0.3 |
| Mean corpuscular hemoglobin concentration(g/dl) | 32 \pm 0.9 | 33 \pm 1.1 |
| Platelets ($\times 10^3/\mu\text{l}$) | 307 \pm 41 | 367 \pm 51 |
| Neutrophils (N;%) | 33 \pm 1.7 ^a | 39 \pm 2.1 ^b |
| Lymphocytes (L;%) | 56 \pm 1.5 ^a | 51 \pm 1.8 ^b |
| N/L | 0.59 \pm 0.05 ^a | 0.76 \pm 0.1 ^b |

Means within the same row with different small superscript letters differ significantly $p < 0.05$.

Table 2. Least square means \pm S.E. for serum biochemistry values in Awassi sheep with different genotypes of the *MspI* CAST gene.

| Parameter | CAST Gene Genotypes | |
|-------------------------------|----------------------------|----------------------------|
| | NN (n = 16) | MN (n = 15) |
| Glucose (mg/dl) | 66 \pm 3.3 | 68 \pm 4 |
| Total protein (g/l) | 72 \pm 1.6 | 74 \pm 2 |
| Albumen (mg/l) | 27 \pm 1.3 ^a | 29 \pm 1.6 |
| Cholesterol (mg/dl) | 68 \pm 3.7 | 63 \pm 4.6 |
| Triglycerides (mg/dl) | 23 \pm 3.6 | 24 \pm 4.5 |
| T3 ($\mu\text{g/ml}$) | 3.1 \pm 0.2 ^a | 3.9 \pm 0.3 ^b |
| T4 (ng/ml) | 11 \pm 0.4 | 11 \pm 0.4 |
| Cortisol ($\mu\text{g/ml}$) | 1.7 \pm 0.4 ^a | 2.9 \pm 0.5 ^b |

Means within the same row with different small superscript letters differ significantly $p < 0.05$.

differences were found in the hematology analyses between the two genotypes.

In the serum biochemistry analyses, animals with heterozygous (MN) CAST genotype were found to have significantly ($p < 0.05$) higher triiodothyronine (T3) and cortisol levels than homozygous recessive (NN) genotype (Table 2). No other significant differences were found in the serum biochemistry analyses between the two genotypes.

Discussion

Not only the hematological analysis is a useful clinical tool in the diagnosis of many diseases, it can also be used to understand certain environmental effects on blood components. Furthermore, it was suggested that findings

of various hematological parameters can be used in the selection process of animals that show clinical resistance to important diseases and other environmental conditions [8]. In addition, certain blood biochemistry parameters, such as protein, thyroid hormones, and cortisol, can be useful indicators of the physiologic, metabolic, and welfare status of the animal [6].

In this study, two CAST genotypes were detected with frequencies of 0.65 and 0.35 for MN and NN genotype, respectively. These results are in agreement with previously reported data in different breeds of sheep [11]. In this study, lambs with heterozygous CAST gene were found to have significantly higher percentages of neutrophils and lower percentages of lymphocytes. Whether this difference has a significant effect on disease resistance in MN lambs requires further investigation. Previous research in Awassi sheep has reported superior performance of MN CAST genotype [11].

Appropriately functioning thyroid gland is considered essential to sustain the productivity and performance of lambs. Measurements of the circulating thyroid hormone, therefore, can be considered an important indicator of the metabolic and nutritional status of the animal [12]. In this study, MN CAST lambs were found to have significantly higher levels of T3 than that in NN CAST lambs. Degradation of protein as a result of high levels of T3 may explain the major effect of CAST gene on meat tenderness claimed by many authors [11].

Stress in animals can be assessed by direct measurement of serum cortisol levels [13]. In this study, the heterozygous CAST genotype animals were found to have significantly higher levels of cortisol than homozygous CAST genotype lambs. This may indicate that heterozygous animals, while are superior in performance, they may suffer greater stressful times than their CAST homozygous herd mates. A link also has been reported between the prepartum rise in cortisol levels and post-partum cortisol and T3 levels in newborn lambs [14].

Results of this study showed that lambs with MN *MspI* CAST genotype have significantly high percentage of neutrophils, neutrophil to lymphocytes ratio, serum T3 concentration, and cortisol concentration than lambs with NN CAST genotype. These results indicate that CAST gene heterozygous individuals may be healthier than homozygous individual, which may explain the superiority of the CAST gene heterozygous animals in growth performance. However, further research is warranted to investigate the exact CAST genotype effect on animal resistance to specific disease and other environmental stressful conditions.

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Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

Khaleel Ibrahim Jawasreh designed the experiment and coordinated all research activities, including genotyping and data analysis in addition to manuscript writing. Zuhair Bani Ismail supervised fieldwork, blood analysis, and manuscript writing.

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