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Association between *MspI* calpastatin gene polymorphisms, growth performance, and meat characteristics of Awassi sheep

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ABSTRACT

The association between the *ovine* calpastatin gene (CAST) genotypes, growth performance and meat characteristics of Awassi sheep was investigated. Homozygous (MM) and heterozygous (MN) genotypes of the CAST were obtained by RFLP, using Awassi ram lambs (age=7 to 14 days n= 80).A fattening trial was conducted using 10 ram lambs *MspI* - of CAST genotypes (5 lambs from each genotype) (homozygous (MM) and heterozygous (MN). Lambs were weighed at the beginning of the experiment and the subsequent weights were measured biweekly before the morning feeding throughout the duration of the experiment. At the end of the fattening period, all lambs were slaughtered to evaluate carcass characteristics and meat quality. The results showed a significant association between CAST genotypes and growth rate and final body weight showing that lambs of the MN genotype had a higher average daily gain and final body weight compared to lambs of the MM genotype. The CAST gene genotypes showed a significant effect on some carcass components and meat quality parameters indicating that MN genotype showed lower total bone and higher meat to bone ratio than the MM genotype in the dissected leg cut. Furthermore, the MN genotype had a higher longissimus muscle weight compared to MM animals. Meat quality analysis showed that MN genotype lambs had higher shear force, lower cooking loss and lightness. It can be concluded that the CAST gene can be considered as one of the genes that control growth performance and meat quality traits.

Key words: Awassi, Calpastatin genotypes, Growth performance, Meat characteristics, Sheep

The Awassi is a predominant fat-tailed sheep breed that is found in the Mediterranean countries and also in Spain, Australia and some European countries. Under the same environmental conditions a high variation has been observed in the growth performance of Awassi sheep (Jawasreh and Khasawneh. 2007). Recently, many advances have been made by the identification of chromosomal regions which affect important traits in livestock production through molecular genetics application (Andersson 2001). In order to explain some variations that existed in some economic traits in livestock, some molecular markers such as single nucleotide polymorphisms (SNP's) were used for assisting the selection process in some phenotypic traits by investigating the presence or absence of the targeted gene variants.

One of the genes that may affect the growth and meat characteristics, is the calpastatin gene (CAST) which is a specific inhibiter of calpains, that has a pivotal a role in meat tenderization and myogenesis. This study aimed to investigate the relationship between the CAST gene polymorphisms and growth performance, carcass characteristics and meat quality of Awassi sheep.

MATERIALS AND METHOD

Animals and DNA extraction: All procedures used in this study were approved by the Animal Care and Use Committee at Jordan University of Science and Technology. Approximately 5 mL of blood samples were collected from the jugular vein of 80 ram lambs (aged 1-2- weeks- old) found in Al-Khanasry Station using vacutainer tubes containing EDTA and then the blood was stored at 4°C pending analysis. DNA was extracted from the collected blood using the E. Z. N. A blood DNA kit.The DNA quality was tested using 1.5% agarose gel electrophoresis.

Polymerase chain reaction (PCR) amplification and restriction fragment length polymorphisms (RFLPs)

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analysis: PCR-RFLP genotyping was used to discover the polymorphism in the region between exons (61bp 1C and 88bp 1D) and 473 bp of CAST gene using Life Pro Thermal Cycler.The following 2 primers (forward and reverse) were used for genotyping the regions. CAST F: 5'-TGGGGCCCAATGACGCCATCGATG-3' and CAST R: 5'- GGTGGAGCAGCACTTCTGATCACC-3'.The PCR reactions were carried out in 20 µL volumes, including 10µL of nuclease free water, 100 ng of extracted DNA as a template, 0.5 µM of each Primer forward and 5 unit/µl of HOT FIRE Pol® DNA polymerase. The optimum annealing temperature was determined empirically using the gradient PCR, and then specific PCR protocols were applied. Genotyping results were screened by ultraviolet light.

PCR protocol included an initial step of 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 a final extension at 72°C for 10 min for RFLP analysis. The mix contained ten microliters of the PCR product that was digested with 20 units of the restriction endonuclease *MspI*enzyme at 37°C for 12 h and 2 µL of nuclease free water, 1 µL of bovine serum albumen and 6µL of buffers. The digested products were resolved in 2% agarose gels, stained with ethidium bromide and viewed under UV light.

Fattening trial: The fattening experiment was conducted at Al-Khanasry Research Station, National Center for Agriculture Research and Extension (NCARE). Depending on CAST gene detected genotypes, and because of the low genotype frequency of NN relatively to MM and MN genotypes, 2 groups (MM and MN genotypes) with 5 animals in each were subjected to a fattening period of 70 days to investigate growth performance and meat characteristics. During the fattening period, lambs were housed individually in shaded pens (1.5 m × 0.75 m). All lambs were introduced slowly to *ad lab.* access to a diet containing 16% CP and 2.78Mcal metabolizable energy/kg for 70 days (National Research Council 1985), and free access to clean water. The ingredients of the totally mixed ration were soybean 15%, barley 61.4%, wheat straw 21%, salt 1.5%, limestone 0.1% and minerals and vitamins 0.1%. Feed and orts were weighed daily for the calculation of feed intake and feed efficiency. The live weights of the lambs were recorded biweekly.

Slaughtering procedures, carcass composition and meat quality: At the end of fattening period, all lambs were slaughtered at the same time for the evaluation of carcass traits and meat quality after a 14 h period of fasting with free access to water. Lambs were slaughtered using a standard slaughter procedure described by Abdullah *et al.* (1998). Briefly, carcasses cuts were divided into 4 parts (shoulder, rack, loin and leg cuts), in addition to the fat tail, and all their weights were recorded. After sectioned each carcass, the rib-eye area, fat depths, tissue depth (GR), rib fat depth (J), eye muscle width (A), eye muscle depth (B), eye muscle area, fat depth (C) and leg fat depth (L3) were measured on chilled cuts and longissimus muscles (Abdullah *et al.* 1998). Each major cut was separated into right and left sides using an electrical saw. The right side of each cut was sealed in a plastic bag and frozen at (–20°C), then after that dissection right leg to determine their muscle, bone, subcutaneous fat and inter muscular fat components. Longissimus muscles were excised from the right side of loin cuts, cleaned from the subcutaneous fat, vacuum-packaged and frozen at (–20°C) for meat quality measurements as described by Abdullah and Qudsieh (2009), included Warner-Bratzler shear force values on cooked meat samples, water holding capacity, cooking loss and color coordinates (L*, a*, and b*).

Statistical analysis: The CAST gene genotype was inserted as the only fixed effect in the model. Initial body weight was used as a covariate for analyzing differences in body weight gain. For loin and leg measurements, loin and leg weights were included as covariates. Least square means of MIXED procedures of SAS (VERSION 8. 1, 2000, SAS Inst. Inc., Cary, NC) software was used to further identify significant differences among means at $P<0.05$.

RESULTS AND DISCUSSION

PCR-RFLP of calpastatin gene: The refinement in meat quality is one of the main objectives of livestock production and meat tenderness and one of the most essential factors for quality assessment of meat. The amplification of the targeted *MspI*Calpastatin gene obtained a gene fragment of 622 bp (Fig. 1), which was digested with an *MspI* restriction enzyme. Two *MspI*CAST gene genotypes were found (Fig. 2); MM genotype was of 2 fragments (336 bp and 286 bp), while the MN genotype was found to be of 3 fragments (622 bp, 336 bp, 287 bp) (Fig. 2).

The M and N allele frequencies of the CAST *MspI* genotypes were 0.486 and 0.514, respectively, while the genotypic frequencies of NN, MN and MM were 0.00, 0.648 and 0.352, respectively. Similar findings were found by Gabor *et al.* (2009) who indicated the absence of the NN

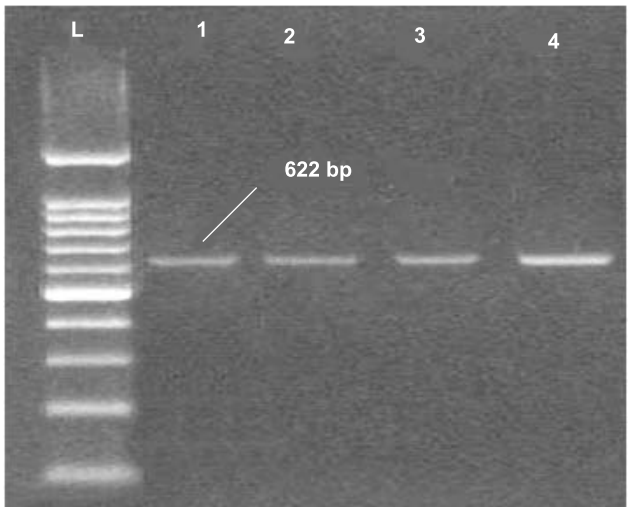

Fig. 1. PCR product of calpastatin gene (622 bp) visualized by1.5% Agarose, L, ladder 100 bp; 1– 4, PCR product of CAST gene.

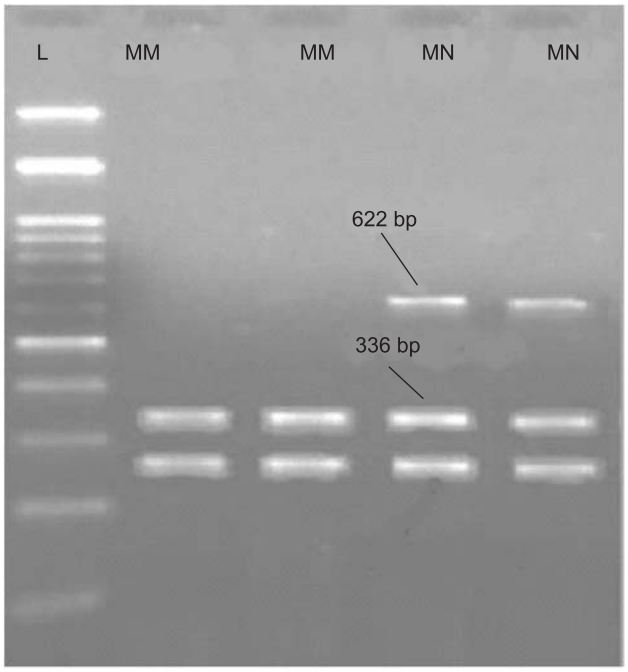

Fig. 2. PCR-RFLP results for CAST gene by *MspI* restriction enzyme on 2% agarose gel. L= ladder 100 bp, MM genotype (336 bp, 287 bp), MN genotype (622 bp, 336 bp, 287 bp).

genotype in different sheep breeds in Slovakia with a relative increase in MN (heterozygous genotype) frequency.

Growth performance: The results (Table 1) showed no significant differences between MM and MN genotypes in the dry matter intake (DM) during fattening period. Also, the least square means (LSM) results showed that lambs that hold MNgenotype were about 0.53 kg higher in final body weight, and consumed lower (1.19 kg) feed / kg of live weight (Table 1) than the MM genotype lambs. While, average daily gain was higher in heterozygous MN genotype lambs. Sumantri *et al*. (2014) indicated positive correlations between body weight and calpastatin genotypes. They reported an association between the calpastatin gene and body weight in male sheep of Indonesia. Male lambs that are of the MN genotype were significantly higher in their body weight than male lambs that had the NN genotype (p=0.017).

Chung and Davis (2012) reported a significant difference between Calpastatin genotypes in average daily gain; AA genotype gained more daily weight (0.222 kg) than AB (0.217kg) and BB (0.201 kg) genotypes. The size of skeletal muscle mainly depends on the balance between the rate of degradation and the rate of synthesis, the inhabitation effect of the calpastatin for the calpains (Ilian *et al.* 2004) which results in decreasing the rate of protein degradation and increasing the rate of protein synthesis in the skeletal muscles protein that may be responsible for such significant differences.

Carcass characteristics: The CAST genotype did not significantly influence (P>0.05) hot carcass weight, cold carcass weight, dressing percentage, non-carcass components (heart, liver, spleen, kidney, kidney fat, mesenteric fat, testis, fat tail, lung, and trachea), and the carcass cuts weights (shoulders, leg, loin) (Table 1). Dissected leg cuts were also comparable (P<0.05) in their intermuscular fat, subcutaneous fat, total fat, total lean and meat to fat ratio. The only significant effect for the different CAST genotypes was observed on the total bone and meat to bone ratio (Table 1).

Table 1. Least square means of growth performance and carcass components, carcass of Awassi ram lambs that carry the MN or MM *MspI* Calpastatin gene genotypes

Traits	Genotype		SEM
	MM	MN	
Growth traits			
Initial body weight (kg)	22.6	20.2	1.14
Final body weight (kg)	31.78[b]	32.31[a]	0.96
Average daily gain (kg/d)	0.128[b]	0.167[a]	0.011
Total dry matter intake (kg)	59.56	63.7	3.65
Feed conversion ratio (kg feed/kg weight gain)	6.95	5.72	0.76
Carcass traits			
Fasting body weight (kg)	31.78[b]	32.31[a]	0.96
Hot carcass weight (kg)	16.38	15.49	0.08
Cold carcass weight (kg)	15.40	15.25	0.45
Dressing%	50.77	49.074	1.91
Non-carcass components (g)			
Heart	122	126	7.6
Liver	499	443	26.2
Spleen	65	54	4.9
Kidney	100	105	3.5
Kidney fat	73	78	15.7
Mesenteric fat	236	191	35.6
Testes	90	100	10
Lungs and trachea	495	438	34.3
Fat tail	1170	930	1
Carcass cut weights (kg)			
Shoulder and Rack	7.30	7.46	0.09
Leg	5.25	5.15	0.13
Loin	1.56	1.63	0.059
Dissected leg cut			
Leg weight (g)	2625	2575	6.5
Intermuscular fat (g)	83	84	5.89
Subcutaneous fat (g)	290	249	35.72
Total fat (g)	372	333	38.33
Total lean (g)	1437	1494	73.14
Total bone (g)	552[a]	465[b]	21.85
Meat to bone ratio	2.59[b]	3.25[a]	0.19
Meat to fat ratio	4.70	4.71	0.71

Within the same row, means without a common letter (a and b) differ (P < 0.05).

The *longissimus* muscle area, tissue depth, fat depth, rib fat depth, fat thickness, eye muscle area, width and depth were comparable (P>0.05) among the different CAST genotype. The *longissimus* muscle weight was observed to be significantly affected by CAST *MspI* genotypes (Table 2).

The analysis showed that the variation in calpastatin gene had no effect on all of the studied carcass traits. In Kivircik

Table 2. Least square means for *longissimus* weight and linear dimensions and fat measurements of Awassi ram lambs that carry the MNor MM *MspI*calpastatin gene genotypes

Traits	Genotype		SEM
	MM	MN	
Longissimus muscle weight (g)	192[b]	224[a]	9.95
Tissue depth (GR) (mm)	14.27	11.62	1.23
Rib fat depth (J) (mm)	5.68	5.99	1.06
Eye muscle width (A) (mm)	58.67	62.52	1.57
Eye muscle depth (B) (mm)	24.58	25.61	1.34
Eye muscle area (cm2)	12.20	12.82	1.17
Fat depth (C) (mm)	3.77	3.07	0.61
Fat thickness (L3)	8.22	8.87	1.61

Within the same row, means without a common letter (a and b) differ (P < 0.05)

Table 3. Least square means of meat quality characteristics of Awassi ram-lambs that carry the MN or MM *MspI*calpastatin gene genotypes

Traits	Genotype		SEM
	MM	MN	
Cooking loss (%)	48.45a	45.69b	0.516
Water holding capacity (%)	33.86	31.89	1.158
Shear force (kg/cm$^{3)}$	3.98b	4.36a	0.096
pH	5.75	5.80	0.026
Colour			
a*(redness)	3.89	3.39	0.29
b*(yellowness)	19.22	17.96	0.55
L*(lightness)	37.60a	32.47b	1.03

Within the same row, means without a common letter (a and b) differ (P < 0.05).

sheep Yilmaz *et al.* (2014) reported significant differences among calpastatin genotypes in back fat thickness and skin and backfat thickness values of loin eye muscle. Also found that lambs Kivircik with MN and MM genotype were of low fat carcass than those of NN genotype. Gregu' a-Kania (2012) found that the longest muscle (m. longissimus lumborum) and fat thickness were not affected by CAST genotypes in lambs of the age of 80 and 120 days. Lack of association was observed between the CAST gene variants in carcass weight and dressing percentage of thin tail sheep (Dagong *et al.* 2012).

Jawasreh *et al*. (2012) found that Awassi sheep individuals that carried the MN genotype (heterozygous) had a higher neutrophil to lymphocyte ratio than MM genotypes and they also indicated that lambs that carry the MN genotype had higher (P<0.05) albumin and triiodothyronine (T3) concentrations than the MM genotype. These may explain the higher meat to bone ratio found in the MN genotypes. Triiodothyronine (T_3) acts to increase the basal metabolic rate that affects protein synthesis and helps in regulating long bone growth.

Meat quality: The least squares means of meat quality characteristics measured on *Longissimus* muscle are presented in Table 3. Meat quality parameters were similar (P>0.05) among MM and MN CAST genotypes except for cooking loss, shear force, and lightness (L*). Shear force measurement tended to be significantly higher (P>0.09) for the MN genotype compared to the MM genotype, while cooking loss and lightness (L*) were significantly lower in MN genotype than in MM genotype (Table 3). These results were in agreement with Schenkel *et al.* (2006), results who reported a significant association between variants of CAST and beef tenderness. Also, Ciobanud *et al.* (2004) reported a significant association between the CAST and cooking loss in Pig'smeat. A nonsignificant difference among calpastatin genotypes in meat tenderness, pH, water holding capacity and cooking loss of thin tail sheep were reported by Dagong *et al.* (2012).

The size of skeletal muscle fibers, the muscle protein synthesis rate, and the muscle protein degradation rate are among those claimed changes. Calpain activity may play an imperative role that is necessary for cell proliferation, myoblast fusion, fibers growth, and fiber numbers. The Calpain system is important in fibers growth because the growth rate increases as protein degradation decreases and this is associated with the Calpain system activity that decreases with the increase in CAST gene activity. The Calpain system influences can also be seen in fiber numbers by the modulation of myoblast fusion and changing the myoblast proliferation rate. Calpastatin is an inhibitor factor of the calcium dependent calpain protease enzymes and it plays a regulatory role in muscle growth and meat tenderization post-slaughtering (Ciobanud *et al.* 2004).

It could be concluded that the MN *MspI* CAST genotype performs much better than the MM *MspI* CAST genotype. The MN individuals showed a higher final body weight, higher daily gain with a low conversion ratio and heavier *Longissimus* muscle than the MM genotype. However, the meat tenderness of the MN meat was lower than the MM genotype. The MN genotype could be used as a marker for improving the above mentioned traits.

The study was designed for investigating the effect of CAST gene genotypes on growth performance and meat quality of Awassi sheep. The MN individuals showed a higher final body weight, higher daily gain with a low conversion ratio and heavier *Longissimus* muscle than the MM genotype. However, the meat tenderness of the MN meat was lower than the MM genotype. The MN genotype could be used as a marker for improving the above mentioned traits.

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REFERENCES

Abdullah A Y, Purchas R W and Davies A S. 1998. Patterns of change with growth for muscularity and other composition characteristics of Southdown rams selected for high and low back depth. *New Zealand journal of Agricultural Research*

41(3): 367–76.

Abdullah A Y and Qudsieh R I. 2009. Effect of slaughter weight and aging time on the quality of meat from Awassi ram lambs. *Meat Science* **82**: 309–16.

Andersson L. 2001. Genetic dissection of phenotypic diversity in farm animals. *Nature Reviews Genetics* **2**(2): 130–38.þ

Chung H and Davis M. 2012. PCR-RFLP of the ovine calpastatin gene and its association with growth. *Asian Journal of Animal and Veterinary Advances* ***7***(8): 641–52.

Ciobanud D C, Bastiaansen J W M, Lonergan S M, Thomsen H, Dekkers, J C M, Plastow G S and Rothscgild M F. 2004. New alleles in calpastatin gene are associated with meat quality traits in pigs. *Journal of Animal Science* **82**(10): 2829–39.

Dagong M I A, Herman R, Sumantri C, Noor R R and Yamin M. 2012. Carcass and physical meat characteristics of thin tail sheep (TTS) based on calpastatin gene (CAST) (Locus intron 5–exon 6) genotypes variation. *Indonesian Journal of Animal and Veterinary Sciences* ***17***(1): 13–24.þ

Gabor M, Trakovicka A and Miluchova M. 2009. Analysis of polymorphism of CAST gene and Clpg gene in sheep by PCR-RFLP method. *Scientific Papers Animal Science and Biotechnologies* **42**(2):470–76.

Gregu³a-Kania M. 2012. Effect of calpastatin gene polymorphism on lamb growth and muscling. *Annals of Animal Science* **12**(1): 63–72.þ

Ilian M A, Bekhit A E D and Bickerstaffe R. 2004. The relationship between meat tenderization, myofibril fragmentation and autolysis of calpain 3 during postmortem aging. *Meat Science* **66**: 387–97.

Jawasreh K I Z, Al-Majali A, Al-Ramamneh D and Alqaisi A A. 2012. The effect of MspI calpastatin gene genotypes in some hematological and biochemical blood values in Awassi sheep. *Proceeding of 15th AAAP Animal Science Congress, Thammasat University, Rangsit Campus, Thialand.* p300. 26–30 November 2012.

Jawasreh K I Z and Khasawneh A Z. 2007. Studies of some economic characteristics on Awassi lambs in Jordan. *Egyptian Journal of Sheep And Goat Sciences* **2**: 101–10.

SAS Institute Inc. 2000. SAS/STST User Guide: Version 8.1 1st ed. SAS institute INC., Cary, NC.

Schenkel F S, Miller S P, Jiang Z, Mandell I B, Ye X, Li H and Wilton J W. 2006. Association of a single nucleotide polymorphism in the calpastatin gene with carcass and meat quality traits of beef cattle. *Journal of Animal Science* **84**(2):291–99.þ

Sumantri C, Diyono R, Farajallah A and Inounu I. 2014. Polymorphism of Calpastatin gene and its effect on body weight of local sheep. *Indonesian Journal of Animal and Veterinary Sciences* **13**(2):117–26.þ

Yilmaz O, Cemal I, Karaca O, and Ata N. 2014. Association of Calpastatin (CAST) gene polymorphism with weaning weight and ultrasonic measurements of loin eye muscle in Kývýrcýk lambs. *Journal of the Faculty of Veterinary Medicine, Kafkas University* **20**(5): 675–80.þ