CAST/MspI gene polymorphism and its impact on growth performance and carcass traits of Shami goats breed in Iraq

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Abstract. The identification of allelic and genotypic polymorphisms of the calpastatin gene and verify the effect of these polymorphisms on growth and non-carcass components traits of Shami goats breed were The objectives of this study. Seventy males of Shami goats were phenotyped for growth performance (birth weight, weaning weight, slaughter weight), carcass weights (hot and chill carcass weights) and non-carcass components traits (head, legs, skin, heart, lungs, testes, spleen and kidney weights). Male goats were weighed at the beginning of the experiment and the subsequent weights were measured. All goats were slaughtered to evaluate carcass characteristics. Two alleles (M and N) with frequencies of 0.84 and 0.16, respectively, and two genotypes (MM, MN) with 68.6% and 31.4% frequencies successively, were detected. The association of calpastatin genotype was significant with weaning weight, slaughter weight, carcass hot and chill weights (P < 0.05) by the superiority of MN genotype with 16.964, 27.70, 11.28 and 11.01 kg respectively. The same superiority of MN genotype was found in non-carcass components traits (P < 0.05) with 2081.07, 801.78, 1699.29, 109.28, 326.07 and 187.64 gm., for head, legs, skin, heart, lungs and testes respectively.

Keyword: Calpastatin gene, PCR-RFLP, growth performance, non-carcass components, Shami goats.

1. Introduction

There is a worldwide trend for rapid accession in require for goat meat (23) consequent to several reasons including; the increased consumer demand to leaner meat compared to other types of red meat (19), subcutaneous fat is slow development in goat (26) and goat deposit higher polyunsaturated fatty acids than other ruminants (5). The purpose of studying carcass traits is to evaluate the objective parameters related to qualitative and quantitative aspects (9). Thus, the value of a carcass depends, among other factors, on the body weight: slaughter age ratio, whose objective is to obtain higher weights at younger ages so as to meet the demands of the consumer market (17). The non-carcass components traditionally served only to cover parts of the costs generated during slaughter. Studies aiming at the development of the local meat sector should take into account the cultural habits of the consumer such as in Africa (1), Texas (20) or in Brazil (21). As a the cultural context, the non-carcass components (offal) may be considered as waste material that is thrown away, or as delicacies that can command an interesting price such as in Jamaica, Antigua and French West Indies (2). Otherwise, the quality of the animal yield does not depend only on the carcass yield and its cuts, but also on the proportion and quality of the other components (non-carcass components) (18), and the lower proportion of non-carcass components may have contributed to higher dressing percentages (15 in
goats and 16 in sheep). Recently, there has been remarkable progress in identifying chromosomal regions that affect important traits in livestock production through molecular genetics application (3). One of the genes that may affect the growth and meat characteristics, is the calpastatin gene (CAST). The calpain-calpastatin system plays a critical role in the expansion and evolution of skeletal muscles in goats and other mammals (10). The action of calpastatin is quite correlated with muscle growth rate through its role to repress the activity of calpains that result in detraction the rate of protein decay (11). This study aimed to investigate the relationship between the CAST gene polymorphisms and growth performance and carcass characteristics of Shami goats in Iraq.

2. Material and methods

A total of 70 Shami goat males were used to investigate the associations between CAST gene marker with growth performance and carcass traits in Ruminants Researches Station /Ministry of Iraqi Agriculture west of Baghdad. The diets in the Ruminants Researches Station were mainly composed of Concentrate which offered ad lib. at 07.00 am daily after quantifying and discarding the residue of the previous day. The concentrate mixture consisted of 53% barley, 25% wheat bran, 15% soybean meal, 6% wheat straw, 0.5% salt. Goat's males were slaughtered when reached the assigned period (10 months). They were fasted for 12-h, and weighed immediately prior to slaughter. The dressed carcass comprised the body after removing the skin, head and fore and hind feet and the viscera. The digestive tract was removed and weighed then emptied of its content, washed, drained and weighed to facilitate calculation of empty body weight. After carcasses chilling (24 h at 4-6 °C) they weighed, then the kidney and kidney fat were separated and weighed.

2.1. Blood samples and DNA isolation

Blood samples were collected from 70 goat males before slaughtering. Approximately 4.5 mL blood samples were gathered from the vena jugulars in K3-EDTA tubes and transferred to a (–20 C0) freezer. The DNA was isolated with a DNA isolation kit (Bioneer Column-Pure Blood Genomic DNA Kit, South Korea) from blood samples as per the Manufacturer’s instructions in Al-Nahrain University, Biotechnology Researches Center. Quantity and quality of the DNA were checked using a Nano Drop 2000 spectrophotometer (Thermo Scientific, USA).

2.2. Amplifications of DNA fragments and PCR-RFLP

The DNA amplification of the CAST gene was achieved by PCR-RFLP. Two primer pairs [5’-CCT TGT CAT CAGACT TCA CC-3’ (forward) and 5’-ACT GAG CTT TTAAAG CCT CT-3’ (reverse)] targeting a fragment of 565bp were employed as described by(14) for identification of the M and N alleles of CAST gene.

The PCR amplification reaction solution was performed in total volume of 25 L (containing 14 L Pre Mix Kit, 7 L DNA, 1 L forward primer, 1 L reverse primer, 2 L deionized water). The PCR cycling condition was a preliminary denaturizing at 95 °C for 5 min, followed by 1 cycle of denaturing at 94 °C for 0.5 min, annealing at 55 0C for 0.5 min, and extension at 72 °C for 0.5 min by 35 cycles and followed by 7 min at 72 °C as a final extension. The PCR reactions were performed on an ABI Veriti thermo cycler.

The amplified fragment of CAST gene was digested by the restriction endonuclease MspI (BROMIGA CO.). Digestion was conducted at 37 °C for 3h and in a 20- L reaction solution (including 4 L deionized water, 3 L RE10X Buffer, 3 L of MspI, and 10 L of PCR product).

Digested products were separated by electrophoresis on 2% (v/w) agarose gel stained with Safe View (NBS Biologicals, UK) Electrophoresis was performed in a 1X TBE buffer at room temperature and constant 70 V for 90 min.
2.3. Statistical analysis

Allelic and genotypic frequencies, chi-square and Hardy–Weinberg equilibrium were calculated using SAS program(22). The only fixed effect inserted in the model was the CAST gene genotype. Birth, weaning, slaughter, hot carcass and chill carcass weights were used as a covariates. Also the non-carcass components traits were analyzed as covariates. Least square means of MIXED model of SAS software was used to identify the significant differences among traits means.

3. Results and discussion

PCR-RFLP of CAST gene: The amplification of the targeted MspI Calpastatin gene obtained a gene fragment of 565 bp (Fig. 1), which was digested with an MspI restriction enzyme. Two MspICAST gene genotypes were observed; MM genotype was of two fragments (306 bp and 259 bp), and the MN genotype was found to be of three fragments (565 bp, 306 bp, 259 bp) (Fig. 1). Allele frequencies, genotype frequencies values obtained from our study and the results of chi-square tests performed for Hardy–Weinberg equilibrium are presented in Table (1) and the figure(1) of gel. The highest frequency value was found for the M allele (0.84), and the lowest value frequency was for N allele with 0.16 in Shami goat population. According to genotype frequency assessments, the MM genotype was determined as the most common genotype among population in this study with 68.6%, and the less genotype was the MN with 31.4%, While the NN genotype could not be identified (0.00) (Table 1). Hardy–Weinberg equilibrium was examined, and was significant (P < 0.01) which was 16.345. Frequency values obtained from M and N alleles in studied population were similar or nearby to those in the relevant literature (8, 25and 4). The obtained data for these population in light of the literature raises suspicion that there is selection developing against the NN genotype. Thus, it may be thought that inbreeding has increased in Shami goat's population over the course of time and raised certain genotype frequencies.
Table 1. CAST gene Genotype frequencies and allelic frequencies for Shami goats breed.

| Breed          | No. | M allele freq. | N allele freq. | Genotype | Chi-square |
|----------------|-----|----------------|----------------|----------|------------|
| Shami goats    | 70  | 0.84           | 0.16           | MM 48    | 0 16.354** |
|                |     |                |                | MN 22    |            |
|                |     |                |                | NN 0     |            |

**: (p<0.01)

**Growth performance traits & carcass weights:** Table 2 revealed the association of CAST gene polymorphism with some growth performance and carcass weights traits. The results showed significant differences (p < 0.05) among MM and MN genotypes in all studied growth traits and carcass weights except birth weight which was not significant. The least square means (LSM) values of goats with MN genotype were higher in all weights compare with goats holding the MM genotype. Similar results on male sheep are obtained in the study of (24) in Indonesia. Other results confirm our findings, which revealed significant effect for the variation in calpastatin gene on birth weight in New Zealand Romney sheep (6). Other else, Lack of association was observed between the CAST gene variants in carcass weight and dressing percentage of thin tail sheep (7). Also, The CAST genotype did not significantly influence hot carcass weight and cold carcass weight in Awassi sheep in Jordan (13). The skeletal muscle size depends on the scales between the rates of both degradation and synthesis, the inhabitation effect of the calpastatin for the calpains which may be responsible for such significant differences (12).

**Non-carcass components:** The effects of CAST gene polymorphism in non-carcass components ranged between significant (p < 0.05) to non-significant (table 3). The results indicated that there were significant differences between the two genotypes (MM & MN) in head, legs, skin, heart, lungs and testes weight traits, by the superiority of the MN genotype on the MM genotype significantly with 2081.07, 801.78, 1699.29, 109.28, 326.07 and 187.64 grams

Table 2. Least square means of growth performance and carcass weights traits (kg) depends on genotype (MM or MN)

| Traits        | Genotype | S.L. |
|---------------|----------|------|
|               | MM 48    | MN 22|
| Birth weight  | 3.171±0.144 a | 3.450±0.251 a | N.S. |
| Weaning weight| 15.515±0.614 a | 16.964±0.65 a | *     |
| Slaughter weight| 22.578±0.976 b | 27.707±1.08 a | *     |
| Hot carcass weight | 8.718±0.443 b | 11.282±0.55 a | *     |
| Chill carcass weight | 8.439±0.435 b | 11.017±0.55 a | *     |

*: (P<0.05),  N.S.:non-significant
Table 3. Least square means of non-carcass components traits (gm) depends on genotype (MM or MN)

| Traits    | Genotype | MM        | MN        | S.L.  |
|-----------|----------|-----------|-----------|-------|
| NO.       | 48       | 22        |           |       |
| Head      |          | 1652.66±59.350 b | 2081.07±129.87a * |       |
| Legs      |          | 659.43±28.833 b | 801.78±34.95 a   * |       |
| Skin      |          | 1333.13±57.899 b | 1699.29±138.30 a * |       |
| Heart     |          | 90.156±4.185 b | 109.28±4.021 a   * |       |
| Lungs     |          | 271.09±11.785 b | 326.07±13.240 a  * |       |
| Liver     |          | 531.87±109.87 a | 503.92±13.948 a  N.S. |       |
| Spleen    |          | 47.96±10.599 a  | 45.00±3.740 a    N.S. |       |
| Testes    |          | 122.50±14.766 b | 187.64±12.170 a  * |       |
| Kidney    |          | 90.31±4.908 a   | 100.35±3.76 a    N.S. |       |

*: (P<0.05), N.S.:non-significant

Respectively, compared with 1652.66, 659.43, 1333.13, 90.156, 271.09 and 122.50 grams on the same order for MM genotype. Otherwise, the observed differences between MM and MN genotypes were not significant in spleen and kidney weights traits.

It could be concluded that the MN MspI CAST genotype Performs much better than the MM MspI CAST genotype in most studied traits. The MN individuals showed a higher values than the MM genotype. Therefore, The MN genotype could be used as a marker for improving the above mentioned traits. Further studies concerning the polymorphisms and effects of calpastatin gene in Shami goats would be highly relevant to the influence of this genomic region in relation to meat production traits and the other economic traits.

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