Genomic markers in beef tenderness

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The effectiveness of genomic markers in predicting the meat tenderness in pure beef genotypes under South African production and slaughter conditions

Industry Sector: Cattle and Small Stock

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Aims of the project

- 3.1.1 To determine the expression of genomic markers in five South African purebred genotypes – Bos indicus (Brahman), Sanga type (Nguni), British Bos taurus (Angus), European Bos taurus (Charolais) and the composite (Bonsmara) for genes associated with beef tenderness in meat.
- 3.1.2 To determine the relationship between the actual physiological tenderness characteristics under South African production and slaughter conditions of the meat from these five main South African genotypes and the known DNA-marker information.
- 3.1.3 To assess the phenotypic variation in meat tenderness within South African selected pure beef genotypes under the same environmental conditions and to build a tenderness prediction model.

Executive Summary

Purebred South African bulls of 5 breeds (n=166) were finished on a grain diet at the Animal Production Institute of the Agricultural Research Council (API-ARC), Irene. Breeds included Angus (n=27; representative of British *Bos taurus*), Brahman (n=35; Zebu type *Bos*

indicus), Bonsmara (n=35; South African composite breed with large Sanga contribution), Charolais (n=34; European *Bos taurus*) and Nguni (n=35; Sanga type *Bos taurus africanus*). Animals were sampled over 3 slaughter periods in 2011 (50 animals), 2012 (50 animals) and 2014/2015 (66 animals). Bulls were sourced from breeders that are registered with the appropriate breeders' associations and were progeny of registered pure breed bulls and cows. Bulls were ≃9 months old when entering the feedlot and reared under feedlot conditions for ≃120 days to ≃12 months old. Bullas were slaughtered to yield A2/3 carcasses (zero permanent incisors, lean to medium fatness). Bulls were penned overnight with access to water before slaughter following captive bolt immobilization at the abattoir of the API-ARC. All treatments and procedures were approved by the Ethics Committee of the Agricultural Research Council (ARC AEC-I 2010 001).

To determine whether the effects of genotype were additive to electrical stimulation, the right half of the carcass was electrical stimulated for 15 seconds at 500V peak, using 5 ms pulses at 15 pulses per second and directly chilled at 4 °C. The left half of the carcass was not electrically stimulated (served as a control), while chilling was delayed for 6 hours (at 10 °C) to allow for the full development of metabolic processes within muscle fibers before chilling at 4 °C.

Animal measurements included weights, recorded during the feedlot growth period to determine body weight gain (total gain and average daily gain) and liver body weight (BW) measured on the day before slaughter as a final weight. Carcass measurements included hot carcass weight (HCW; used to calculate dressing percentage), cold carcass weight (used to determine carcass mass loss), EMA (in the thoracic region at T9/10), pH and temperature (measured at the lumbar end of the LTL). Beef quality estimates measured from samples collected directly from the carcass or from LTL excised from the lumbar region (L6) up to the thoracic region (T9/10) included myofibrillar fragment length (MFL), Warner-Bratzler shear force (WBSF), calpain enzyme system activities, sarcomere length (SL), colour measurements, energy metabolites, collagen (content and solubility) and water-holding capacity (WHC). Colour was determined using the CIE L*A*b* colour convention with measurements of L*, a*, b*, C* and h_{ab} over the ageing period. Energy metabolites included the concentrations of glycogen, glucose 6-phosphate, glucose, lactate, creatine phosphate and ATP determined at 1 h, 3 h, 6 h and 20 h post-mortem.

The genes that are most likely to affect beef quality, specifically tenderness, as those of the calpain enzyme system. Calpain-1, calpain-2, calpain-3 and calpastatin are all found in the sarcoplasm and are known to determine post-mortem proteolysis. The genes for these proteins can therefore be identified as causative to proteolysis at least, but potentially also for beef tenderness. We therefore used the 114 SNPs located in these causative genes (*capn1*, *capn2*, *capn3* and *cast* respectively) to determine their genotypic distribution, as well as the association of these genotypes with beef quality traits in order to determine the importance of these genes in determining the quality (tenderness) phenotype. These data were used to identify possible markers for genomic selection (GS), once they were validated for tenderness in South African beef breeds.

- The *capn1* gene (on BTA29) was validated for beef tenderness, with a large number of strong associations (relatively high correlations) with estimates of beef tenderness, found in both the ES and the NS treatment groups. It correlated

especially with MFL as a measure of physical tenderness (r^2= 0.07 to 0.15), with fewer SNPs explaining the phenotypic variation in WBSF (r^2 = 0.09 to 0.10). Almost no associations occurred with calpain-1 enzyme activity itself, but the effects of the SNPs in *capn1* was rather a change in the responsiveness of the enzyme to calpastatin inhibition, as shown by several relatively strong correlations (r^2 = 0.07 – 0.12) to the relative calpastatin inhibition per calpain(-s).

- The *capn2* gene (on BTA16) was validated for beef tenderness, explaining the phenotypic variation in, especially, the activities of calpain-1 and calpain-2 (r^2 = 0.07 – 0.11). Although effects on enzyme activities were evident, these changes only resulted in a few significant associations of the genotypes with physical tenderness MFL (r^2 = 0.07 – 0.09).
- The *capn3* gene (on BTA10) exhibited very few associations with beef quality. The protein coded by this gene is responsible for background proteolysis and does not cause variation in tenderness. The lack of an effect of these SNPs on tenderness is therefore unsurprising.
- The *cast* gene (on BTA7) is quite large (136,434 bp) and contained a large number of SNPs (63), of which only 4 exhibited extensive effects on tenderness. Many of the correlations with MFL ranged between 0.07 – 0.11, although a few SNPs exhibited strong phenotypic correlations with MFL (r^2 = 0.12 – 0.16), while associations with WBSF were less common and less pronounced (r^2 = 0.07 – 0.11). These differences in physical tenderness were only in part explained by differences in the total and /or relative inhibition of calpastatin of protease enzyme activities (r^2 = 0.07 – 0.12).

Using SNPs of the Illumina Bovine HD SNP BeadChip the *capn1*, *capn2* and *cast* genes were verified for tenderness in SA purebred beef cattle. The amount of phenotypic variation in tenderness estimates explained by some of these SNPs were large, making them useful targets for genomic selection in these breeds. Both Nguni and Bonsmara exhibited high allelic frequencies for alleles that were favorable for tenderness, giving them the genetic potential to produce tender beef.

Popular Article

Inheemse rasse soos die Nguni en Bonsmara het die genetiese potensiaal om sagte vleis te produseer

Basson, A

Inleiding

Hierdie proef is onderneem om vleisbeesgenetika in Suid-Afrikaanse (SA) rasse te ondersoek. As deel van die proef is daar getoets of die rasse wat algemeen vir kruisteling in SA gebruik word, verskil in die verspreiding van voordelige gene vir sagtheid (en ander vleiseienskappe), met spesifieke fokus op die inheemse Bonsmara en Nguni. Die karkasse is gehalveer om die een helfte elektries te stimuleer en dadelik te verkoel, terwyl die ander helfte as kontrole gedien het. Hier is verkoeling vir 6 ure uitgestel om die normale perimortem prosesse soos energieverskaffing in metabolisme, genoeg tyd te gee om te ontwikkel, voordat hierdie nie-gestimuleerde karkas-helftes verkoel is.

Daar is verskeie vrae waarvoor ons antwoorde soek met hierdie navorsing. Ons weet dat die Nguni oor die genetiese en biochemiese potensiaal beskik om sagte vleis te produseer (Frylinck *et al.*, 2009), maar hoe vergelyk dit met Bonsmara, Angus, Charolais en Brahman? Kan die Nguni onder die regte slagtoestande, sagte vleis produseer? Kan ons deur middel van genomiese seleksie (GS) die kwaliteit van beesvleis verbeter in die industrie, waar elektriese stimulering dalk die invloed van voordelige gene sou uitkanselleer, of is verbeterde genetika se positiewe invloed op kwaliteit steeds waargeneem na stimulering?

Die Proef

Vyf vleisbeesrasse is in die proef ingesluit; Angus en Charolais as *Bos taurus* rasse, Brahman as Zebu-tipe *Bos indicus*, Bonsmara as 'n inheemse kruisbeesras met 'n groot Sanga-tipe bydra en Nguni as inheemse Sanga-tipe *Bos taurus africanus*. Die stoetbulle is afgerond in die voerkraal tot naastenby 12 maande oud voor slagting, of 'n karkasklassifisering van A2/3. 'n Groot aantal monsters is versamel van die *Longissimus lumborum et thoracis* spier (lende) om die toestande rondom slagting te bepaal, asook lendeskywe wat vakuum-verseël is en verouder is vir 3, 9, 14 en 20 dae, om die invloed van veroudering op vleiskwaliteit te bepaal (met of sonder elektriese stimulering).

Vleis se Kwaliteitseienskappe

Vir kwantitatiewe eienskappe is daar 'n baie groot aantal gene wat 'n eienskap bepaal en elkeen van hierdie gene dra slegs 'n klein proporsie by tot die uiteindelike resultaat, byvoorbeeld sagte vleis. Elkeen van hierdie gene kan honderde (selfs duidende) variasies toon op 'n molekulêre vlak. Enkel-nukleotied polimorfismes (single nucleotide polymorphisms = SNPs) wat die verskil in een enkele DNA molekule is, kan soms 'n relatiewe groot invloed op die fenotipe hê. Hierdie SNPs (uitgespreek "snips") is wat ons geïdentifiseer en getoets het binne-in gene wat sagtheid behoort te beïnvloed.

Genetika en Fisiologie

Spier in die lewendige dier het 'n baie rigiede proteïenstruktuur wat hoogs ge-orden is, terwyl die omskakelings na vleis in die karkas 'n ontwrigting van hierdie orde behels – hoe meer die speirstrukture ontwrig word, hoe sagter is die vleis. Die kalpaïen ensiem-sisteem (spesifieke proteases) dra grootliks by tot die ontwikkeling van die finale sagtheid van vleis. Alhoewel kalpaïen-1 en kalpastatien (die inhibeerder van kalpaïen) die grootste bydra lewer tot die degradering van die proteïene in vleis om dit sagter te maak, kan kalpaïen-2 en kalpaïen-3 dalk ook hiertoe bydra. Ons het dus diere met die Bovine-HD SNP BeadChip van Illumina genotipeer vir die gene van die ensieme kalpaïen-1 (*capn1* in chromosoom 29), kalpaïen-2 (*capn2* in chromosoom 16), en kalpaïen-3 (*capn3* in chromosoom 10), asook die ensiem-inhibeerder, kalpastatien (*cast* in chromosoom 7). Ons bepaal dus eerstens watter gene fisiologies belangrik is en analiseer dan al die geen-variante (of SNPs) om die korrelasie tussen hierdie variante en vleiskwaliteit van die diere te bepaal. 'n Groot voordeel van hierdie navorsing, wat dit onderskei van ander werk, is dat ons 'n baie gedetaileerde prentjie van die fisiologie van die vleis het, deur meting van verskeie eienskappe (met of sonder behandeling), gekoppel aan redelik indiepte inligting omtrent die genotipes van hierdie funksionele gene.

Resultate

Brahman bulle (rooi in die grafiek) het deurgaans die hoogste vlakke van kalpastatien per kalpaïene getoon, wat bygedra het tot meer intakte spierveselstrukture (langer miofibril fragment lengtes – MFL) asook verhoogde taaiheid (hoë Warner-Bratzler snyweerstande of WBSW gemeet in kg). In teenstelling het die Nguni (turquois in die grafiek) heelwat laer inhibering van ensiemwerking deur kalpastatien getoon, wat in sommige gevalle die laagste van al die rasse was, met ander woorde die Nguni was die ras met die mees voordelige biochemie. In die Bonsmara was die patroon vir biochemiese en strukturele veranderinge baie soortgelyk aan dié van Nguni's en die sagtheid van die lendeskywe (verlaging in snyweerstande) het vinnig verbeter tussen dag 3 en 9 van veroudering. Teen 14 dae se veroudering het die snyweerstande gestabiliseer en Bonsmara bulle het nie dieselfde sagtheid as die Nguni bereik nie, inteendeel, hulle snyweerstande was soortgelyk aan Brahman en Charolais.

Kalpaïen-1 is die belangrikste protease wat sagtheid bepaal en die kalpaïen-1 geen (*capn1*) behoort dus by te dra tot vleiskwaliteit. Die grootste invloed van *capn1* was om die proteïenstruktuur te ontwrig, deur middel van laer relatiewe kalpastatien inhibisie per kalpaïen aktiwiteit. Ons het sterk korrelasies vir verskeie SNPs in hierdie geen geïdentifiseer waar veral MFL (maar ook party van die snyweerstande), sowat 15-20% laer was in die "voordelige" genotipe (voordelig vir sagtheid).

Die kalpaïen-2 ensiem is verantwoordelik vir die ontwikkeling van agtergrond-sagtheid en die geen (*capn2*) was ge-assosieer met sowat 12 – 15% hoër protease ensiemaktiwiteit, wat in sommige SNPs met soveel as 38% hoër ensiem aktiwiteit geassosieer was. Dit was egter tot 'n kleiner mate met die bevordering van sagtheid en die ontwrigting van vesels geassosieer.

Kalpastatien aksie kan 'n groot invloed op sagtheid hê. In die lewendige dier funksioneer dit om die kalpaïen protease ensiemaktiwiteit, wat sellulêre proteïene groot skade sou kon aanrig, in beheer te hou. In die prosesse wat spier omskakel na vleis toe, verhoed dit ook die afbraak van spierproteïene, maar in dié geval sal dit dan die ontwikkeling van sagtheid benadeel. In die kalpastatien geen (*cast*) was daar 'n relatief klein aantal SNPs wat 'n redelike groot invloed op die ontwrigting van spiervesel proteïene gehad het. Die MFL was nagenoeg 10 – 15% laer, terwyl sommige van die SNPs se "voordelige" genotipes tot meer as 'n 20% verbetering in die MFL gelei het (i.e. korter lengtes). Dit was gedeeltelik verduidelik deur 'n verlaging in die totale eenhede kalpastatien werking, met soveel as 20% laer inhibisie vanaf kalpastatien, gekoppel aan 'n redelike verbetering in die sagtheid van die vleis, veral in die vroeë tot intermediêre stadiums van veroudering.

Bespreking

Uit die 4 gene wat hier getoets is, is die kalpaïen-1 en kalpastatien gene veral geskik vir genomiese seleksie in Suid-Afrikaanse vleisbeesrasse, terwyl 'n paar van die SNPs in die kalpaïen-2 geen ook potensiaal toon. Rasverskille in sagtheidseienskappe (fisiese en biochemies) word gereflekteer in verskille in die verspreiding van genotipes tussen die verskillende rasse (sien tabel hier onder)..

	Totale Aantal Voordelige Allele*

cast	*capn1*	*capn2*	
Angus (n=27)	189	146	220
Bonsmara (n=35)	270	209	174
Brahman (n=35)	237	39	141
Charolais (n=34)	217	147	215
Nguni (n=35)	256	233	241

* Die groen blok dui die ras met die grootste aantal voordelige allele vir sagtheid aan

Nguni's hét die genetiese potensiaal om sagte vleis te produseer, maar die noemenswaardige ligter karkasse is geneig om te vinnig te verkoel wat beteken die vleis raak te koud vir metaboliese ensieme om energie optimaal te benut, terwyl die struktuur binne miofibrille ook sub-optimaal word vir die proteases se ensiemwerking. In hierdie proef het Nguni's die "beste genetika" gehad en die allele wat voordelige is vir sagtheid in die gene wat hier getoets is, was volop in Nguni's.

Please contact the Primary Researcher if you need a copy of the comprehensive report of this project – Lorinda Frylinck on lorinda@arc.agric.za