

Appendix A. Table A1. The genotyping techniques used in this study.

Gene	Genotyping technique	Primer Sequence (5'→3')	Amplicon size (bp)	Reference
ESR	PCR-RFLP	F: CCTGTTTTTACAGTGACTTTTTACAGAG R: CACTTCGAGGGTCAGTCCAATTAG	120 bp	(16) Rothschild <i>et al.</i> (1991)
PRLR	PCR-RFLP	F: CGTGGCTCCGTTTGAAGAACC R: CTGAAAGGAGTGCATAAAGCC	163 bp	(17) Serrano <i>et al.</i> (2009)
LIF	PCR-RFLP	F: ATGTGGATGTGGCCTACGG R: GGGAACAAGGTGGTGATGG	407 bp	(18) Spotter <i>et al.</i> (2001)
MYOG	PCR-RFLP	F: TCTTGACCTTGTTCATTGTGG R: CTTCTCACACCACCTTAC	353 bp	(19) Te Pas <i>et al.</i> (1996)
LPL	PCR-RFLP	F: CAGGGAACCTTGCATACTTTGTG R: AGCATGTAATACTTCCAGAGGCT	693 bp	(20) Flores <i>et al.</i> , 2022
IGF2 in 3	PCR-RFLP	F: GACCGAGCCAGGGACGAG R: CGCGCCCCACGCGCTCCCACGCTG	85 bp	(21) Buys (2003)
IGF2 in 7	MS PCR	F: CTCCGAGGGTCTGAGACTTCAGAG R: CAGGCACATGGCAGGTGCCAATCAAG Mutant: TCTCTGTCTCTCTGTTTCTCTCCCG AGGGTCTGAGACTTCATAC	90 bp 70 bp	(21) Buys (2003)
CAST	PCR-RFLP	F: AGGCTGTAAAAACAGAACCTG R: ATTTCTCTGATGTTGGCTGCTC	bp	(22) Kuryl <i>et al.</i> (2003)
CTSD	PCR-RFLP	F: GGC TGT GCA CCC TAG GAA C R:TCG TCA GGT CCA GGA CAA AC	184 bp	(23) Russo <i>et al.</i> 2008
LEPR	PCR-RFLP	F: GGA AGG CAT TTG TTT CAG CAG TAA R: CAA GTC CTC TTT CAT CCA GCA CTG CF: CCT GTG TGT GTG CAA TGG TGT GGC CGT CC	2kb	(24) Perez-Montarelo <i>et al.</i> (2012)
HAL	MS-PCR	TF: GTG CTG GAT GTC CTG TGT TCA ATG TGT GTG TGC AAT GGT GTG GCC GGC T R: CTG GTG ACA TAG TTG ATG AGG TTT GTC TGC	114 bp and 134 bp	(15) Matias <i>et al.</i> , 2023
FUT1	PCR-RFLP	F: CCA ACG CCT CCG ATT CCT GT R: GTG CAT GGC AGG CTG GAT GA	161 bp	(25) Bao <i>et al.</i> (2011)
RN	PCR-RFLP	F: AAA TGT GCA GAC AAG GAT CTC G	249 bp	(26) Manalaysay <i>et al.</i> , 2019

Genotyping of animals using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) and Mutagenically Separated Polymerase Chain Reaction (MS-PCR). Insulin like growth factor 2 intron 7 (IGF2 in 7) and Halothane (HAL) genes were genotyped using MS-PCR. Other gene markers were genotyped using PCR-RFLP. These include gene markers that are associated with meat quality traits are rendement napole (RN), cathepsin D (CTSD), leptin receptor (LEPR), lipoprotein lipase (LPL), calpastatin (CAST), insulin-like growth factor 2 intron 3 (IGF2 in 3). PCR-RFLP was also applied to gene markers associated with litter size (*PRLR*, *ESR*, *LIF*), resistance to colibacillosis (*FUT1*), and growth (*MYOG*). These targeted loci represent critical genetic determinants of productivity, disease resistance, and carcass quality in the closed nucleus population of Markad.