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Introgression of the *callipyge* mutation into the Assaf fat tail breed

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SUMMARY – Introgression of the *callipyge* (*CLPG*) mutation into the fat-tail Assaf breed was initiated by inseminating Assaf ewes with Dorset-Hampshire semen from a ram that was heterozygous for the *callipyge* mutation. Presence of the mutation in the F1 generation and the first backcross generation (BC1) was validated using molecular markers linked to the *CLPG* locus. Carcasses of callipyge BC1-*CN* lambs had significantly ($P < 0.01$) better conformation than carcasses of normal BC1-*NN* lambs and straightbred Assaf lambs. Computerized tomography analysis of live BC1 and Assaf lambs showed that carrying the *callipyge* mutation increased *Longissimus dorsi* and Q*uadriceps femoris* muscles by about 30% along with increased tissue density. Taste panel evaluation of oven-roasted neck and leg cuts indicated that meat from callipyge lambs was less palatable than that of Assaf lambs.

Key words: Callipyge, Assaf, genetic markers, computerized tomography.

RESUME – *"Introgression de la mutation* callipyge *dans la race Assaf à queue grasse". L'introgression de la mutation* callipyge *dans la race Assaf à queue grasse a été initiée en inséminant des brebis Assaf avec de la semence d'ovins Dorset-Hampshire hétérozygotes pour la mutation* callipyge*. L'existence de la mutation chez les agneaux de la génération F1 et de la 1ère génération de croisement en retour (BC1) a été validée en utilisant des marqueurs moléculaires liés au locus* CLPG*. Les carcasses d'agneaux hétérozygotes* CN *BC1 étaient mieux conformées que celles d'agneaux non porteurs de la mutation* NN *BC1 ($P < 0,01$). L'analyse par tomographie digitale d'agneaux Assaf et BC1 vivants a montré que la mutation* callipyge *accroît la taille du* Longissimus dorsi *et du* Quadriceps femoris *d'environ 30%, ainsi que la densité tissulaire. La valeur gustative de morceaux de cou et de cuisse cuisinés au four était inférieure chez les agneaux callipyge, par comparaison avec les Assaf.*

Mots-clés : *Callipyge, Assaf, marqueurs génétiques, tomographie digitale.*

Introduction

Expression of the *callipyge (CLPG*) locus in sheep causes muscle hypertrophy and significant increase in the weight of leg and loin muscles, the most valuable retail cuts of the lamb carcass (Koohmaraie *et al*., 1995; Freking *et al*., 1998). The inheritance of the *callipyge* phenotype has a non-Mendelian pattern termed polar overdominance (Cockett *et al*., 1996). *Callipyge* phenotype is only displayed in heterozygous individuals that inherit the mutation from their sire, while both homozygous (*NN* and *CC*), and individuals that inherit the mutation from their mother have normal muscle development. The *callipyge* locus has been mapped to the telomeric region of sheep chromosome 18 (Cockett *et al*., 1994) and DNA markers linked to the *CLPG* locus are available (Cockett *et al*., 1997). The use of genetic markers that flank the *callipyge* mutation can help in early identification of homozygous *CC* rams and ewes which have normal phenotype. As the *callipyge* phenotype is not evident until about 3 weeks of age, no dystocia problems are associated with expressing the gene, contrary to the situation in the double muscling gene in cattle. The disadvantage of the *callipyge* phenotype, as was found in several studies (Shackelford *et al*., 1997; Duckett *et al*., 2000) is the increased toughness of the *Longissimus* muscle and the reduction of meat tenderness and juiciness. The relatively high toughness of meat from callipyge lambs can be partly overcome by applying various *post mortem* treatments (Carpenter *et al*., 1997; Koohmaraie *et al*., 1998; Duckett *et al*., 2000).

The fat tailed Assaf and Awassi breeds suffer from inferior carcass merit and poor leg muscling (Goot *et al*., 1991). Crossbreeding the Awassi and the Assaf with exotic mutton breeds may improve carcass composition but may have undesired effects on adaptability and high milk production. Introgression of the *C* allele of the *CLPG* gene into these breeds has been suggested as a way of

improving their meat production without affecting other economically important traits. However, information on the effect of the *callipyge* gene on growth traits and meat quality in fat tail breeds is missing as all the results regarding the *callipyge* mutation thus far have been done with non-fat tailed breeds. The results of our first studies on the effect of the *callipyge* gene on meat quality and carcass characteristics of Assaf lambs are described within.

Materials and methods

Introgression of the *callipyge* mutation into the Assaf

The Volcani Center Animal Experimentation Ethics Committee approved the use of animals in this study. Semen from a heterozygous *CN* Dorset-Hampshire crossbred ram was collected at the University of Wisconsin-Madison, frozen and shipped to the Volcani Center, Israel in 1998. F1 crossbred Assaf lambs were born following intra-uterine insemination of Assaf ewes. Four F1 rams expressing the *callipyge* mutation, verified by physical examination and molecular genotyping, were crossed on Assaf ewes to produce the first backcross generation (BC1).

Genotyping for markers linked to the *CLPG* locus

Genotyping of F1 lambs and their dams for markers linked to the *callipyge* locus (CSSM18, IDVGA30, BMS1561, OY3, OY15, ILSTS54, TGLA122 and MCM38; Cockett *et al*., 1994; Lien *et al*., 1999) was carried out at Prof. Noelle Cockett's laboratory at Utah State University. Genotyping BC1 lambs with OY15 and CSSM18 that flank the *callipyge* locus was carried out at our laboratory at the Volcani Center.

Feeding and housing

Lambs were born indoors and stayed with their mothers until weaning at about one month of age. After weaning, lambs were provided with *ad libitum* concentrates (16% crude protein) and 0.1 kg/d/head of hay.

Carcass classification

Carcass classification for conformation and fatness was carried out according to the British Meat and Livestock Commission method (1999). Results were subjected to Chi-square analysis.

Taste panel

Two tasting sessions on different days were carried out on meat samples collected from callipyge and normal lambs. In the first session, samples of oven-roasted leg muscle from two five-month-old Assaf male lambs and two five-month old heterozygous *CN* crossbred BC1 male lambs were compared. In the second session, samples of oven-roasted neck muscles from the same lambs included in the first session were compared. Following slaughter, the legs and necks were aged (2°C) for 48 h, frozen and stored (–20°C) for 2 months. After thawing over night, the cuts were oven-roasted and meat slices were served following warming. During the tasting sessions, each of the 48 panellists received three leg or neck fat-free meat samples, with two being of the same type (Assaf or BC1-*CN*). The six possible testing orders of the three meat samples were applied randomly. Panellists indicated their acquaintance with sheep meat (1 = familiar; 5 = not familiar) and rated each sample for taste, texture (juiciness and tenderness) and overall acceptability on five-point scales (1 = excellent; 5 = very poor). Results were subjected to analysis of variance. The model included genotype (Assaf, BC1-*CN*), testing order (six combinations) and panellists experience as fixed effects. Statistical analyses were conducted using the JMP computer package (SAS, 2001). Results were expressed as least squares means and standard errors. Differences of $P < 0.05$ were considered significant.

In vivo study of body composition by computerized tomography analysis

Examination took place at the Neuro-Radiology Unit, Shiba Medical Center, using CT 8200 machine (Elscint). Lambs were scanned after a 24 h fast. Five minutes before the examination, animals were sedated by IV injection of 6 mg Xylazin (Rompun 2%, Bayer). The animals were placed on the computerized tomography (CT) table in sternal recumbency with their legs in full extension. Fifty-sixty images at 10 mm distances were taken for each animal covering the hind part of the body. Information was stored on CD. Muscle area and tissue densities expressed in Hounsfield units (Hounsfield, 1979) were measured in selected cross-sections.

Results and discussion

Applying molecular markers

Eighteen out of 45 Assaf ewes that were inseminated with Dorset-Hampshire semen from a male carrier for the *callipyge* mutation conceived and produced 27 lambs. All F1 progeny, their Assaf dams and the imported semen were genotyped for eight markers (Table 1). Combining information from physical examination of the F1 progeny and information from the genetic analysis indicated that nine ram lambs and three ewe lambs were heterozygous *CN*. In some cases, the markers helped to solve ambiguity in lamb classification as the contribution of the Dorset-Hampshire genetic background to the F1 lambs also improved their muscularity.

Table 1. Identification of microsatelite markers associated with the *callipyge* mutation. Markers are listed according to their relative position to the *CLPG* locus

Marker	Sire alleles (semen)	Alleles in Assaf dams	Alleles in F1 lambs in phase with the *callipyge* mutation
CSSM18	4,5	1,2,3,4	5
IDVGA30	1,2	1,2	2
BMS1561	2,2	2,3	2
Relative location of the *CLPG* locus			
OY3	2,6	1,2,4,5,7,8	6
OY15	1,3	3,4,5	1
ILSTS54	1,4	1,2,3	1
TGLA122	4,8	2,4,6,7,8,9,10	8
MCM38	1,7	2,4,6,7,11	1

In four of the eight marker loci examined (CSSM18, OY3, OY15 and MCM38), the marker allele that was linked to the *callipyge* mutation was not present in the Assaf population. Two of these markers (CSSM18 and OY15) were selected to be used in subsequent genotyping of Assaf crosses.

Classification for conformation and fatness

Four *CN* F1 rams were selected as sires for the BC1 generation. Lambs born in the March/April 2000 lambing season were genotyped and carcasses of BC1 male lambs and contemporary Assaf male lambs were evaluated for conformation and fatness after slaughter at five months of age (Table 2). BC1-*CN* Assaf lambs showed improved carcass confirmation ($P < 0.01$) compared to BC1-*NN* and Assaf lambs, which were similar for carcass composition. All carcasses had a high degree of fatness with no significant differences among genotypes.

Taste panel

Least squares analysis (Table 3) showed that genotype had highly significant ($P < 0.001$) effect on

all meat quality criteria, and panellist experience had significant effect (P < 0.05) in most cases, with the less experienced panel members scoring relatively lower grades. Testing order had no significant effects on panel meat evaluation results.

Table 2. Classification for conformation and fatness of five-month-old Assaf, BC1-*CN* and BC1-*NN* lamb carcasses

Genotype	n	Carcass weight	Dressing (%)	Conformation class (%)†					Fatness class (%)†		
				E	U	R	O	P	4L	4H	5
BC1-*CN*	7	24.6	48	86	14	–	–	–	–	100	–
BC1-*NN*	7	26.5	47	–	29	57	14	–	–	72	28
Assaf	26	26.6	47	–	42	42	18	–	4	73	23

†According to the British MLC classification system.

Table 3. Analysis of variance for the effect of genotype, tasting system and panellist experience on grading of meat samples from Assaf and BC1-*CN* lambs

Model	Texture	Taste	Acceptability
Leg			
Genotype	***	***	***
Tasting order	ns	ns	ns
Panellist experience	*	*	ns
Neck			
Genotype	***	***	***
Testing order	ns	ns	ns
Panellist experience	*	***	**

*P < 0.05; ***P < 0.001; ns = non significant.

Assaf meat samples, both from neck and leg cuts received higher ranking for texture, taste and general acceptability than meat samples from BC1-*CN* lambs (Table 4).

Table 4. Least squares means for grades given to meat quality traits of Assaf and BC1-*CN* lambs

	Texture	Taste	Acceptability
Leg			
Assaf	4.0 ± 0.1^{a}	3.9 ± 0.1^{a}	4.1 ± 0.1^{a}
BC1-*CN*	3.1 ± 0.1^{b}	2.8 ± 0.1^{b}	3.1 ± 0.1^{b}
Neck			
Assaf	4.3 ± 0.3^{a}	3.9 ± 0.4^{a}	4.1 ± 0.3^{a}
BC1-*CN*	3.4 ± 0.3^{b}	3.0 ± 0.4^{b}	3.2 ± 0.3^{b}

[a,b]Within column, means with different superscript differ significantly (P < 0.01).

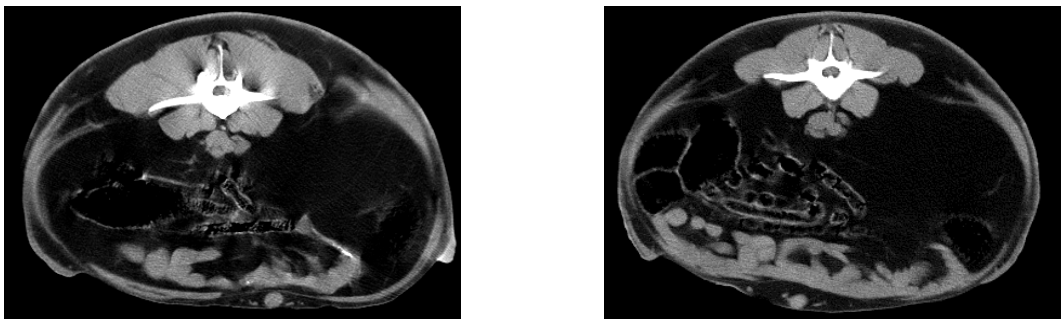
Computerized tomography results

Studies on the effect of the *callipyge* mutation on body conformation and muscle growth characteristics have been mainly performed after slaughter. In the present study, we selected an *in vitro non-invasive* approach by using CT scanning. Two Assaf and two BC1-*CN* lambs of similar body

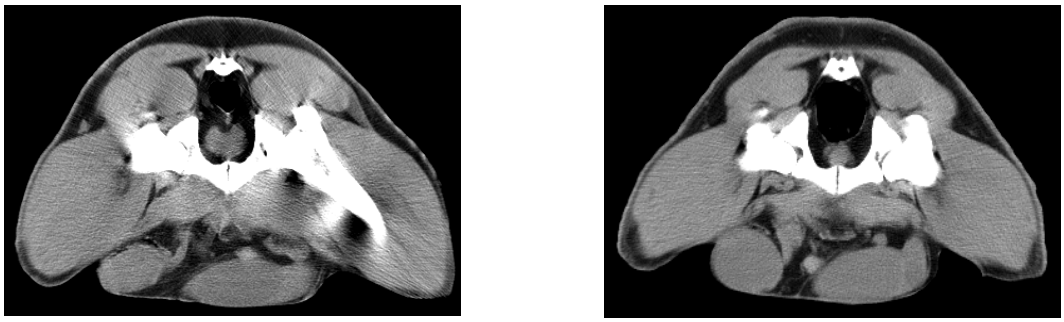
weight were selected and the following transverse sections were analysed: (i) section through the 6th lumber vertebra at the level of the dorsal spine; (ii) section through the caudal acetabular rim; (iii) section through the mid-thigh region; and (iv) section through the stifle joint (Fig. 1).

BC1-*CN* Assaf Assaf

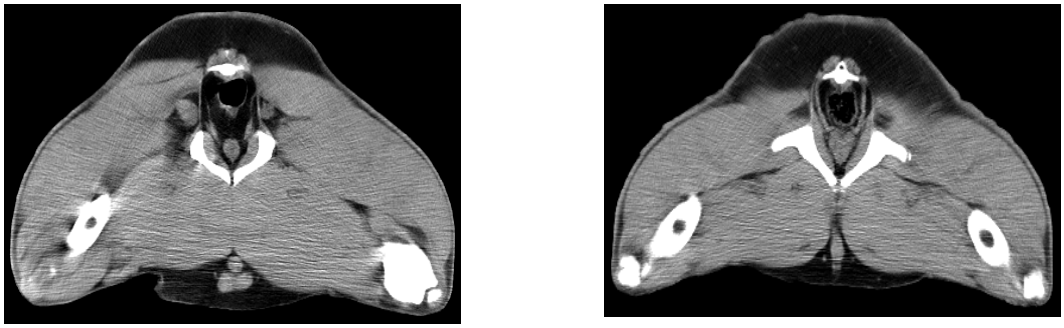
A. Transverse section through the 6th lumber vertebra at the level of the dorsal spine.



B. Transverse section through the caudal acetabular rim.



C. Transverse section through the mid-thigh region.



D. Transverse section through the stifle.

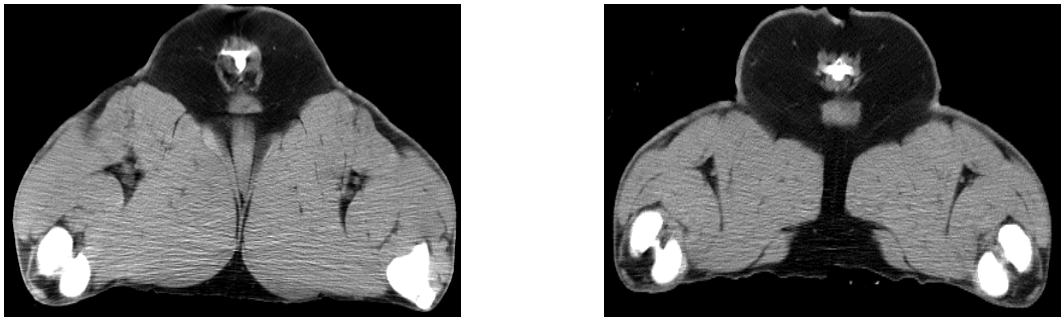


Fig. 1. Computerized tomography evaluation of Assaf and BC1-*CN* crossbred lambs carrying the *callipyge* mutation.

The average cross-section area of the *Longissimus dorsi* (Table 5) was 28% larger in BC1-*CN* lambs then in normal Assaf lambs. A similar difference of 34% between *NN* and *CN* was noted in Dorset lambs (Koohmaraie *et al.*, 1995). *Longissimus dorsi* of BC1-*CN* lambs had on the average higher density (+24%) measured in Hounsfield units. In several studies (Shackelford *et al.*, 1997; Fahmy *et al.*, 1999; Duckett *et al.*, 2000), *Longissimus dorsi* post mortem tenderness as measured by Warner-Bratzler shear force values was higher by 76-123% in *CN* lambs then in contemporary *NN* lambs. The higher differences found between *CN* and *NN* lambs in the other studies can be attributed to genotype differences in post-mortem degradation of myofibrillar muscles proteins (Duckett *et al.*, 2000).

Table 5. Cross section areas (mm^2) and tissues density (Hounsfield units) of *Longissimus dorsi* muscles measured in transverse section through the 6th lumber vertebra at the level of the dorsal spine in Assaf lambs and BC1-*CN* lambs

Lamb no.	Live weight (kg)	*Longissimus dorsi*	
		Area (mm^2)	Density
BC1-*CN*			
2589	61.2	5633	63
2483	65.2	5242	57
Assaf			
2436	62.0	4179	52
2533	62.2	4238	45

Quadriceps femoris cross-section areas of BC1-*CN* lambs were 25-31% larger than similar cross-section areas of Assaf lambs (Table 6). Tissue density of BC1 lambs was only 3-6% higher than that of Assaf lambs.

Table 6. Cross-section areas (mm^2) and tissues density (Hounsfield units) of *Quadriceps femoris* measured in transverse section through the caudal acetabular rim (A), mid-thigh region (B) and transverse section through the stifle (C) of Assaf lambs and BC1-*CN* Assaf lambs

Lamb no.	Cross-section (A)		Cross-section (B)		Cross-section (C)	
	Area (mm^2)	Density	Area (mm^2)	Density	Area (mm^2)	Density
BC1-*CN*						
2589	10,315	56	21,028	54	18,697	58
2483	11,411	55	19,546	56	17,792	54
Assaf						
2436	6,154	54	16,078	53	13,416	55
2533	10,573	53	16,433	50	15,025	54

Conclusions

Our results show that the effect of the *callipyge* mutation on body composition and meat quality in the fat tail Assaf breed is similar to the effect of the mutation on other genetic backgrounds namely improvement of carcass conformation and reduction in meat palatability. Both factors have to be considered before introduction of the mutation into commercial flocks can be justified. The CT scan was found to be rapid and effective way to evaluate body conformation and tissue density of callipyge lambs.

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References

Carpenter, C.E., Solomon, M.B., Snowder, G.D., Cockett, N.E. and Busboom, J.R. (1997). Effect of electrical stimulation and conditioning, calcium chloride injection and aging on the acceptability of callipyge and normal lambs. *Sheep and Goat Res. J.*, 13: 127-134.

Cockett, N.E., Berghams, S., Beckers, M.C., Shay, T.L., Jackson, S.P., Snowder, G.D. and Georges, M. (1997). The *callipyge* gene of sheep. *Animal Biotechnology*, 8: 23-30.

Cockett, N.E., Jackson, S.P., Shay, T.L., Farnir, F., Berghmans, S., Snowder, G.D., Nielsen, D.M. and Georges, M. (1996). Polar over dominance at the ovine *callipyge* locus. *Science*, 273: 236-238.

Cockett, N.E., Jackson, S.P., Shay, T.L., Nielsen, D., Moore, S.S., Steele, M.R., Barendse, W., Green, R.D. and Georges, M. (1994). Chromosomal location of the *callipyge* gene in sheep (*Ovis aries*) using bovine DNA markers. *Proc. Natl. Acad. Sci. USA*, 91: 3019-3023.

Duckett, S.K., Snowder, G.D. and Cockett, N.E. (2000). Effect of the *callipyge* gene on muscle growth, calpastatin activity, and tenderness of three muscles across the growth curve. *J. Anim. Sci.*, 78: 2836-2841.

Fahmy, M.H., Gariepy, C. and Fortin, J. (1999). Carcass quality of crossbred lambs expressing the *callipyge* phenotype born to Romanov purebred and crossbred ewes. *Anim. Sci.*, 69: 525-533.

Freking, B.A., Keele, J.W., Nielsen, M.K. and Leymaster, K.A. (1998). Evaluation of the ovine *callipyge* locus. II. Genotypic effects on growth, slaughter, and carcass traits. *J. Anim. Sci.*, 76: 2549-2559.

Goot, H., Bor, A., Hasdai, A., Zenou, A. and Gootwine, E. (1991). Body and carcass composition of Awassi, Assaf, Booroola × Awassi and Booroola × Assaf ram lambs. In: *Major Genes for Reproduction in Sheep*, Elsen, J.M., Bodin, L. and Thimonier, J. (eds). INRA, Paris, pp. 335-340.

Hounsfield, G.E.N. (1979). Computed medical imaging. Nobel lecture, Dec. 8, 1979. *J. Comput. Assist. Tomogr.*, 4: 665-674.

Koohmaraie, M., Shackelford, S.D. and Wheeler, T.L. (1998). Effect of prerigor freezing and postrigor calcium chloride injection on the tenderness of callipyge *longissimus*. *J. Anim. Sci.*, 76: 1427-1432.

Koohmaraie, M., Shackelford, S.D., Wheeler, T.L., Lonergan, S.M. and Doumit, M.E. (1995). A muscle hypertrophy condition in lamb (callipyge): Characterization of effects on muscle growth and meat quality traits. *J. Anim. Sci.*, 73: 3596-3607.

Lien, S., Cockett, N.E., Klungland, H., Arnheim, N., Georges, L. and Gomez-Raya, L. (1999). High-resolution gametic map of the sheep callipyge region: Linkage heterogeneity among rams detected by sperm typing. *Anim. Genet.*, 30: 42-46.

Meat and Livestock Commission (1999). *Sheep Year Book 1999*.

SAS (2001). *JMP Statistical Software*, version 4.0. SAS Institute Inc.

Shackelford, S.D., Wheeler, T.L. and Koohmaraie, M. (1997). Effect of the *callipyge* phenotype and cooking method on tenderness of several major lamb muscles. *J. Anim. Sci.*, 75: 2100-2105.