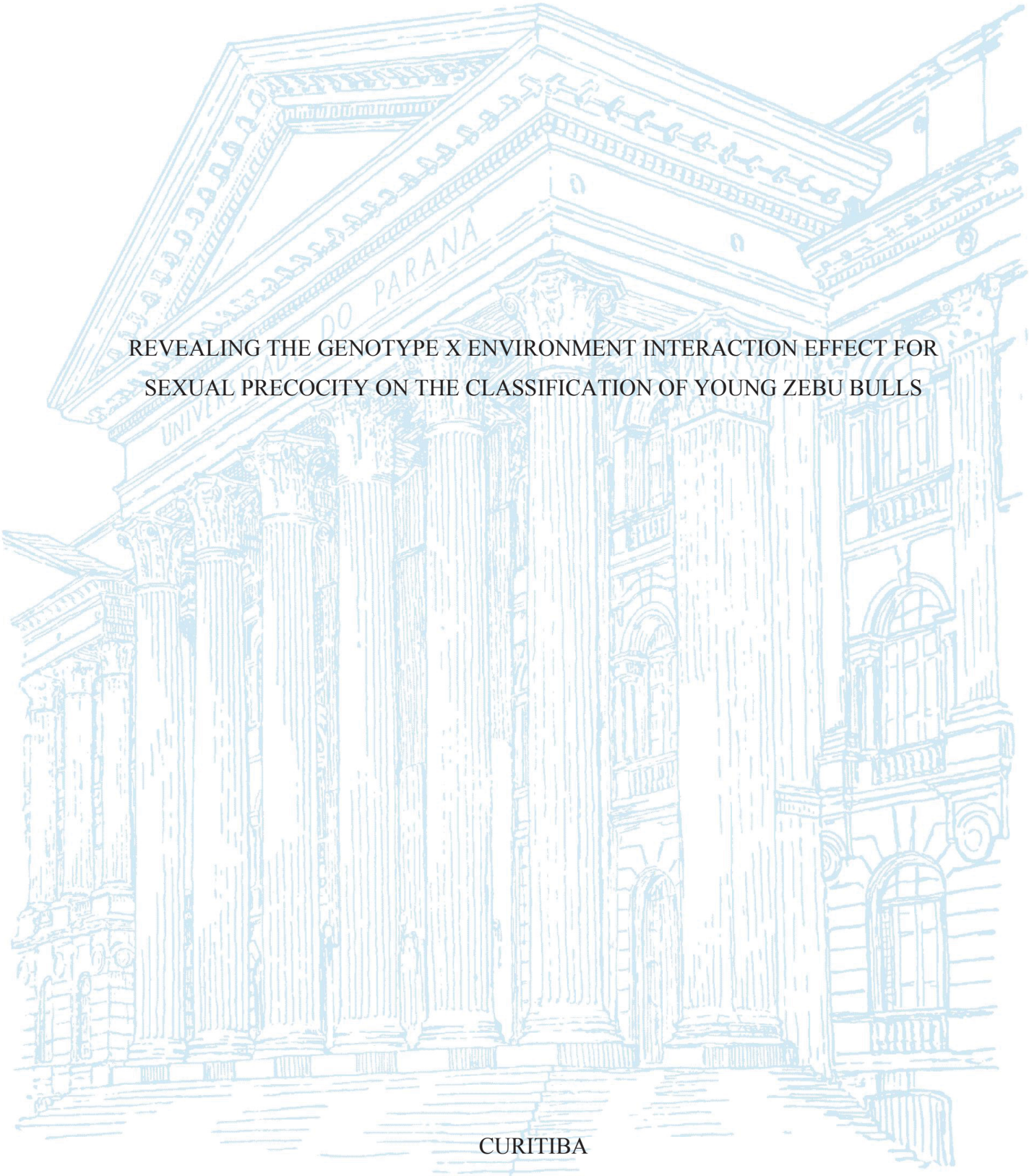
UNIVERSIDADE FEDERAL DO PARANÁ

BÁRBARA MAZETTI NASCIMENTO



REVEALING THE GENOTYPE X ENVIRONMENT INTERACTION EFFECT FOR SEXUAL PRECOCITY ON THE CLASSIFICATION OF YOUNG ZEBU BULLS

CURITIBA

2021

BÁRBARA MAZETTI NASCIMENTO

REVEALING THE GENOTYPE X ENVIRONMENT INTERACTION EFFECT FOR SEXUAL PRECOCITY ON THE CLASSIFICATION OF YOUNG ZEBU BULLS

Tese apresentada ao curso de Pós-Graduação em Zootecnia, Setor de Ciências Agrárias, Universidade Federal do Paraná, como requisito parcial para a obtenção do título de Doutor em Zootecnia.

Orientadora: Profa. Dra. Laila Talarico Dias

Coorientador: Prof. Dr. Rodrigo de Almeida Teixeira

CURITIBA

2021

Nascimento, Bárbara Mazetti
Revealing the genotype x environment interaction effect for sexual precocity on the classification of young zebu bulls. / Bárbara Mazetti Nascimento. - Curitiba, 2021.

Tese (Doutorado) - Universidade Federal do Paraná. Setor de Ciências Agrárias, Programa de Pós-Graduação em Zootecnia.
Orientação: Laila Talarico Dias.
Coorientação: Rodrigo de Almeida Teixeira.

1. Zebu - Fecundidade. 2. Nelore (Zebu). 3. Bovinos de corte - Reprodução. 4. Melhoramento genético. I. Dias, Laila Talarico. II. Teixeira, Rodrigo de Almeida. III. Título. IV. Universidade Federal do Paraná.

Sistema de Bibliotecas/UFPR
Guilherme Luiz Cintra Neves - CRB9/1572

TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em ZOOTECNIA da Universidade Federal do Paraná foram convocados para realizar a arguição da tese de Doutorado de **BARBARA MAZETTI NASCIMENTO** intitulada: **Revealing the genotype x environment interaction effect for sexual precocity on the classification of young zebu bulls.**, sob orientação da Profa. Dra. LAILA TALARICO DIAS, que após terem inquirido a aluna e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do título de doutor está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

CURITIBA, 19 de Março de 2021.

Assinatura Eletrônica
26/03/2021 15:08:45.0
LAILA TALARICO DIAS
Presidente da Banca Examinadora

Assinatura Eletrônica
31/03/2021 20:39:59.0
MARINA RUFINO SALINAS FORTES
Avaliador Externo (THE UNIVERSITY OF QUEENSLAND)

Assinatura Eletrônica
26/03/2021 15:13:34.0
MARSON BRUCK WARPECHOWSKI
Avaliador Interno (UNIVERSIDADE FEDERAL DO PARANÁ)

Assinatura Eletrônica
26/03/2021 16:02:35.0
ROBERTO CARVALHEIRO
Avaliador Externo (UNIVERSIDADE EST.PAULISTA JÚLIO DE MESQUITA FILHO)

Aos meus pais, Augusto e Eloides, a base de tudo!

ACKNOWLEDGMENTS

Agradeço aos meus pais, Augusto e Eloides, e à minha irmã Bruna pelo apoio, paciência e carinho nesses quatro anos. Obrigada pela educação, por me ensinarem a fazer sempre o que é correto, e por não deixarem me faltar nada, nunca! Serei eternamente grata por tudo, e essa conquista é de nós quatro.

Ao meu namorado André, que transformou esse meu último ano. Obrigada por ser companheiro, por me ensinar a ver a beleza nas coisas simples, por ser paciente quando eu testei a paciência de todos. E mais importante, obrigada por não me deixar desistir todas as vezes que ficou difícil demais essa tarefa. Amo você!

Aos meus orientadores, Laila e Rodrigo. Não podia deixar de colocá-los juntos, já que, independente do papel, foram meus orientadores ao longo desses 10 anos de Gama. Tive os melhores "pais" na universidade! Muito obrigada pela oportunidade, mas também por serem pacientes, compreensivos e amigos sempre que eu precisei.

Aos membros da banca, Prof. Marson, Dr. Roberto e Dra. Marina, e às suplentes, Dra. Joslaine e Profa. Alda. Obrigada por aceitarem contribuir com o meu trabalho. Tenho certeza de que escolhi profissionais que só têm a acrescentar e me ajudar a crescer como profissional. Obrigada à Dra. Marina por ter me recebido muito bem durante meu período na Austrália, e ao Dr. Roberto pela ajuda essencial na teoria das análises que formaram esse trabalho.

À Universidade Federal do Paraná, minha segunda casa desde 2006. Além de conhecimento, esses campi, corredores e salas de aula me trouxeram amigos, um amor, e muitas boas lembranças que levarei para sempre comigo.

Aos meus amigos (e agregados) da veterinária, Gi e Nico, Gabi V. e Koval, Gabi M. e Dodo, Duda e Natha, Fer e Leo, Isa e Rodrigo, Mari, Lorran, Ana e Ruben, Lari e Leonardo, Nat e Gus, obrigada por mantermos as amizades depois de tanto tempo. Vocês são especialíssimos!

À minha família GAMA atual, Lorena, Amauri, Julia, Simone, e todos os que passaram por lá nesses 10 anos... Priscilla, Ederson, Gabriele, Juliana, Fernanda, Gisele, Altair, Álida, Bruno, Francisco, Cláudia, Rodrigo, Hendyel, João, Daiane, Denyus, Suelen, Susana, Pâmela, Gustavo, Anna Mikaela, Cristiane, João, e outros que não nominei, mas que estiveram envolvidos de alguma forma no Gama. Eu tive os melhores colegas de trabalho, de café com bolo, de risada, de almoço, que alguém poderia querer. Obrigada por terem feito os dias de trabalho muito melhores.

À minha família “australiana”: Nat, Ju, Bruna, Camila, Alanny, Daniel, Natália, Antomat, Eli, Fran, Mari e Letícia. Vocês fizeram a experiência de Brisbane ser incrível! Obrigada pela acolhida e pela amizade que persiste, mesmo à distância.

À CAPES, pela bolsa de estudo durante o doutorado e o doutorado sanduíche. Esse apoio foi essencial à minha formação pessoal e profissional.

A todos que de alguma forma contribuíram e fizeram parte dessa jornada, muito obrigada!

“A true, selfless act always sparks another.”
Klaus

"Disciplining yourself to do what you know is right and important, although difficult, is the high road to pride, self-esteem, and personal satisfaction."
Margaret Thatcher

RESUMO

Em países de grande extensão territorial, a produção de bovinos de corte é realizada em ambientes diversos, com climas e sistemas de produção distintos. Nesta situação, a existência de interação genótipo x ambiente é esperada, especialmente para características reprodutivas, que sofrem maior influência ambiental, uma vez os filhos de determinados touros podem não ser os melhores em todos os ambientes, ou seu desempenho pode não ser superior em sistemas de criação diferentes dos quais foram selecionados. Porém, em geral, os programas de melhoramento genético não consideram o efeito da interação genótipo x ambiente, o que pode causar viés nas estimativas dos valores genéticos. Em bovinos, a característica mais utilizada como critério de seleção para precocidade sexual é o perímetro escrotal, por ser facilmente obtida e por estar correlacionada com características seminais nos machos e reprodutivas de fêmeas. Entretanto, o perímetro escrotal também está correlacionado com as características de crescimento. Assim, para que o perímetro escrotal reflita apenas precocidade sexual, é necessário ajustá-lo para as características de crescimento. Na literatura, os estudos que avaliaram o efeito da interação genótipo x ambiente para o perímetro escrotal não consideraram o ajuste para o crescimento, o que pode resultar em escolhas equivocadas quanto ao melhor touro para cada propriedade. Assim, o objetivo dessa tese de doutorado foi identificar o efeito da interação genótipo x ambiente sobre a classificação de touros jovens para perímetro escrotal ajustado para idade, peso, altura, e escores visuais de conformação, precocidade e musculatura, através da análise de normas de reação. Para isso, foram utilizados dados de rebanhos comerciais de bovinos Nelore pertencentes à base de dados do grupo Aliança Nelore. A caracterização do ambiente foi realizada pela padronização das soluções dos grupos contemporâneos, obtidas através do Modelo Animal, no qual o peso ao sobreano foi utilizado como variável dependente. Em seguida, as normas de reação foram determinadas através do Modelo de Regressão Aleatória linear, considerando-se as variâncias ambientais heterogêneas. Posteriormente, estimou-se a correlação genética entre o intercepto e o coeficiente de inclinação da curva de norma de reação e a correlação de Spearman entre a classificação dos touros quanto ao valor genético estimado para os ambientes extremos e médio. Observou-se aumento nas variâncias genéticas aditivas e ambientais para todos os perímetros escrotais ajustados conforme o ambiente tornou-se menos restritivo, exceto quando o ajuste do perímetro escrotal considerou o peso ao sobreano. O coeficiente de herdabilidade foi maior com a melhoria do gradiente ambiental para todas as características estudadas. A correlação de ranking mostrou mudança no posicionamento dos touros quando classificados pelo valor genético estimado, principalmente quando o ranqueamento em ambientes extremos foi comparado. Por essa razão, recomenda-se considerar o efeito da interação genótipo x ambiente nos modelos de avaliação genética de reprodutores, quando o critério de seleção for o perímetro escrotal ajustado para crescimento. Assim, a escolha dos reprodutores será mais assertiva. Durante o doutorado foi possível participar do Programa Doutorado Sanduíche no Exterior, da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), na Universidade de Queensland, na Austrália, e desenvolver o trabalho apresentado no último capítulo desta tese. O objetivo deste trabalho foi identificar o efeito da interação genótipo x ambiente sobre o perímetro escrotal medido aos 6 meses, 12 meses, 18 meses e 24 meses, utilizando as matrizes de parentesco baseadas no pedigree e em informações genômicas, em bovinos Brahman. Para tanto, foi utilizado o banco de dados de rebanhos experimentais pertencentes ao Cooperative Research Centre for Beef Genetic Technologies (Beef CRC). O ambiente foi caracterizado pela padronização das soluções dos grupos contemporâneos obtidos pela análise do Modelo Animal utilizando a matriz de relacionamento genômica, com o peso corporal, medido nas idades em que o perímetro escrotal foi avaliado, como variável dependente. Em seguida, as normas de

reação foram determinadas através do Modelo de Regressão Aleatória utilizando a matriz de parentesco baseada apenas no pedigree ou a matriz de parentesco genômica. Posteriormente, foi estimada a correlação de Spearman entre a classificação dos touros quanto ao valor genético estimado para os ambientes extremos e o ambiente mediano, de forma a avaliar a existência ou não de mudança no ranqueamento dos animais. Com o aumento do gradiente ambiental, a variância ambiental para as medidas tomadas aos 12 meses e 18 meses diminuiu, enquanto que, para o perímetro escrotal mensurado aos 6 meses e 24 meses, houve aumento dessa estimativa. Já para a variância genética aditiva e para herdabilidade, conforme o ambiente se tornou mais favorável, tais estimativas aumentaram para as medidas avaliadas aos 12 meses e 18 meses e diminuíram para o perímetro escrotal tomados aos 6 meses e 24 meses. Entretanto, a alteração na variância dos valores genéticos estimados em ambientes extremos pelas normas de reação não foi suficiente para alterar significativamente o ranqueamento, conforme resultados próximos à unidade em todas as correlações de Spearman procedidas. Em relação às medidas de 12 meses e 18 meses, consideradas mais acuradas para identificar precocidade sexual em bovinos da raça Brahman devido à proximidade da idade à puberdade, a existência de interação genótipo x ambiente não foi observada. Para essas idades, não foi observado mudança no ranqueamento dos animais e a variação foi pouco significativa entre as estimativas dos valores genéticos dos touros nos ambientes extremos. Já para o perímetro escrotal medido aos 6 meses e 24 meses, é possível afirmar que existe interação genótipo x ambiente, devido à diferença entre os valores genéticos dos animais avaliados nos ambientes extremos.

Palavras-chave: Bovinos de corte. Crescimento. Modelo de regressão aleatória. Normas de reação. Perímetro escrotal.

ABSTRACT

In countries with a large territorial extension, beef cattle are raised in different environments, with distinct climates and production systems. In this situation, the existence of genotype x environment interaction is expected, especially for reproductive traits, which suffer greater environmental influence, since the offspring of certain bulls may not be the best in all environments, or their performance may not be superior in raising systems different from those in which they were selected. However, in general, breeding programs do not consider the effect of genotype x environment interaction, which may cause bias in the estimate of breeding values. In beef cattle, the most used trait as selection criterion for sexual precocity is the scrotal circumference, because it is easily obtained and it is correlated with seminal traits in males and reproductive traits in females. But the scrotal circumference is also correlated with growth traits. So, to scrotal circumference reflect only sexual precocity, the adjustment for such characteristics is necessary. Studies evaluating genotype x environment interaction effect for scrotal circumference seems to not consider these adjustments, which can lead to wrong choices of the most adequate sires for each property. Thus, the aim of this thesis was to identify the effect of the genotype x environment interaction on the classification of young bulls for scrotal circumference adjusted for age, weight, height, and the visual scores conformation, precocity and muscularity, through the analysis of reaction norms. Data from commercial Nellore cattle herds belonging to the Aliança Nelore group were used. The environment characterization was performed by standardizing the solutions of the contemporary groups, obtained through the Animal Model, where body weight was used as dependent variable. Then, the reaction norms were determined through a linear Random Regression Model, considering the heterogeneous environmental variances. After that, was estimated the genetic correlation between the intercept and the slope coefficient of the reaction norm curve and the Spearman correlation between the classification of bulls regarding the estimated genetic value for extreme and average environments. There was an increase in the additive and environmental genetic variances for all adjusted scrotal circumferences as the environment became less restrictive, except when the scrotal circumference was adjusted for body weight. The heritability coefficient was higher as the environmental gradient improved for all traits studied. The rank correlation showed a change in the positioning of bulls when ranked by the estimated genetic value, especially when comparing the ranking in extreme environments. For this reason, it is recommended to consider the effect of the genotype x environment interaction in the genetic evaluation of bulls, when the selection criterion is the scrotal circumference adjusted for growth. Thus, the choice of sires will be more assertive. During the doctorate, it was possible to participate in the Doctoral Exchange Program, of the Coordination for the Improvement of Higher Education Personnel (CAPES), at The University of Queensland, Australia, and develop the study presented in the last chapter of this thesis. The aim of this study was to identify the effect of the genotype x environment interaction on scrotal circumference measured at 6 months, 12 months, 18 months and 24 months, using pedigree-based and genomic-based kinship matrices in Brahman cattle. An experimental dataset belonging to the Cooperative Research Centre for Beef Genetic Technologies (Beef CRC) was used. The environment was characterized by standardizing the contemporary group solutions obtained by Animal Model analysis using the genomic relationship matrix, with weight measured at the evaluated ages as the dependent variable. Then, the reaction norms were determined through the Random Regression Model using the pedigree-based kinship matrix or the genomic kinship matrix. Subsequently, Spearman's correlation was estimated between the ranking of the bulls regarding the genetic value estimated for the extreme environments and the median environment in order to evaluate the existence or not of re-ranking of the animals. With the increase in the environmental gradient, the

environmental variance for the measurements taken at 12 months and 18 months decreased, while for the scrotal circumference measured at 6 months and 24 months, there was an increase in this estimate. For the additive genetic variance and heritability, as the environment became more favorable, such estimates increased for the measures evaluated at 12 months and 18 months and decreased for the scrotal circumference taken at 6 months and 24 months. However, the change in variance of genetic values estimated in extreme environments by the reaction norms was not enough to significantly alter the ranking, according to results close to unity in all Spearman's correlations performed. Regarding the measurements at 12 months and 18 months, considered more accurate to identify sexual precocity in Brahman cattle due to the proximity of the age at puberty, the existence of genotype x environment interaction was not observed. For these ages, there was no change in the ranking of animals and the variation was not very significant between the estimates of genetic values of bulls in extreme environments. For the scrotal circumference measured at 6 months and 24 months, it is possible to state that there is a genotype x environment interaction, due to the difference between the genetic values of the animals evaluated in the extreme environments.

Keywords: Beef cattle. Growth. Random regression model. Reaction norms. Scrotal circumference.

FIGURES

FIGURE 1 - (A) LOCATION OF THE HERDS BELONGING TO ALIANÇA NELORE DATABASE IN BRAZIL, ELABORATED USING GOOGLE MAPS (2021), (B) BRAZILIAN CLIMATE DISTRIBUTION, (C) BRAZILIAN BIOMES DISTRIBUTION, (D) BRAZILIAN RELIEVES DISTRIBUTION 24
FIGURE 2 - OCCURRENCE OF GENOTYPE X ENVIRONMENT INTERACTION 26
FIGURE 3 - AVERAGE YEARLING WEIGHT AND THE NUMBER OF RECORDS OVER THE ENVIRONMENTAL GRADIENT IN NELLORE CATTLE 29
FIGURE 4 - DISTRIBUTION OF PHENOTYPES FROM A SINGLE GENOTYPE ACCORDING TO THE ENVIRONMENT BY ANALYZING THE REACTION NORM 32
FIGURE 5 - REACTION NORMS ALONG THE ENVIRONMENTAL GRADIENT FOR SCROTAL CIRCUMFERENCE OBTAINED FOR 10 SIRES WITH THE HIGHEST (A) AND LOWEST (B) BREEDING VALUE FOR LONG-YEARLING WEIGHT IN NELLORE CATTLE 33
FIGURE 6 - ESTIMATED BREEDING VALUES OF FIVE BEST AND FIVE WORST BULLS FOR YEARLING WEIGHT IN NELLORE CATTLE, USING INVERSES OF PEDIGREE (AM-BLUP) AND PEDIGREE–GENOMIC (SSGBLUP) RELATIONSHIP MATRICES OVER THE ENVIRONMENTAL GRADIENTS 34
FIGURE 7 - REACTION NORMS FOR SCROTAL CIRCUMFERENCE FOR A RANDOM SAMPLE OF 10 MONTANA SIRES 35
FIGURE 8 - REACTION NORMS FOR SCROTAL CIRCUMFERENCE (SC) EVALUATED USING PEDIGREE RELATIONSHIP MATRIX (RNM_A) AND GENOMIC RELATIONSHIP MATRIX (RNM_H) FOR THE 30 ANIMALS WITH HIGHER GENOMIC BREEDING VALUES 35
FIGURE 10 - AVERAGE BODY WEIGHT AT POST-YEARLING IN NELLORE CATTLE BY ENVIRONMENTAL GRADIENTS 58
FIGURE 11 - ADDITIVE GENETIC AND ENVIRONMENTAL VARIANCE ESTIMATES OVER THE ENVIRONMENTAL GRADIENTS FOR SCROTAL CIRCUMFERENCE ADJUSTED FOR AGE AT POST-YEARLING (A), BODY WEIGHT AT POST-YEARLING (B), AND HIP HEIGHT (C) IN NELLORE CATTLE. 59
FIGURE 12 - ADDITIVE GENETIC AND ENVIRONMENTAL VARIANCE ESTIMATES OVER THE ENVIRONMENTAL GRADIENTS FOR SCROTAL CIRCUMFERENCE

ADJUSTED FOR AGE AND BODY WEIGHT AT POST-YEARLING (A), AGE AND HIP HEIGHT AT POST-YEARLING (B), AND BODY WEIGHT AND HIP HEIGHT AT POST-YEARLING (C) IN NELLORE CATTLE. 60
FIGURE 13 - HERITABILITY COEFFICIENT ESTIMATES OVER THE ENVIRONMENTAL GRADIENT FOR SCROTAL CIRCUMFERENCE ADJUSTED FOR AGE AT POST-YEARLING (SCA), BODY WEIGHT AT POST-YEARLING (SCW), AND HIP HEIGHT (SCH) IN NELLORE CATTLE. 61
FIGURE 14 - HERITABILITY COEFFICIENT ESTIMATES OVER THE ENVIRONMENTAL GRADIENT FOR SCROTAL CIRCUMFERENCE ADJUSTED FOR AGE AND HIP HEIGHT AT POST-YEARLING (SCAH), AGE AND BODY WEIGHT AT POST-YEARLING (SCAW), AND BODY WEIGHT AND HIP HEIGHT AT POST-YEARLING (SCWH) IN NELLORE CATTLE. 62
FIGURE 15 - REACTION NORMS FOR THE TOP 1% BULLS FOR SCROTAL CIRCUMFERENCE ADJUSTED FOR AGE AT POST-YEARLING IN NELLORE CATTLE. 63
FIGURE 16 - REACTION NORMS FOR THE TOP 1% BULLS FOR SCROTAL CIRCUMFERENCE ADJUSTED FOR HIP HEIGHT IN NELLORE CATTLE. 64
FIGURE 17 - REACTION NORMS FOR THE TOP 1% BULLS FOR SCROTAL CIRCUMFERENCE ADJUSTED FOR BODY WEIGHT AT POST-YEARLING IN NELLORE CATTLE. 65
FIGURE 18 - REACTION NORMS FOR THE TOP 1% BULLS FOR SCROTAL CIRCUMFERENCE ADJUSTED FOR AGE AND BODY WEIGHT AT POST-YEARLING IN NELLORE CATTLE. 66
FIGURE 19 - REACTION NORMS FOR THE TOP 1% BULLS FOR SCROTAL CIRCUMFERENCE ADJUSTED FOR BODY WEIGHT AND HIP HEIGHT AT POST-YEARLING IN NELLORE CATTLE. 67
FIGURE 20 - REACTION NORMS FOR THE TOP 1% BULLS FOR SCROTAL CIRCUMFERENCE ADJUSTED FOR AGE AND HIP HEIGHT AT POST-YEARLING IN NELLORE CATTLE. 68
FIGURE 21 - AVERAGE BODY WEIGHT AT POST-YEARLING IN NELLORE CATTLE BY ENVIRONMENTAL GRADIENTS 87
FIGURE 22 - ADDITIVE GENETIC AND ENVIRONMENTAL VARIANCE ESTIMATES OVER THE ENVIRONMENTAL GRADIENTS FOR SCROTAL CIRCUMFERENCE

ADJUSTED FOR CONFORMATION (A), PRECOCITY (B), AND MUSCULATURE (C) IN NELLORE CATTLE. 88
FIGURE 23 - ADDITIVE GENETIC AND ENVIRONMENTAL VARIANCE ESTIMATE OVER THE ENVIRONMENTAL GRADIENTS FOR SCROTAL CIRCUMFERENCE ADJUSTED FOR CONFORMATION AND PRECOCITY (A), CONFORMATION AND MUSCULATURE (B), AND PRECOCITY AND MUSCULATURE (C) IN NELLORE CATTLE. 89
FIGURE 24 - ADDITIVE GENETIC AND ENVIRONMENTAL VARIANCE ESTIMATE OVER THE ENVIRONMENTAL GRADIENTS FOR SCROTAL CIRCUMFERENCE ADJUSTED FOR AGE AND CONFORMATION (A), AGE AND PRECOCITY (B), AND AGE AND MUSCULATURE (C) IN NELLORE CATTLE. 90
FIGURE 25 - HERITABILITY COEFFICIENT ESTIMATES OVER THE ENVIRONMENTAL GRADIENT FOR SCROTAL CIRCUMFERENCE ADJUSTED FOR CONFORMATION (SCC), PRECOCITY (SCP), AND MUSCULATURE (SCM) IN NELLORE CATTLE. 91
FIGURE 26 - HERITABILITY COEFFICIENT ESTIMATES OVER THE ENVIRONMENTAL GRADIENT FOR SCROTAL CIRCUMFERENCE ADJUSTED FOR CONFORMATION AND PRECOCITY (SCCP), CONFORMATION AND MUSCULATURE (SCCM), AND PRECOCITY AND MUSCULATURE (SCPM) IN NELLORE CATTLE. 92
FIGURE 27 - HERITABILITY COEFFICIENT ESTIMATES OVER THE ENVIRONMENTAL GRADIENT FOR SCROTAL CIRCUMFERENCE ADJUSTED FOR AGE AND CONFORMATION (SCAC), AGE AND PRECOCITY (SCAP), AND AGE AND MUSCULATURE (SCAM) IN NELLORE CATTLE. 93
FIGURE 28 - REACTION NORMS FOR THE TOP 1% BULLS FOR SCROTAL CIRCUMFERENCE ADJUSTED FOR CONFORMATION IN NELLORE CATTLE. 94
FIGURE 29 - REACTION NORMS FOR THE TOP 1% BULLS FOR SCROTAL CIRCUMFERENCE ADJUSTED FOR PRECOCITY IN NELLORE CATTLE. 95
FIGURE 30 - REACTION NORMS FOR THE TOP 1% BULLS FOR SCROTAL CIRCUMFERENCE ADJUSTED FOR MUSCULATURE IN NELLORE CATTLE. 96
FIGURE 31 - REACTION NORMS FOR THE TOP 1% BULLS FOR SCROTAL CIRCUMFERENCE ADJUSTED FOR CONFORMATION AND PRECOCITY IN NELLORE CATTLE. 97

FIGURE 32 - REACTION NORMS FOR THE TOP 1% BULLS FOR SCROTAL CIRCUMFERENCE ADJUSTED FOR CONFORMATION AND MUSCULATURE IN NELLORE CATTLE. 98
FIGURE 33 - REACTION NORMS FOR THE TOP 1% BULLS FOR SCROTAL CIRCUMFERENCE ADJUSTED FOR PRECOCITY AND MUSCULATURE IN NELLORE CATTLE. 99
FIGURE 34 - REACTION NORMS FOR THE TOP 1% BULLS FOR SCROTAL CIRCUMFERENCE ADJUSTED FOR AGE AND CONFORMATION IN NELLORE CATTLE. 100
FIGURE 35 - REACTION NORMS FOR THE TOP 1% BULLS FOR SCROTAL CIRCUMFERENCE ADJUSTED FOR AGE AND PRECOCITY IN NELLORE CATTLE. 101
FIGURE 36 - REACTION NORMS FOR THE TOP 1% BULLS FOR SCROTAL CIRCUMFERENCE ADJUSTED FOR AGE AND MUSCULATURE IN NELLORE CATTLE. 102
FIGURE 37 - MEAN OF SCROTAL CIRCUMFERENCE MEASURED AT 6 MONTHS (A), 12 MONTHS (B), 18 MONTHS (C) AND 24 MONTHS (D) ALONG THE ENVIRONMENTAL GRADIENT FOR BRAHMAN CATTLE IN AUSTRALIA ... 126
FIGURE 38 - ADDITIVE VARIANCES FOR SCROTAL CIRCUMFERENCE MEASURED AT 6 MONTHS (A), 12 MONTHS (B), 18 MONTHS (C) AND, 24 MONTHS (D) ALONG THE ENVIRONMENTAL GRADIENT USING THE A MATRIX AND THE H MATRIX FOR BRAHMAN CATTLE IN AUSTRALIA 127
FIGURE 39 - ENVIRONMENTAL VARIANCES FOR SCROTAL CIRCUMFERENCE MEASURED AT 6 MONTHS (A), 12 MONTHS (B), 18 MONTHS (C), AND 24 MONTHS (D) ALONG THE ENVIRONMENTAL GRADIENT USING THE A MATRIX AND THE H MATRIX FOR BRAHMAN CATTLE IN AUSTRALIA 128
FIGURE 40 - HERITABILITIES FOR SCROTAL CIRCUMFERENCE MEASURED AT 6 MONTHS (A), 12 MONTHS (B), 18 MONTHS (C), AND 24 MONTHS (D) ALONG THE ENVIRONMENTAL GRADIENT USING THE A MATRIX AND THE H MATRIX IN BRAHMAN CATTLE IN AUSTRALIA 129
FIGURE 41 - REACTION NORMS FOR THE ESTIMATED BREEDING VALUES VALUE USING THE A MATRIX (A) AND THE H MATRIX (B) OF SCROTAL CIRCUMFERENCE MEASURED AT 6 MONTHS IN BRAHMAN CATTLE IN AUSTRALIA 130

FIGURE 42 - REACTION NORMS FOR THE ESTIMATED BREEDING VALUES VALUE USING THE A MATRIX (A) AND THE H MATRIX (B) OF SCROTAL CIRCUMFERENCE MEASURED AT 12 MONTHS IN BRAHMAN CATTLE IN AUSTRALIA 131
FIGURE 43 - REACTION NORMS FOR THE ESTIMATED BREEDING VALUES VALUE USING THE A MATRIX (A) AND THE H MATRIX (B) OF SCROTAL CIRCUMFERENCE MEASURED AT 18 MONTHS IN BRAHMAN CATTLE IN AUSTRALIA 132
FIGURE 44 - REACTION NORMS FOR THE ESTIMATED BREEDING VALUES VALUE USING THE A MATRIX (A) AND THE H MATRIX (B) OF SCROTAL CIRCUMFERENCE MEASURED AT 24 MONTHS IN BRAHMAN CATTLE IN AUSTRALIA. 133

TABLES

TABLE 1 - MEANS, STANDARD DEVIATION, MINIMUM, AND MAXIMUM OF THE HERITABILITY COEFFICIENT ESTIMATES FOR SCROTAL CIRCUMFERENCE ADJUSTED FOR AGE (SC_A), BODY WEIGHT (SC_W), HIP HEIGHT (SC_H), AGE AND BODY WEIGHT (SC_{AW}), AGE AND HIP HEIGHT (SC_{AH}), BODY WEIGHT AND HIP HEIGHT (SC_{WH}), IN NELLORE CATTLE 55

TABLE 2 - ESTIMATES OF VARIANCE (DIAGONAL), COVARIANCE (ABOVE DIAGONAL), AND CORRELATION (BELOW DIAGONAL) BETWEEN INTERCEPT AND SLOPE OF REACTION NORM MODELS FOR ADDITIVE EFFECT FOR THE SCROTAL CIRCUMFERENCE ADJUSTED FOR AGE (SC_A), BODY WEIGHT (SC_W), HIP HEIGHT (SC_H), AGE AND BODY WEIGHT (SC_{AW}), AGE AND HIP HEIGHT (SC_{AH}), BODY WEIGHT AND HIP HEIGHT (SC_{WH}) IN NELLORE CATTLE 56

TABLE 3 - SPEARMAN'S CORRELATION AMONG ESTIMATED BREEDING VALUES FOR SCROTAL CIRCUMFERENCE ADJUSTED FOR AGE (SC_A), BODY WEIGHT (SC_W), HIP HEIGHT (SC_H), AGE AND BODY WEIGHT (SC_{AW}), AGE AND HIP HEIGHT (SC_{AH}), BODY WEIGHT AND HIP HEIGHT (SC_{WH}), ACROSS ENVIRONMENTAL GRADIENTS OF THE TOP 1% NELLORE BULLS 57

TABLE 4 - MEANS, STANDARD DEVIATION, MINIMUM, AND MAXIMUM OF THE HERITABILITY COEFFICIENT ESTIMATES FOR SCROTAL CIRCUMFERENCE ADJUSTED FOR CONFORMATION (SC_C), PRECOCITY (SC_P), MUSCULATURE (SC_M), CONFORMATION AND PRECOCITY (SC_{CP}), CONFORMATION AND MUSCULATURE (SC_{CM}), PRECOCITY AND MUSCULATURE (SC_{PM}), AGE AND CONFORMATION (SC_{AC}), AGE AND PRECOCITY (SC_{AP}), AGE AND MUSCULATURE (SC_{AM}) IN NELLORE CATTLE 84

TABLE 5 - ESTIMATES OF VARIANCE (DIAGONAL), COVARIANCE (ABOVE DIAGONAL), AND CORRELATION (BELOW DIAGONAL) BETWEEN INTERCEPT AND SLOP OF REACTION NORM MODELS FOR ADDITIVE EFFECT FOR SCROTAL CIRCUMFERENCE ADJUSTED FOR CONFORMATION (SC_C), PRECOCITY (SC_P), MUSCULATURE (SC_M), CONFORMATION AND PRECOCITY (SC_{CP}), CONFORMATION AND MUSCULATURE (SC_{CM}), PRECOCITY AND MUSCULATURE (SC_{PM}), AGE AND CONFORMATION (SC_{AC}),

AGE AND PRECOCITY (SC_{AP}), AGE AND MUSCULATURE (SC_{AM}) IN NELLORE CATTLE 85
TABLE 6 - SPEARMAN'S CORRELATION AMONG ESTIMATED BREEDING VALUES FOR SCROTAL CIRCUMFERENCE ADJUSTED FOR CONFORMATION (SC_C), PRECOCITY (SC_P), MUSCULATURE (SC_M), CONFORMATION AND PRECOCITY (SC_{CP}), CONFORMATION AND MUSCULATURE (SC_{CM}), PRECOCITY AND MUSCULATURE (SC_{PM}), AGE AND CONFORMATION (SC_{AC}), AGE AND PRECOCITY (SC_{AP}), AGE AND MUSCULATURE (SC_{AM}), ACROSS ENVIRONMENTAL GRADIENTS OF THE TOP 1% NELLORE BULLS 86
TABLE 7 - STATISTICS FOR AGE AT 6 MONTHS (AGE6), AGE AT 12 MONTHS (AGE12), AGE AT 18 MONTHS (AGE18), AGE AT 24 MONTHS (AGE24), BODY WEIGHT AT 6 MONTHS (BW6), BODY WEIGHT AT 12 MONTHS (BW12), BODY WEIGHT AT 18 MONTHS (BW18), BODY WEIGHT AT 24 MONTHS (BW24), SCROTAL CIRCUMFERENCE AT 6 MONTHS (SC6), SCROTAL CIRCUMFERENCE AT 12 MONTHS (SC12), SCROTAL CIRCUMFERENCE AT 18 MONTHS (SC18), AND SCROTAL CIRCUMFERENCE AT 24 MONTHS (SC24) FOR BRAHMAN CATTLE IN AUSTRALIA 120
TABLE 8 - AVERAGE BODY WEIGHT, IN KILOGRAMS, AT THE MINIMUM, INTERMEDIATE, AND MAXIMUM ENVIRONMENTAL GRADIENT (EG) MEASURED AT 6 MONTHS, 12 MONTHS, 18 MONTHS, AND 24 MONTHS IN BRAHMAN CATTLE 121
TABLE 9 - ESTIMATES OF HERITABILITY IN THE MINIMUM AND MAXIMUM ENVIRONMENTAL GRADIENT (EG) FOR SCROTAL CIRCUMFERENCE AT 6 MONTHS (SC6), SCROTAL CIRCUMFERENCE AT 12 MONTHS (SC12), SCROTAL CIRCUMFERENCE AT 18 MONTHS (SC18), AND SCROTAL CIRCUMFERENCE AT 24 MONTHS (SC24) USING A MATRIX AND H MATRIX IN BRAHMAN CATTLE 122
TABLE 10 - ESTIMATES OF VARIANCE (DIAGONAL), COVARIANCE (ABOVE DIAGONAL), AND CORRELATION (BELOW DIAGONAL) BETWEEN INTERCEPT AND SLOP OF REACTION NORM MODELS FOR ADDITIVE EFFECT FOR SCROTAL CIRCUMFERENCE MEASURED AT 6 MONTHS (SC6), 12 MONTHS (SC12), 18 MONTHS (SC18), AND 24 MONTHS (SC24) ESTIMATED USING A MATRIX AND H MATRIX IN BRAHMAN CATTLE 123

TABLE 11 - RANK CORRELATION AMONG ESTIMATED BREEDING VALUES (EBV) FOR SCROTAL CIRCUMFERENCE MEASURED AT 6 MONTHS (SC6), 12 MONTHS (SC12), 18 MONTHS (SC18), AND 24 MONTHS (SC24) OBTAINED USING A MATRIX IN DIFFERENT ENVIRONMENTAL GRADIENT (EG) IN BRAHMAN CATTLE 124
TABLE 12 - RANK CORRELATION AMONG ESTIMATED BREEDING VALUES (EBV) FOR SCROTAL CIRCUMFERENCE MEASURED AT 6 MONTHS (SC6), 12 MONTHS (SC12), 18 MONTHS (SC18), AND 24 MONTHS (SC24) OBTAINED USING H MATRIX IN DIFFERENT ENVIRONMENTAL GRADIENT (EG) IN BRAHMAN CATTLE 125

CONTENTS

1 GENERAL INTRODUCTION 22

2 LITERATURE REVIEW 24

2.1 GENOTYPE X ENVIRONMENT INTERACTION 25

2.2 ENVIRONMENTAL DESCRIPTOR 27

2.3 RANDOM REGRESSION AND REACTION NORMS 29

2.4 LITERATURE 36

3 EFFECT OF GENOTYPE X ENVIRONMENT INTERACTION FOR SCROTAL CIRCUMFERENCE ADJUSTED FOR GROWTH TRAITS IN NELLORE CATTLE 41

ABSTRACT 42

INTRODUCTION 43

MATERIAL AND METHODS 44

RESULTS 47

DISCUSSION 49

LITERATURE CITED 51

4 GENOTYPE X ENVIRONMENT INTERACTION FOR SCROTAL CIRCUMFERENCE ADJUSTED FOR VISUAL SCORES IN NELLORE CATTLE USING REACTION NORMS 69

ABSTRACT 70

INTRODUCTION 72

MATERIAL AND METHODS 73

RESULTS 76

DISCUSSION 78

LITERATURE CITED 80

5 GENOTYPE X ENVIRONMENT INTERACTION FOR SCROTAL CIRCUMFERENCE USING GENOMIC REACTION NORM MODEL IN BRAHMAN CATTLE 103

ABSTRACT 104

INTRODUCTION 106

MATERIAL AND METHODS 107

RESULTS 111

DISCUSSION 113

LITERATURE CITED 116

6 FINAL CONSIDERATIONS 134

7 APPENDIX 135

1 GENERAL INTRODUCTION

In beef cattle breeding programs, the inclusion of traits that reflect sexual precocity as selection criteria is important, since reproductive traits influence directly the generation interval, the selection intensity, and profit (ABREU et al., 2017). However, those characteristics are considered difficult to measure and usually present low heritability coefficient.

The scrotal circumference, an indicator trait of sexual precocity for males and the females related to them, is simple to measure and presents moderate heritability (TERAKADO et al., 2015; BOLIGON et al., 2017; SCHMIDT et al., 2019; BRUNES et al., 2020). As the scrotal circumference and growth traits are favorably correlated (SCHMIDT et al., 2019), in order to express only sexual precocity, usually this measure is adjusted for age and body weight simultaneously (ORTIZ-PEÑA et al., 2000). However, since the body weight may not properly distinguish biotypes, adjust the scrotal circumference for visual scores can remove growth effect more adequately.

In large countries as Australia, Brazil, and United States, the beef cattle genetic breeding programs usually use information from properties distributed over the country, that adopt different production systems according to the environmental conditions. Thus, the occurrence of genotype x environment interaction is expected, especially for reproductive traits, which are more influenced by the environmental effects. However, this effect is usually disregarded in the estimation of breeding values, which can lead to bias, decreasing the effectiveness of selection by an inappropriate choice of parents of the following generations (CALUS et al., 2002).

Studies of the genotype x environmental interaction effect on scrotal circumference usually do not consider the adjustments for growth traits and age of measure. However, the adjustments are important so scrotal circumference can be properly used as an accurate selection criterion for sexual precocity. And account the genotype x environment interaction effect allows to indicate the best animal for each environment according to where their parents were selected, improving profit (SANTANA JR et al., 2014; AMBROSINI et al., 2016).

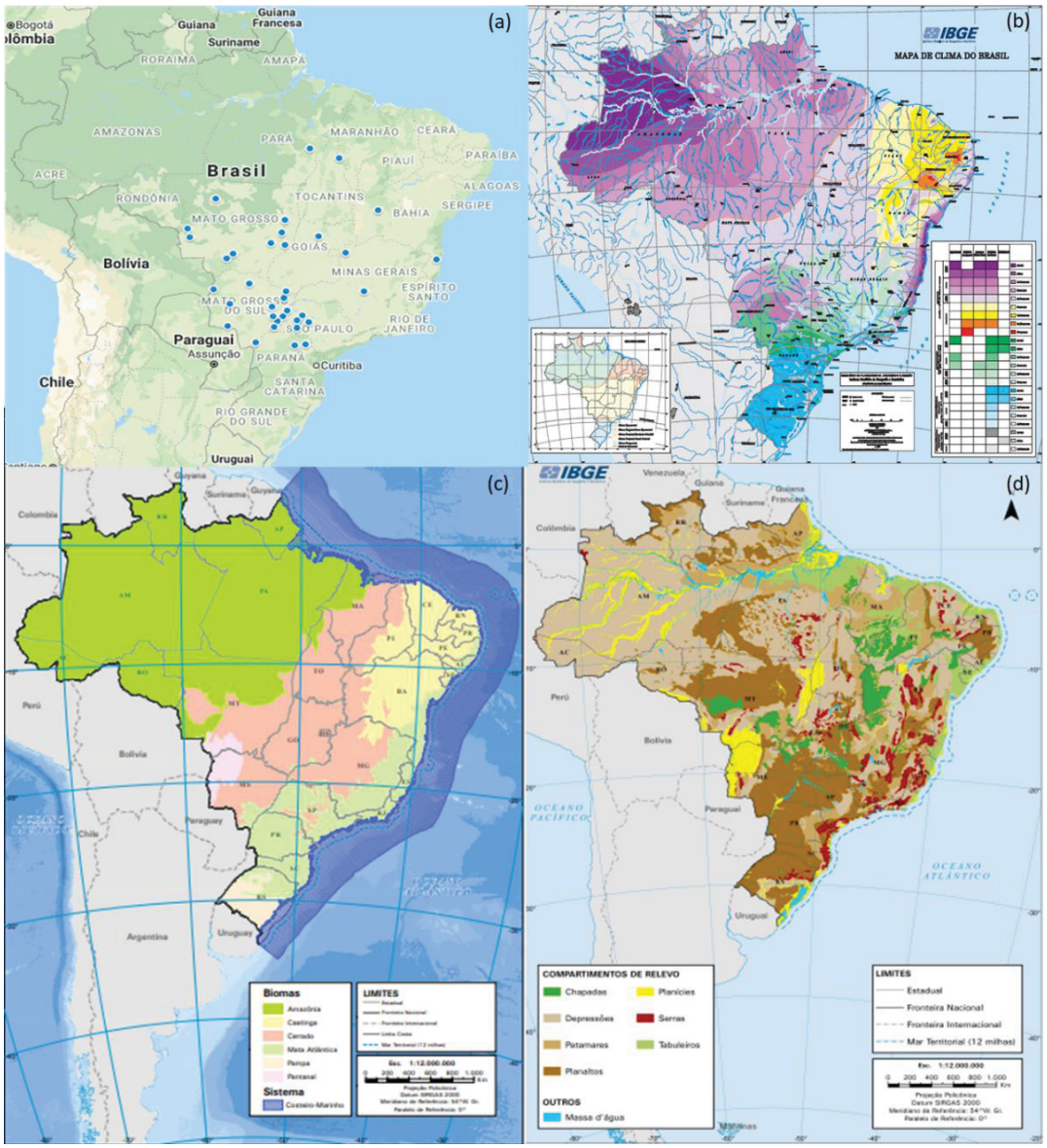
Thus, the aim of this thesis was to identify the effect of genotype x environment interaction for scrotal circumference adjusted to different growth traits. To achieve this, the first step was to verify the occurrence of genotype x environment interaction effect on the scrotal circumference adjusted for age, body weight, hip height, and the visual scores conformation, precocity, and musculature in Nellore cattle evaluated in Brazil. Then, the

occurrence of genotype x environment interaction for the scrotal circumference measured at different ages in Brahman cattle raised in Australia was verified.

2 LITERATURE REVIEW

Countries that have a huge territorial extension and use pasture system to raise beef cattle, as Brazil and Australia, have a distinct breeding environment. As an example, it is pointed at Figure 1 the location of the farms belonging to Aliança Nelore database (GENSYS, 2021). The distribution in nine different States indicates that the animals are raised in different climates, relieves, biomes, production system, among other factors.

FIGURE 1 - (a) LOCATION OF THE HERDS BELONGING TO ALIANÇA NELORE DATABASE IN BRAZIL, ELABORATED USING GOOGLE MAPS (2021), (b) BRAZILIAN CLIMATE DISTRIBUTION, (c) BRAZILIAN BIOMES DISTRIBUTION, (d) BRAZILIAN RELIEVES DISTRIBUTION



FONT: (a) the author (2021), (b) IBGE (2002), (c) IBGE (2019), (d) IBGE (2006).

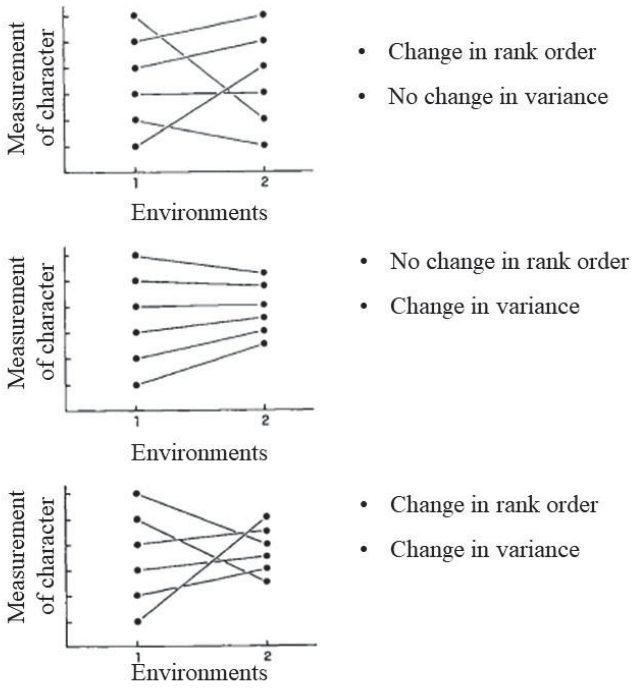
However, the interaction between the genotypes raised in those different environments are generally not considered in the genetic evaluation. According to de Jong and Bijma (2002), modeling the environment as fixed effect and them consider the performance in different environments as the same trait, the usual approach on genetic evaluation, induce selection of plastic phenotypes, that is, different phenotypes from the same genotype bred in different environment. So, there is a possibility that the best bull evaluated for a selection criterion in a region/environment is not superior in all regions where its offspring will be raised, since the genotype x environment interaction component is ignored in such evaluations.

2.1 GENOTYPE X ENVIRONMENT INTERACTION

A phenotype of an individual is a combination of the effect of its genes and the effect of nongenetic factors, i.e. the environment (BOURDON, 2000). Those effects will influence performance at the same time, so different genotypes at the same environment will have different phenotypes, likewise two identical genotypes may perform differently in different environments (GRIFFITHS et al., 1996).

Thus, the change in performance for a given trait of two or more genotypes evaluated in two or more environments is defined as genotype x environment interaction (BOWMAN, 1972). These changes can be relative both to the positioning in the classification of genotypes in different environments and to the change in genetic, environmental and phenotypic variances between environments. So, when the changes are an indicative of genotype x environment interaction, they can be represented graphically, as shown in Figure 2, and they may occur together or not.

FIGURE 2 - OCCURRENCE OF GENOTYPE X ENVIRONMENT INTERACTION



FONT: Adapted from Bowman (1972).

However, the phenotype measured in different environments is usually considered as being the same trait. But, as different groups of genes may act on these phenotypes depending on the environment where the individuals are evaluated, it may be necessary to consider those measures as different, since physiology and performance will be, somehow, influenced by different set of genes (BOWMAN, 1972; FALCONER, 1990). So, those phenotypes may be genetically correlated, and the magnitude can indicate the portion of similar genes on the traits (FALCONER, 1990). When individuals from the same population are created under different environmental conditions, the genotype x environment interaction must be considered (FALCONER; MACKAY, 1996). However, usually this effect is not taken into account in the estimation of breeding values, which can cause bias in that estimate, reducing the effectiveness of selection, since changes in classification may occur (CALUS et al., 2002).

When there is no genotype x environment interaction, the best genotype for one environment is the same for the others. However, when this effect is observed, genotypes should be chosen according to the environment where the animals will be raised (FALCONER; MACKAY, 1996). The change in the classification of genotypes may be greater or smaller depending on the species, the trait evaluated, and the size of the variation between environments (BOURDON, 2000). The genotype x environment interaction should be especially considered when there is a change in the positioning of the animals, because the

selection in the environments chooses distinct animals. Thus, raising the offspring of these reproducers in very different locations from which they were selected may result in loss of performance (CARDELLINO; ROVIRA, 2013).

For traits that present phenotypic plasticity, that is, variation in the phenotype of a genotype in response to environmental change, it is important to understand how heritability varies with environmental change (DE JONG, 1990; THOMPSON, 1991). As the existence of genotype x environment interaction can alter genetic, environmental and phenotypic variances, the genetic parameters will also be modified according to the breeding environments (ALENCAR et al., 2005). In general, traits of low heritability are more susceptible to genotype x environment interaction (BOURDON, 2000).

Most of the studies describe the effect of genotype x environment interaction for growth traits in beef cattle, as body weight and weight gain (ALENCAR et al., 2005; AMBROSINI et al., 2016; OLIVEIRA et al., 2018). But, in the last years, papers dealing with the effect of genotype x environment interaction over reproduction traits are increasing (MATOS et al., 2013; SANTANA JR et al., 2013; CHIAIA et al., 2015; LEMOS et al., 2015; MOTA et al., 2020). Furthermore, over the years, the methodology of environmental description, essential for the study of genotype x environment interaction, has been modified to better describe the differences on raising animals.

2.2 ENVIRONMENTAL DESCRIPTOR

In animal production, environments represent the quality of resources offered to those animals (AMBROSINI et al., 2016), and the influence on their performance.

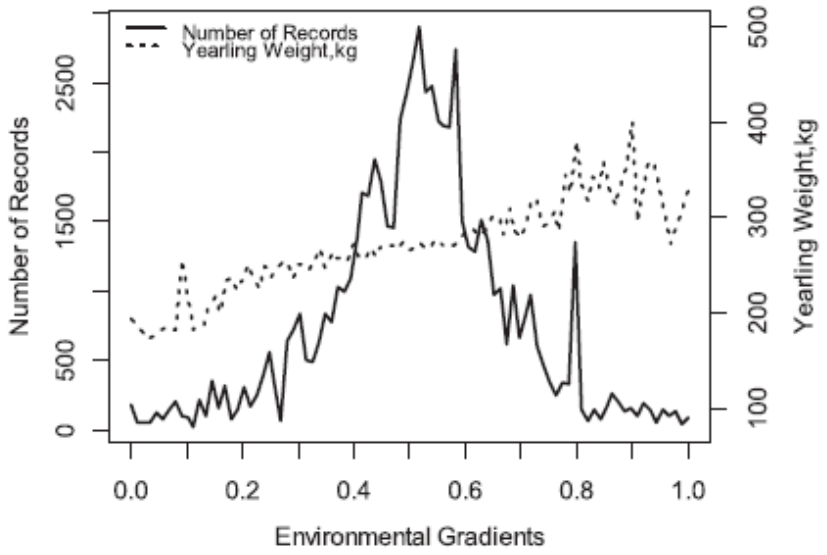
One of the most famous attempts to consider the genotype x environmental interaction on breeding analysis is the Interbull (International Bull Evaluation Service - Sweden). In this center, the geneticists analyze the genetic merit for milk production from six breeds from over 30 countries using the MACE (Multiple Across-Country Evaluation) methodology. Through a de-regression of the national breeding value, the genotype x environmental interaction is evaluated by genetic correlation among countries, and the result is a list of breeding values for all bulls according to the genetic basis from each country (INTERBULL, 2021).

Brazil is a large country, so properly represent the environment where animals are raised is important to avoid biased evaluations. A common way to represent the environment is to divide geographically the area where the animals evaluated are raised, as presented by Toral et al. (2004). These authors studied the genotype x environmental interaction for weight

at birth, at weaning, at yearling, and at post-yearling in Nellore cattle and determined microregions according to the official division by Brazilian Institute of Geography and Statistics (IBGE). Climate can be also used as an indicator of environment, as demonstrated by Santana Jr. et al. (2014) in their study of genotype x environment interaction for post-weaning weight gain, scrotal circumference and muscling in Montana cattle. Averages of minimum and maximum temperature, and average annual rain from the cities where the farms are located were used to group animals, as well as their latitude, longitude and altitude. In order to describe the environment, the authors used a cluster analysis. This methodology, where the performance of a genotype in different environments are treated as different traits, is known as Multitrait Models, and it considers environments as having discrete distribution, that is, the number of environments is limited, without the possibility of ranking them, since they cannot be quantified according to their quality (DE JONG; BIJMA, 2002; HAYES et al., 2016).

Another way to describe an environment is to quantify it in more or less favorable for the expression of a trait. According to Falconer and Mackay (1996), this can be done by considering the average performance of all phenotypes in each environment, that is, determining its environmental value. In more recently studies, the environmental value was set considering the contemporary group (CHIAIA et al., 2015; AMBROSINI et al., 2016). To use this approach, an analysis based on animal model is performed to obtain the solutions for the contemporary group based on a trait, usually body weight (PÉGOLO et al, 2011) or weight gain (CHIAIA et al., 2015), to indicate if the improve of the environment results in an improve of the performance. This solution for each contemporary group is standardized to be expressed in deviations of the mean solution, that is, the environmental gradient. This trend can be observed at Figure 3, extracted from Oliveira et al. (2018).

FIGURE 3 - AVERAGE YEARLING WEIGHT AND THE NUMBER OF RECORDS OVER THE ENVIRONMENTAL GRADIENT IN NELLORE CATTLE



FONT: Adapted from Oliveira et al. (2018).

To determine genotype x environmental interaction, the genetic correlation through the same trait evaluated in different environments can be performed. However, more recently, reaction norms models, estimated through random regression models, are used to describe the existence of genotype x environmental interaction by graphically showing how the phenotypes from a genotype vary through the environments (KOLMODIN et al., 2002; CHIAIA et al., 2015). The great advantage of this method over the Multitrait Model is that allows to predict changes by selection in all environments, not only those used in the evaluation (DE JONG; BIJMA, 2002).

2.3 RANDOM REGRESSION AND REACTION NORMS

In studies for repeated measurements over time, three methodologies are commonly used. The first one deals with the repeatability model, where it is considered that the genetic correlation between records is equal to unity and the variances are the same across observations. Another methodology is the multivariate analysis, where multiple records of several traits are considered at the same time, assuming the existence of correlation among them. Finally, covariance functions allow data to be analyzed on a trajectory, taking into account the variance and covariance structure of the various observations (GAMA et al., 2004).

The random regression model is one of the covariance functions usually used to analyze traits evaluated repeatedly over time and to estimate growth trajectories

(KIRKPATRICK et al., 1990; MEYER, 1998; GAMA et al., 2004). This methodology is advantageous, since it can predict an infinite number of measurements based on those taken on farms (KIRKPATRICK et al., 1990). In the random regression model, each evaluated animal has its own regression, which has random and normal distribution around a mean regression (GAMA et al., 2004).

To predict the growth trajectory, two continuous functions are considered: one for the additive genetic component, and another for environmental effects, being independent from each other (KIRKPATRICK et al., 1990). Those functions can be represented in a single equation, as demonstrated by Schaeffer (2004):

$$y_{ijkn:t} = F_i + g(t)_j + r(a, x, m1)_k + r(pe, x, m2)_k + e_{ijkn:t}$$

where $y_{ijkn:t}$ is the n-th observation on the k-th animal at time t belonging to the i-th fixed factor and the j-th group; F_i is a fixed effect that is independent of the time scale for the observations, such as cage effect, location effect or herd-test date effect; $g(t)_j$ is a function or functions that account for the phenotypic trajectory of the average observations across all animals belonging to the j-th group; $r(a, x, m1)_k = \sum_{l=0}^{m_1} a_{kl} x_{ijk:l}$ is the random regression function, where a is the additive genetic effects of the k-th animal, x is the vector of time covariates, and $m1$ is the order of the regression function. So $x_{ijk:l}$ are the covariables related to time t, and a_{kl} are the animal additive genetic regression coefficients to be estimated; $r(pe, x, m2)_k = \sum_{l=0}^{m_2} p_{kl} x_{ijk:l}$ is a similar random regression function for the permanent environmental (pe) effects of the k-th animal; and $e_{ijkn:t}$ is a random residual effect with mean null and with possibly different variances for each t or functions of t.

The random regression methodology can be used to analyze infinite-dimension traits, where the phenotypes are a continuous function, such as growth trajectories and reaction norms (KIRKPATRICK; HECKMAN, 1989; KIRKPATRICK et al., 1990). Reaction norms are functions that describe the variation on the phenotype produced by a genotype in each environment (KIRKPATRICK; HECKMAN, 1989). This methodology is used in genotype x environmental interaction studies, since the environments where a genotype is evaluated can be considered as a continuous gradient. So, a covariance function can be used to evaluate how the phenotypes from this genotype vary according to the environment (KOLMODIN et al., 2002; AMBROSINI et al., 2016). The model to estimate breeding values using this methodology is formed by a fraction independent from the environment and a fraction

depending on the environment. The first part is the random intercept of the reaction norm, and the second part is random linear coefficient of random regression over the environment, or slope of the reaction norm (CALUS et al., 2002). So, the model for random regression can be re-written to be used in reaction norms as:

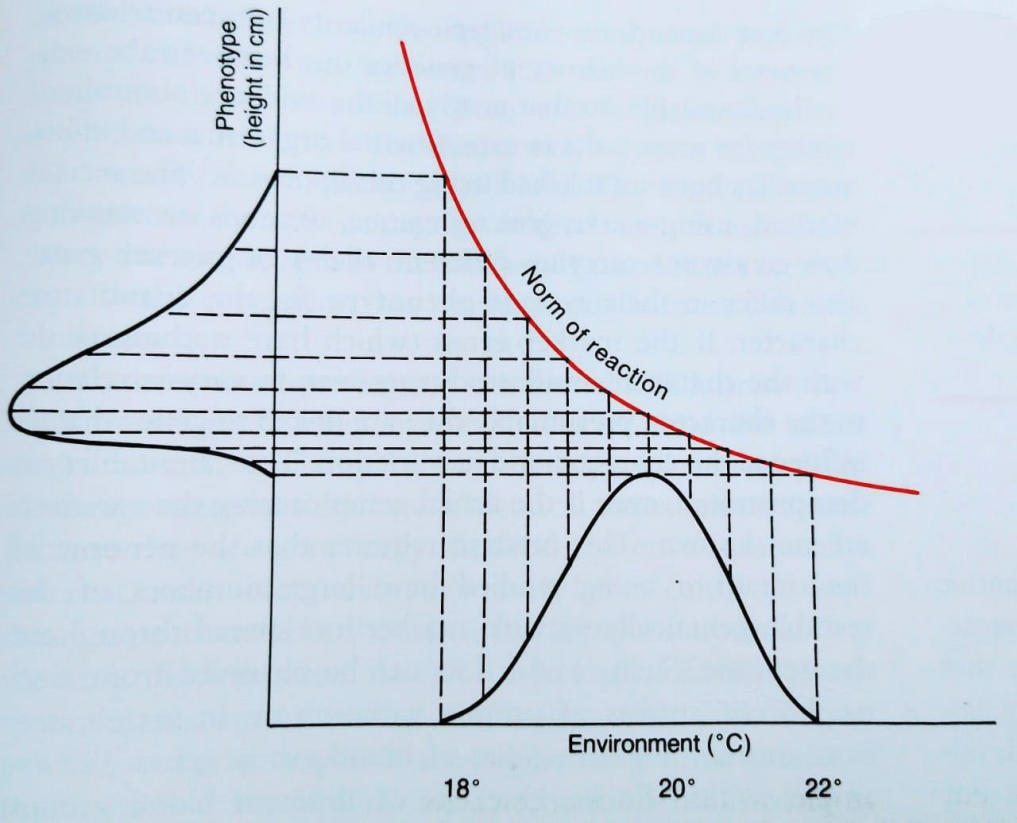
$$Y_{ij} = F_{ij} + \sum_{m=0}^{kb-1} \beta_m \varphi_m(t_{ij}) + \sum_{m=0}^{ka-1} \alpha_{im} \varphi_m(t_{ij}) + e_{ij}$$

where Y_{ij} is the observation of i-th animal in the j-th environment; F_{ij} is the vector of fixed effects; β_m is the average trajectory of the population, t_{ij} is the levels of environments, φ_m is the regression function; α_{im} is the individual random regression coefficient of direct genetic effect, kb and ka are the order of the correspondent polynomials; and e_{ij} is the random residual effect.

As result of random regression analysis, the regression curve, or reaction norm, indicates the genetic sensitivity of a genotype (FALCONER; MACKAY, 1996). Therefore, this methodology can consider differences in environmental sensibility on the variance components, which is not the case in traditional methods to estimate genetic parameters (CALUS et al., 2004). This sensitivity may be higher for some genotypes compared to others (FALCONER; MACKAY, 1996), which means that some genotypes will suffer more with changes in environmental conditions than others. This measurement is made by observing the slope of the reaction norm: the higher the slope, the more sensitive the genotype is to environmental changes.

Reaction norms are a simple way to interpret the effect of the environment over a genotype. At Figure 4, extracted from Griffiths et al. (1996), it is possible to notice how the reaction norm determine the distribution of phenotypes over a range of environments. The format of the reaction norm defines the distortion of environmental distribution over the phenotype axis. So, in this example, at low temperatures, the phenotype changes rapidly, noticed by the abrupt decrease of reaction norm. However, in higher temperatures, the reaction norm is flat, indicating that the environment has little influence over the that genotype, so the phenotypes are more similar.

FIGURE 4 - DISTRIBUTION OF PHENOTYPES FROM A SINGLE GENOTYPE ACCORDING TO THE ENVIRONMENT BY ANALYZING THE REACTION NORM



FONT: Adapted from Griffiths et al. (1996).

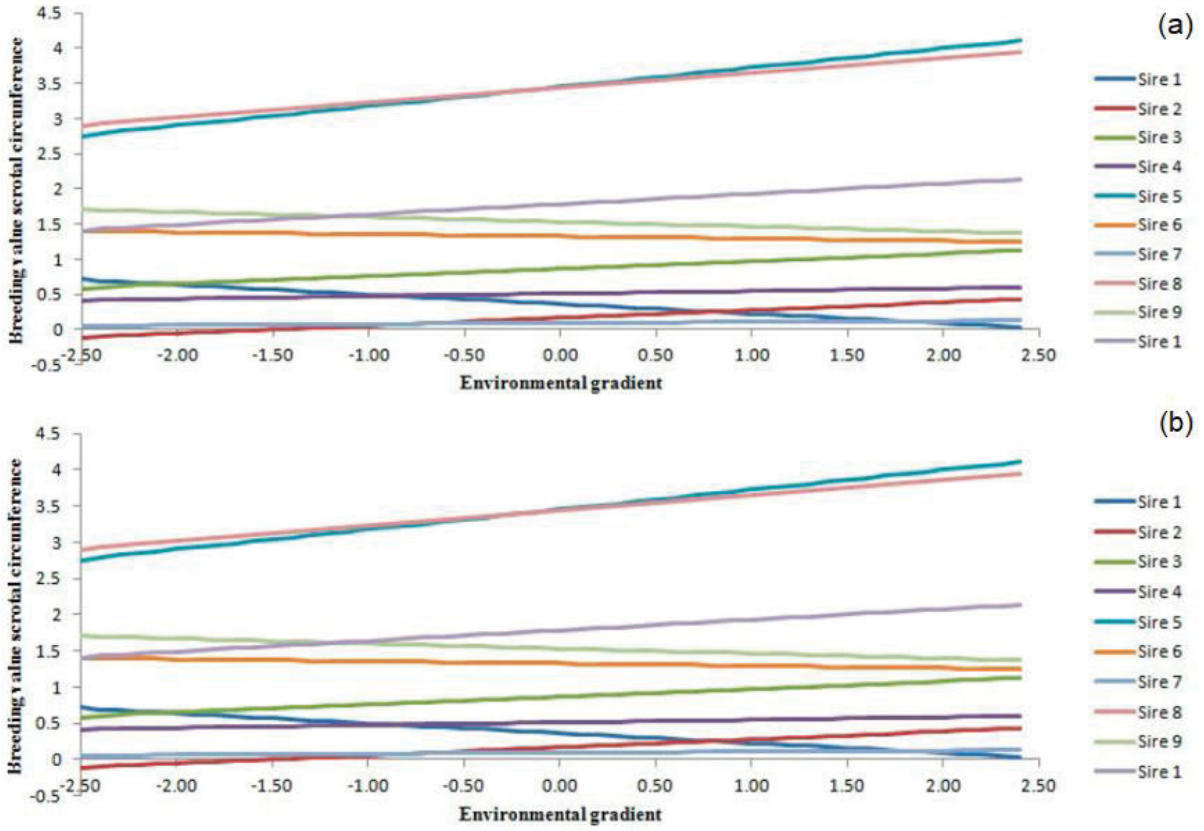
In animal breeding, the reaction norms are estimated for a range of different genotypes evaluated in different environments, since multiple animals from several farms are evaluated together. In order to classify those environments, the average performance of all genotypes in each environment is commonly used to divide them into more or less favorable, which is called the environmental value. Thus, the performance is an indicative of the environmental quality available to the animals. The environmental sensitivity will be the regression of the genotype performance in the environment over the environmental value and can be represented graphically by the slope of the regression curve (FALCONER, 1990; FALCONER; MACKAY, 1996; AMBROSINI et al., 2016).

The reaction norms allow to evaluate the existence or not of genotype x environment interaction. So, if the regression curves representing the environmental sensitivity are not parallel, then the evaluated genotypes do not react at the same way to the environments, therefore, there is genotype x environment interaction and those genotypes are considered sensitive to environmental changes. However, when the reaction norms are parallel to each other, low slope is observed, characterizing a robust genotype, where the genetic variance is

independent of the environment (DE JONG, 1990, HAYES et al., 2016). For genotype x environment interaction studies, the genotypes must be evaluated in a large environmental gradient, specifying the amount of genetic variation in the environments studied, because the variation can be observed in some environments but not in others (THOMPSON, 1991).

The evaluation of genotype x environment interaction using reaction norms in animal production is widely used for production traits. When observing the reaction norms for the 10 Nellore sires with highest and lowest estimated breeding value for yearling weight predicted by single-trait analysis, Lemos et al. (2015) demonstrated an upward trajectory, with small slope and almost no crosses among the reaction norms (Figure 5). However, the change in variance indicated the presence of genotype x environment interaction, although the authors noticed that the rank of the sires should no change when selected in the best or worst environment.

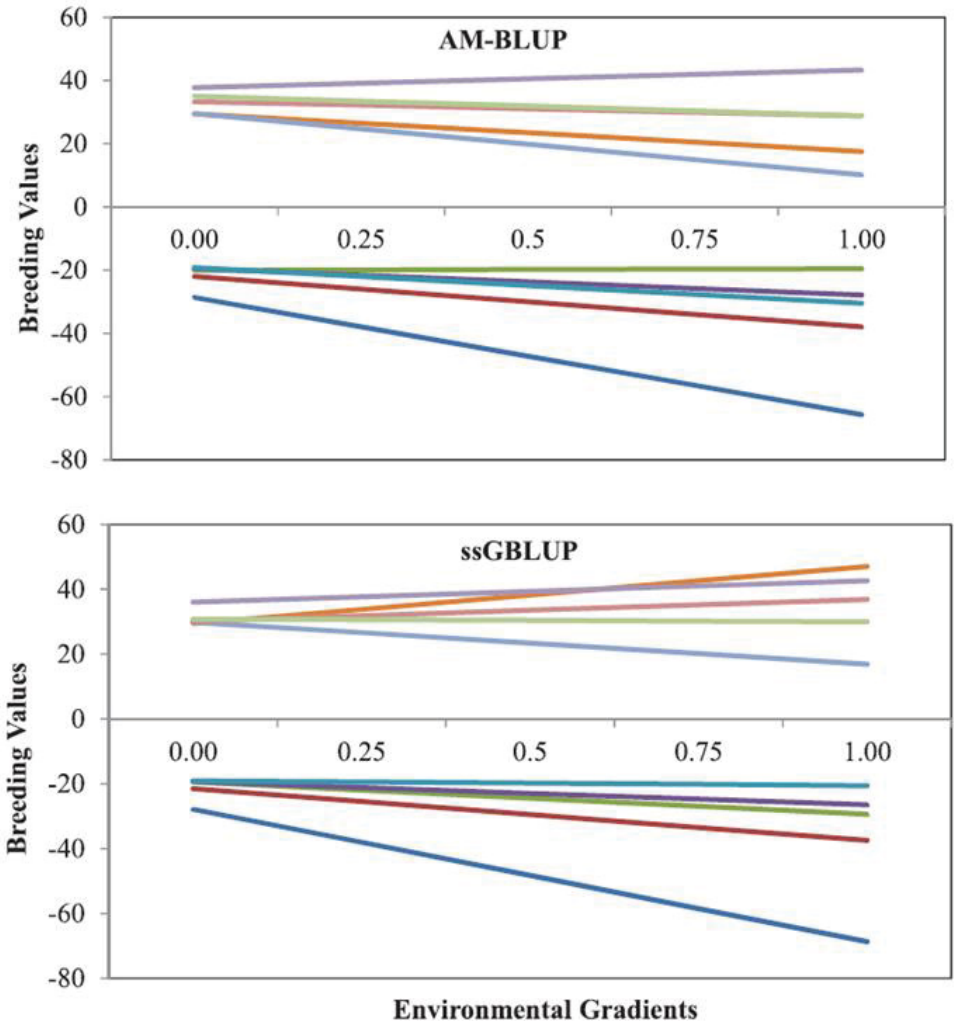
FIGURE 5 - REACTION NORMS ALONG THE ENVIRONMENTAL GRADIENT FOR SCROTAL CIRCUMFERENCE OBTAINED FOR 10 SIRES WITH THE HIGHEST (a) AND LOWEST (b) BREEDING VALUE FOR LONG-YEARLING WEIGHT IN NELLORE CATTLE



FONT: Adapted from Lemos et al. (2015).

In comparison of reaction norm models using pedigree-based (A matrix) and combination of pedigree and genomic (H matrix) relationship matrices for weight at yearling, Oliveira et al (2018) estimated the curves for the best five and worst five animals presented at Figure 6. The authors notice changing of ranking when using the A matrix and H matrix, since the use of the H matrix ranked two sires with no progeny data available among the top five bulls. All animals presented are sensitive to environmental changes, since variance in breeding value was observed through the environmental gradients evaluated. Also, according to the authors, using genomic information increase the accuracy of predicting breeding values, which can lead to better choices of bulls according to the environment.

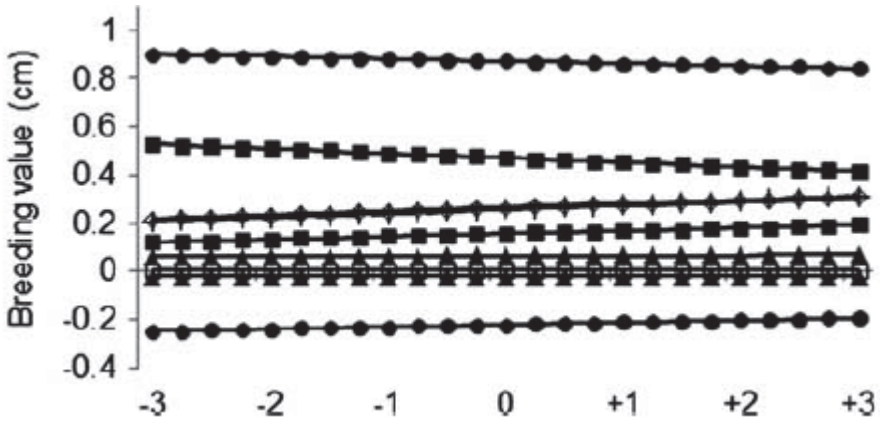
FIGURE 6 - ESTIMATED BREEDING VALUES OF FIVE BEST AND FIVE WORST BULLS FOR YEARLING WEIGHT IN NELLORE CATTLE, USING INVERSES OF PEDIGREE (AM-BLUP) AND PEDIGREE–GENOMIC (SSGBLUP) RELATIONSHIP MATRICES OVER THE ENVIRONMENTAL GRADIENTS



FONT: Adapted from Oliveira et al. (2018).

Other studies estimated reaction norm models for reproductive traits such as scrotal circumference. Santana Jr et al. (2013), analyzing the effect of genotype x environment interaction by reaction norm model for composite beef cattle observed that, for a random sample of 10 sires, the reaction norms for scrotal circumference were almost parallel, as demonstrate in Figure 7.

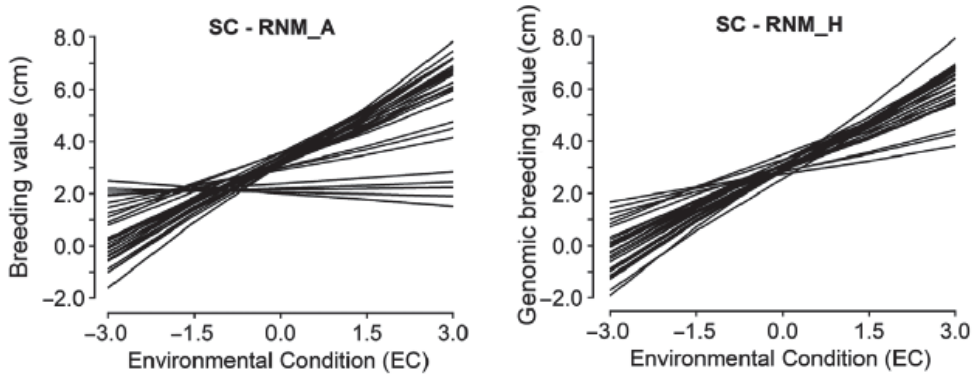
FIGURE 7 - REACTION NORMS FOR SCROTAL CIRCUMFERENCE FOR A RANDOM SAMPLE OF 10 MONTANA SIRES



FONT: Adapted from Santana Jr et al. (2013).

The variance of the slope in reaction norm models are related with the positioning of the curve. In their study, Santana Jr et al. (2013) observed a slope near to zero, which explain the parallelism observed at Figure 7. Opposite results were found by Mota et al. (2020), analyzing reaction norm models for scrotal circumference using pedigree-based and genomic-based relationship matrices in Nellore cattle. (Figure 8).

FIGURE 8 - REACTION NORMS FOR SCROTAL CIRCUMFERENCE (SC) EVALUATED USING PEDIGREE RELATIONSHIP MATRIX (RNM_A) AND GENOMIC RELATIONSHIP MATRIX (RNM_H) FOR THE 30 ANIMALS WITH HIGHER GENOMIC BREEDING VALUES



FONT: Adapted from Mota et al. (2020)

Because of genetic correlation among environmental levels lower than 0.80 and moderate magnitude genetic correlation between intercept and slope, crossing in reaction norms were expected by the authors. Regardless of the matrix used, the existence of genotype x environment interaction was observed by changing in ranking of evaluated sires. Besides that, according to the authors, the use of genomic-based relationship matrix seems to increase accuracy of the estimative of breeding values.

Thus, the reaction norms model obtained through random regression analysis is an accurate way to demonstrate the existence of genotype x environment interaction. Despite the existence of studies considering this effect for scrotal circumference, those analyses do not consider the adjustment of this measure. However, to properly represent sexual precocity, it is necessary to remove from the scrotal circumference the component related to growth, especially in Zebu cattle, which are known for being late in their reproductive life (DAL-FARRA, 2003; BRITO et al., 2004). Furthermore, the use of genomic information seems to help improve the accuracy of estimating breeding values for this trait, as well as allowing to select sires at younger ages, which is interesting in the case of reproductive traits. So, more studies are necessary to identify if genotype x environment interaction effect is important in those traits, and consequently, should be considered in genetic evaluation.

2.4 LITERATURE

ABREU, L. R. A.; MOTA, L. F. M.; FERREIRA, T. A.; PEREIRA, I. G.; PIRES, A. V.; VILLELA, S. D. J.; MERLO, F. A.; MARTINS, P. G. M. A. Genetic evaluation of bodyweight, scrotal circumference, and visual appraisal scores in Bos indicus cattle. **Animal Production Science**, v. 58, n. 9, p. 1584-1594, 2017.

ALENCAR, M. M.; MASCIOLI, A. S.; FREITAS, A. R. Evidências de interação genótipo x ambiente sobre características de crescimento em bovinos de corte. **Revista Brasileira de Zootecnia**, v. 34, n. 2, p. 489-495, 2005.

AMBROSINI, D. P.; MALHADO, C. H. M.; MARTINS FILHO, R.; CARNEIRO, P. L. S. Interação genótipo x ambiente via modelos de normas de reação para características de crescimento em bovinos Nelore. **Pesquisa Agropecuária Brasileira**, v. 51, n. 2, p. 177-16, fev. 2016.

BOLIGON, A. A.; FARIAS, P. P.; ROSO, V. M.; SANTANA JR, M. L.; BIGNARDI, A. B.; SOUZA, F. R. P. Genetic relations and indirect response to selection based on indices

for scrotal circumference, visual scores and weight gain in beef cattle. **Animal Production Science**, v. 58, p. 1210-1217, 2017.

BOURDON, R. M. **Understanding animal breeding**. 2. ed. New Jersey: Prentice-Hall, 2000.

BOWMAN, J. Genotype × environment interactions. **Genetic Selection Evolution**, v. 4, n. 1, p. 117-123, 1972. doi:10.1186/1297-9686-4-1-117.

BRITO, L. F. C.; SILVA, A. E. D. F.; UNANIAN, M. M.; DODE, M. A. N.; BARBOSA, R. T.; KASTELIC, J. P. Sexual development in early- and late-maturing *Bos indicus* and *Bos indicus* x *Bos taurus* crossbread bulls in Brazil. **Theriogenology**, v. 62, p. 1198-1217, 2004.

BRUNES; L. C.; BALDI, F.; E COSTA, M. F.O.; LOBO, R. B.; LOPES, F. B.; MAGNABOSCO, C. U. Genetic-quantitative analysis for reproductive traits in Nellor cattle selected for sexual precocity. **Animal Production Science**, v. 60, p. 896–902, 2020.

CALUS, M. P. L.; BIJMA, P.; VEERKAMP, R. F. Effects of data structure on the estimation of covariance functions to describe genotype by environment interactions in a reaction norm model. **Genetic Selection Evolution**, v. 36, p. 489-507, 2004.

CALUS, M. P. L.; GROEN, A. F.; DE JONG, G. Genotype × environment interaction for protein yield in Dutch dairy cattle as quantified by different models. **Journal of Dairy Science**, v. 85, p.3115–3123, 2002.

CARDELLINO, R. ROVIRA, J. **Mejoramiento genético animal**., Buenos Aires: Hemisferio Sur, 2013

CHIAIA, H. L. J.; LEMOS, M. V. A.; VENTURINI, G. C.; ABOUJAOUDE, C.; BERTON, M. P.; FEITOSA, F. B.; CARVALHEIRO, R.; ALBUQUERQUE, L. G.; OLIVEIRA, H. N.; BALDI, F. Genotype × environment interaction for age at first calving, scrotal circumference, and yearling weight in Nellore cattle using reaction norms in multi-trait random regression models. **Journal of Animal Science**, v. 93, p. 1503-1510, 2015. doi:10.2527/jas2014-8217.

DAL-FARRA, R. A. As tecnologias e a pecuária brasileira: o caso da seleção genética para precocidade sexual de bovinos. **Veterinária em foco**, v. 1, n. 1, p. 11-27, 2003.

DE JONG, G. Quantitative genetics of reaction norms. **Journal of Evolutionary Biology**, v. 3, p. 447-468, 1990.

DE JONG, G.; BIJMA, P. Selection and phenotypic plasticity in evolutionary biology and animal breeding. **Livestock Production Science**, n. 78, p. 195-214, 2002.

FALCONER, D. S. Selection in different environments: effects on environmental sensitivity (reaction norm) and on mean performance. **Genetic Research**, v. 56, p. 57-70, 1990.

FALCONER, D. S.; MACKAY, T. F. C. **Introduction to quantitative genetics**. 4. ed. United Kingdom: Pearson, 1996.

GAMA, L. T.; MATOS, C. P.; CAROLINO, N. **Modelos mistos em melhoramento animal**. Lisboa: Direcção Geral de Veterinária, 2004.

GENSYS. Sumário Aliança Nelore 2020/2021. Disponível em < https://gensys.com.br/sumario/sumario-alianca-nelore-2020-2021/>. Acesso em: 09 mar. 2021.

GOOGLE MAPS. **Meus Mapas**. Disponível em: < https://www.google.com/intl/pt-BR/maps/about/mymaps/>. Acesso em: 17 mar. 2021.

GRIFFITHS, A. J. F.; MILLER, J. H.; SUZUKI, D. T.; LEWONTIN, R. C.; GELBART, W. M. **An introduction to genetic analysis**. 6. ed. United States: W. H. Freeman, 1996.

HAYES, B. J.; DAETWYLER, H. D.; GODDARD, M. E. Models for genome x environment interaction: examples in livestock. **Crop Science**, v. 56, p. 1-9, 2016.

IBGE. Instituto Brasileiro de Geografia e Estatística. **Mapa de biomas e sistema costeiro-marinho do Brasil**. 2019. Escala: 1:12.000.000. Disponível em < https://geoftp.ibge.gov.br/informacoes_ambientais/estudos_ambientais/biomas/mapas/biomas_e_sistema_costeiro_marinho_250mil.pdf>. Acesso em: 07 abr. 2021.

IBGE. Instituto Brasileiro de Geografia e Estatística. **Mapa de clima**. 2002. Escala: 1:5.000.000. Disponível em < https://geoftp.ibge.gov.br/informacoes_ambientais/climatologia/mapas/brasil/Map_BR_clima_2002.pdf>. Acesso em: 07 abr. 2021.

IBGE. Instituto Brasileiro de Geografia e Estatística. **Mapa de unidades de relevo do Brasil**. 2006. Escala: 1:12.000.000. Disponível em < https://geoftp.ibge.gov.br/informacoes_ambientais/geomorfologia/mapas/brasil/macrocaracterizacao_compartimentos_relevo.pdf>. Acesso em: 07 abr. 2021.

INTERBULL. MACE Explained. Disponível em < https://interbull.org/ib/interbullactivities>. Acesso em: 18 fev. 2021.

KIRKPATRICK, M.; HECKMAN, N. A quantitative genetic model for growth, shape, reaction norms, and other infinite-dimensional characters. **Journal of Mathematical Biology**, v. 27, p. 429-450, 1989.

KIRKPATRICK, M.; LOFSVOLD, D.; BULMER, M. Analysis of the inheritance, selection and evolution of growth trajectories. **Genetics**, v. 124, p. 979-993, abr. 1990.

KOLMODIN, R.; STRANDBERG, E.; MADSEN, P.; JENSER, J.; JORJANI, H. Genotype by environment interaction in Nordic dairy cattle studied using reaction norms. **Acta Agriculturae Scandinavica, Section A – Animal Science**, v. 52, n. 1, p. 11-24, 2002.

LEMOS, M. V. A.; CHIAIA, H. L. J. BERTON M. P.; FEITOSA, F. L. B.; ABOUJAOUDE, C.; VENTURINI, G. C.; OLIVEIRA, H. N.; ALBUQUERQUE, L. G.; BALDI, F. Reaction norms for the study of genotype-environment interaction for growth and indicator traits of sexual precocity in Nellore cattle. **Genetic and Molecular Research**, v. 14, n. 2, p. 7151-7162, 2015.

MATOS, A. S.; SENA, J. S. S.; MARCONDES, C. R.; BEZERRA, L. A. F.; LÔBO, R. R.; RORATO, P. R. N.; CUCCO, D. C.; ARAÚJO, R. O. Interação genótipo-ambiente em rebanhos Nelore da Amazônia Legal. **Revista Brasileira de Saúde e Produção Animal**, v. 14, n. 3, p. 599-608, 2013.

MEYER, K. Estimating covariance functions for longitudinal data using a random regression model. **Genetic Selection Evolution**, v. 30, p. 221-240, 1998.

MOTA, L. F. M.; FERNANDES JR, G. A.; HERRERA, A. C.; SCALEZ, D. C. B.; ESPIGOLAN, R.; MAGALHÃES, A. F. B.; CARVALHEIRO, R.; BALDI, F.; ALBUQUERQUE, L. G. Genomic reaction norm models exploiting genotype x environment interaction on sexual precocity indicator traits in Nellore cattle. **Animal Genetics**, v. 51, n. 2, p. 210-223, 2020.

OLIVEIRA, D. P.; LOURENÇO, D. A. L.; TSURUTA, S.; MISZTAL, I.; SANTOS, D. J. A.; ARAÚJO NETO, F. R.; ASPILCUETA-BORQUIS, R. R.; BALDI, F.; CARVALHEIRO, R.; CAMARGO, G. M. F.; ALBUQUERQUE, L. G.; TONHATI, H. Reaction norm for yearling weight in beef cattle using single-step genomic evaluation. **Journal of Animal Science**, v. 96, p.27-34, 2018.

ORTIZ PEÑA, C. D.; QUEIROZ, S. A.; FRIES, L. A. Estimação de fatores de correção do perímetro escrotal para idade e peso corporal em touros jovens da raça Nelore. **Revista Brasileira de Zootecnia**, v. 29, n. 6, p. 1667-1675, 2000.

PÉGOLO, N. T.; ALBUQUERQUE, L. G.; LÔBO, R. B.; OLIVEIRA, H. N. Effects of sex and age on genotype × environment interaction for beef cattle body weight studied using reaction norm models. **Journal of Animal Science**, v.89, p. 3410-3425, 2011.

SANTANA JR, M. L.; ELER, J. P.; CARDOSO, F. F.; ALBUQUERQUE, L. G.; BALIEIRO, J. C. C.; PEREIRA, R. J.; FERRAZ, J. B. S. Genotype by environment interaction for post-weaning weight gain, scrotal circumference, and muscling score of composite

beef cattle in different regions of Brazil. **Genetics and Molecular Research**, v. 13, n. 2, p. 3048-3059, 2014.

SANTANA JR, M. L.; ELER, J. P.; CARDOSO, F. F.; ALBUQUERQUE, L. G.; FERRAZ, J. B. S. Phenotypic plasticity of composite beef cattle performance using reaction norms model with unknown covariate. **Animal**, v. 7, n. 2, p. 202-210, 2013.

SCHAEFFER, L. R. Application of random regression models in animal breeding. **Livestock Production Science**, v. 86, 35–45, 2004.

SCHMIDT, P. I.; CAMPOS, G. S.; ROSO, V. M.; SOUZA, F. R. P.; BOLIGON, A. A. Genetic analysis of female reproductive efficiency, scrotal circumference and growth traits in Nelore cattle. **Theriogenology**, v. 128, p.47-53, 2019.

TERAKADO, A. P. N.; BOLIGON, A. A.; BALDI, F.; SILVA, J. A. II V.; ALBUQUERQUE, L. G. Genetic associations between scrotal circumference and female reproductive traits in Nelore cattle. **Journal of Animal Science**, v. 93, p. 2706–2713, 2015.

THOMPSON, J. D. Phenotypic plasticity as a component of evolutionary change. **Trends in Ecology and Evolution**, v. 6, n. 8, p. 247-249, ago. 1991.

TORAL, F. L. B.; SILVA, L. O. C.; MARTINS, E. N.; GONDO, A.; SIMONELLI, S. M. Interação genótipo x ambiente em características de crescimento de bovinos da raça Nelore no Mato Grosso do Sul. **Revista Brasileira de Zootecnia**, v. 33, n. 6, p. 1445-1455, 2004.

3 EFFECT OF GENOTYPE X ENVIRONMENT INTERACTION FOR SCROTAL CIRCUMFERENCE ADJUSTED FOR GROWTH TRAITS IN NELLORE CATTLE[1]

Running title: Genotype x environment interaction in Nellore

Effect of genotype x environment interaction for scrotal circumference adjusted for growth traits in Nellore cattle[2]

Bárbara M. Nascimento*[3], Roberto Carvalheiro†, Rodrigo de A. Teixeira*, Laila T. Dias*

*Department of Animal Science, Federal University of Paraná, Curitiba, Paraná, Brazil, 80035-060.

†Department of Animal Science, Paulista State University, FCAV, Jaboticabal, São Paulo, Brazil, 14884-900.

[1] This chapter was written according to the Authors Guideline from Journal of Animal Science (Appendix).

[2] The authors acknowledge CAPES for the scholarship granted and Gensys Consultores Associados Ltda and Aliança Nelore for the data concession.

[3] Corresponding author.

ABSTRACT

The aim of this study was to identify the existence of genotype x environment interaction (GxE) effect on scrotal circumference (SC) adjusted for growth traits in Nellore cattle. We analyzed post-yearling measurements of SC adjusted for age (SC_A), weight (SC_W), hip height (SC_H), age and weight (SC_{AW}), age and hip height (SC_{AH}), and weight and hip height (SC_{WH}) from 119,271 Nellore males. The environment gradient (EG) was estimated by standardizing the solutions of the contemporary groups obtained by Animal Model with weight at post-yearling as the dependent variable. Then, the Reaction Norm (RN) model was determined through a linear Random Regression Model with environmental variances considered heterogeneous. In addition, the genetic correlation ($r_{a,b}$) between the intercept and the slope of the RN and the Spearman's correlation between the ranking of bulls according to the estimated breeding value (EBV) were estimated. The decrease in additive genetic variance in the low environments observed for SC_A, SC_H, and SC_{AH} indicated that animals had difficulty to express their genetic potential when raised in challenging environments. On the other hand, for SC_W, SC_{AW}, and SC_{WH}, decrease on additive genetic variance with the improvement of the environment was observed. It is likely that those adjustments truly represent the GxE between sexual precocity and environmental gradient. The medium to high magnitude of heritability (h^2) observed for all traits through the EG indicated that SC could respond to direct selection in any environment. The h^2 vary through the environments, being higher in better EG for all traits evaluated. So, better EG can increase the chance to express genetic potential, and consider the environment seems to be important to estimate more precisely the h^2. The high $r_{a,b}$ for SC_A and SC_{AH} indicated higher sensibility to environmental variation, especially in animals with higher EBV, and existence of GxE by scaling effect. However, low to medium $r_{a,b}$ were observed for SC_W, SC_H, SC_{AW}, and SC_{WH}, showing the possibility of re-ranking according to EBV in different environments. The negative $r_{a,b}$ estimated for SC_W and SC_{WH} imply in downward curve of RN, from the worst to the best environment, while for SC_{AW}, the RN were almost parallels. Spearman's correlation among high, medium, and low environments vary from 0.30 to 0.86 for all traits evaluated. The lower correlations were observed between the extreme environments for all traits evaluated, since the differences in management tend to be higher in those environments. It means that the best animal selected for one environment may not the best for another. Thus, the existence of GxE in SC adjusted to growth traits is evident.

Keywords: *Bos indicus*, cattle breeding, environmental gradient, estimated breeding values, reaction norms, reproductive traits

INTRODUCTION

In Brazil, beef cattle are raised in a huge variety of production systems that are adapted to local realities of climate, geography, and quality of pasture, among other environmental factors. However, not always the environment where the animal is raised allows it to fully express its genetic potential, since factors like poor forage and heat stress can impact in the weight gain, the most economic relevant trait in beef cattle. It happens because the phenotype is basically composed by the genotype and environment, but also by the interaction of these two components, known as genotype x environment interaction (GxE), which is usually not considered in the estimates of breeding value.

Scrotal circumference (SC) is widely used as reproductive trait because it is favorable and genetically correlated to spermatic traits (Boligon et al., 2010; Silva et al., 2011) and female reproductive efficiency (Terakado et al., 2015; Pires et al., 2017). However, an important correlation with growth traits is observed for SC (Boligon et al., 2017; Raidan et al., 2017). Therefore, adjustments for growth traits are necessary in order to better distinguish sexual precocity (Ortiz Peña et al., 2000).

Due to its great importance, identify sires with high breeding value for SC and that also have good performance in growth is paramount. So, GxE studies are important to identify the most suitable bulls for each environment. One way to evaluate GxE effect is by Reaction Norm Models (RNM), which describe the environmental sensitivity of a genotype (Falconer and Mackay, 1996; Kolmodin et al., 2002). In this methodology, it is possible to quantify the environments to determine the environmental value and to estimate the breeding value by environmental gradient (EG). So, the mean performance of the animals is used as a proxy for characterizing their environment (Falconer and Mackay, 1996). The mean performance for body weight (BW) is a useful indicator of the environment, as this trait is largely influenced by the quality and quantity of feed available (i.e. the quality of pasture in grazing systems).

The RNM are widely used in studied with dairy cattle (Calus et al., 2002; Kolmodin et al., 2002) and beef cattle, in this case especially for growth traits such body weight and weight gain (Pégolo et al., 2009; Ambrosini et al., 2016; Carvalheiro et al., 2019). However, few studies evaluated this methodology for reproductive trait such as SC (Santana Jr. et al., 2013; Chiaia et al., 2015; Lemos et al., 2015), usually without considering any adjustment to growth traits. So, the aim of this study was to identify the existence of GxE for SC adjusted for growth traits in Nellore cattle.

MATERIAL AND METHODS

Dataset

Data from 490,324 Nellore males, born between 1984 and 2019 from 10,228 sires and 284,803 dams were used in this study. Those animals belonged to the historical dataset from the "Aliança Nelore" beef cattle database, whose calves were born in the North Region (States of Pará and Tocantins), Northeast Region (State of Bahia), Central-West Region (States of Goiás, Mato Grosso, and Mato Grosso do Sul), Southeast Region (States of Minas Gerais and São Paulo, and South Region (State of Paraná), in Brazil. In this study, were analyzed post-yearling (498.84 ± 53.15 days) measurements of SC after single adjustment for age (SC_A), body weight (SC_W), hip height (SC_H), and double adjustment for age and body weight (SC_{AW}), age and hip height (SC_{AH}), and body weight and hip height (SC_{WH}). The adjustments were performed similarly to the methodology demonstrated by Nascimento et al. (2020). However, in the present study, the linear and the quadratic effects of all traits used in the adjustment were significant. The contemporary groups (CG) were formed by: farm of birth, weaning, and post-yearling, year and season of birth, management group and julian date at weaning and post-yearling.

Data edition

Animals without information for the traits evaluated or used to create the CG, or with measurements above or below three standard deviations from the average for the evaluated traits were removed. Were also deleted CG with less than 15 animals, or less than 10 genetic links among them, verified by the AMC software (Roso and Schenkel, 2006), or with sons of only one sire. After edition, the final dataset was comprised of 119,271 males in 3,376 CG.

Environmental descriptor

The study of GxE was performed using the RN model. The first step was to describe the breeding environments by the estimation of best linear unbiased estimator (BLUE) of the CG through the Animal Model presented as following:

$$Y = X\beta + Za + e$$

where Y is the body weight at post-yearling (BW), β is the vector of fixed effects (CG and linear and quadratic effects of age at post-yearling as covariate), a is the vector of additive genetic effect, represented by animal, X and Z are the incidence matrices of fixed and random effects, respectively, and e is the vector of residual effects.

The environmental gradients (EG) were determined by the solutions of the GC obtained in the Animal Model, described previously, were standardized according to the equation below:

$$EG = \frac{GC_{sol} - GC_{mean}}{GC_{sd}}$$

where EG is the environmental gradient, GC_{sol} is the solution for each CG obtained by Animal Model, GC_{mean} is the average of the solutions from the CG, and GC_{sd} is the standard deviation of the solutions from the CG.

Since BW was used to estimate the EG, is expected that higher EG (+5,08) correspond to less challenging environments, i.e. environments where the animals have better conditions to growth. On the other hand, environments with lower EG (-4,19) are more challenging for the animals, so individuals raised in those places tend to be lighter, as presented at Figure 9.

Reaction Norm Model

The second step was to determine the RN model using a Random Regression Model to study GxE. Because animals present only one observation for each adjustment of SC, based on the results found by Chiaia et al. (2015), linear model was considered and is presented below:

$$Y_{ij} = F_{ij} + \sum_{m=0}^{kb-1} \beta_m \varphi_m(t_{ij}) + \sum_{m=0}^{ka-1} \alpha_{im} \varphi_m(t_{ij}) + e_{ij}$$

where Y_{ij} is the observation of SC adjusted of the sons of the i-th animal in the j-th environment, F_{ij} is the vector of fixed effects (CG), β_m is the average trajectory of the population, t_{ij} is the levels of standardized environments (EG), φ_m is the linear Legendre polynomial, α_{im} is the individual random regression coefficient of direct genetic effect, kb and ka are the order of the correspondent polynomials, fixed in 2 (linear), and e_{ij} is the random residual effect.

The additive genetic variance was obtained using the follow equation:

$$(\mathrm{Var(a)|EG}) = \mathrm{Var}(a_i + b_i \cdot EG) = \sigma_a^2 + \sigma_b^2 \cdot \mathrm{EG}^2 + 2 \cdot \mathrm{EG} \cdot \sigma_{a,b}$$

where $(\mathrm{Var(a)|EG})$ is the additive genetic variance by EG, $\mathrm{a_i}$ and $\mathrm{b_i}$ are the intercept e slope of the RN model, respectively, σ_a^2 is the additive genetic variance for the intercept, σ_b^2 is the additive genetic variance for the slope, EG is the environmental gradient, as defined before, and $\sigma_{a,b}$ is the covariance between intercept and slope.

Considering that heteroscedastic RN model performs better than homoscedastic model (Carvalheiro et al., 2019), the environmental variance was considered as heterogeneous in this analysis, and was obtained using the following equation:

$$(\mathrm{Var(e)|EG}) = \exp(\mathrm{z_0} + \mathrm{z_1} \cdot \mathrm{EG})$$

where $(\mathrm{Var(e)|EG})$ is the residual variance by EG, exp is the exponential function to transform the values of the residual coefficients, obtained by logarithmic function, $\mathrm{z_0}$ is the intercept of the residual function for SC, $\mathrm{z_1}$ is the slope of the residual function for SC in the RN model, considering heterogeneous residual variance, and EG is the environmental gradient.

The heritability estimates ($\mathrm{h^2}$) were given by the following equation:

$$(\mathrm{h^2|EG}) = \frac{(\mathrm{Var(a)|EG})}{(\mathrm{Var(a)|EG}) + (\mathrm{Var(e)|EG})}$$

where $\mathrm{h^2|EG}$ is the heritability by EG, $\mathrm{Var(a)|EG}$ is the additive genetic variance by EG, and $\mathrm{Var(e)|EG}$ is the residual variance by EG.

The genetic correlation between intercept and slope ($\mathrm{r_{a,b}}$) was given by:

$$r_{a,b} = \frac{\sigma_{a,b}}{\sqrt{\sigma_a^2 \sigma_b^2}}$$

where $r_{a,b}$ is the genetic correlation between intercept and slope, $\sigma_{a,b}$ is the covariance between intercept and slope, σ_a^2 is the additive genetic variance for the intercept, and σ_b^2 is the additive genetic variance for the slope.

The estimated breeding values (EBV) for the bulls in each environment were predicted by following equation:

$$\mathrm{EBV_{i|EG}} = \mathrm{b_{0_i}} + \mathrm{b_{1_i}} \cdot \mathrm{EG}$$

where $EBV_{i|EG}$ is the estimated breeding value of the i-th bull in each EG, b_{0_i} is the intercept of the reaction norm for the i-th bull, b_{1_i} is the slope of the reaction norm for the i-th bull, and EG is the environmental gradient. To represent the reaction norms, the top 1% bulls according to general EBV where selected and plotted.

Ranking correlation

The Spearman's correlation among EBV for the top 1% bulls previously selected for each trait evaluated was performed to evaluate changes in ranking in high, medium, and low EG. This analysis was performed using the pspearman function from software R (Savicky, 2014).

All data manipulation, statistics, and additional analysis were performed using software R (R Core Team, 2020) and the following packages: lubridate (Grolemund and Wickham, 2011), naniar (Tierney et al., 2020), and dplyr (Wickham et al., 2021). Also, the figures presented were developed and constructed through ggplot2 (Wickham, 2016) and gridExtra packages (Auguie, 2017) from the same software. The Animal Model and Random Regression analysis were performed using the AIREMLF90 software (Misztal et al., 2018).

RESULTS

Figure 9 presents an increase in the average BW in each EG. At the lower EG (-4.19), animals presented, on average, 202.04 kg, at the EG equal to zero, the average BW was 297.35 kg, and in the higher EG (+5.08), the average BW was 427.30 kg.

The additive genetic and environmental variances estimate for SC_A, SC_W, and SC_H over the EG are presented in Figure 10. For SC_A (Figure 10a) and SC_H (Figure 10c), both variances increased as the environment becomes more favorable. For SC_W (Figure 10b), the additive genetic variance increased while the environmental variance decreased from the worst to the best environment.

When the simultaneous adjustments were proceeded, the environmental variance for SC_{AW} and SC_{WH} decreased and the additive genetic variance presented a slightly increase over the EG, with similar estimates for both variances at the highest EG (Figure 11a and 11c). For SC_{AH}, the genetic additive and the environmental variance increased over the environmental gradients (Figure 11b).

The heritability coefficients (h^2) were moderate for all SC adjusted for a single trait, as presented at Table 1. For SC_A, the h^2 presented small variation between the worst EG (-4,19, $h^2 = 0.39$) and the best EG (5,08, $h^2 = 0.37$). The h^2 estimates decreased until reach its lowest

value close to medium EG (Figure 12). However, because of the standard deviation for this estimate, this difference does not seem to be significant. For SC_W, the best EG was the one with higher h^2 (0.48), while the lowest h^2 was observed in an EG close to -2.50 (Figure 12). Similarly, the highest h^2 for SC_H was estimated in the best EG, while the lowest h^2 was the one at the EG around -1.00 (Figure 12).

When the SC was adjusted for two traits, it was possible to notice similar mean of h^2 for all adjustments (Table 1). However, for SC_{AW} and SC_{WH} the curve of h^2 over the EG were similar (Figure 13), at the best EG (5.08) the h^2 estimates were higher than the others EG. For SC_{AH}, the highest h^2 was observed at the worst environment (EG = -4.19). So, SC_{AW} and SC_{WH} presented an increase of h^2 as the quality of the environment increased, while, for SC_{AH}, the opposite trend was noticed.

The genetic correlation between intercept and slope ($r_{a,b}$) varied in magnitude and direction among traits, as showed in Table 2. SC_A and SC_{AH} had high and positive $r_{a,b}$, while this estimate was moderate and positive for SC_H. Low estimative of $r_{a,b}$ were observed for SC_{AW}, SC_W, and SC_{WH}, being negative for the last two traits.

The EBV of the top 1% sires for SC_A increased in variance from the worst to the best environment (Figure 14). The same trend was observed for SC_H (Figure 15) and SC_W (Figure 16), but with smaller difference between variances in extreme EG. Also, for those traits, it was possible to notice that the rank for the top 1% bulls changed when the animal was evaluated in different environments. Those results suggested existence of GxE for SC_A, SC_H, and SC_W.

When we used the double adjustment SC_{AW} (Figure 17) and SC_{WH} (Figure 18), we observed small differences between EBV of the top 1% animals estimated from the worst to the best EG, but still with change in the rank. Comparatively, for SC_{AH}, the difference between the EBV in the best and in the worst environment were more pronounced, as demonstrated at Figure 19. Change of ranking was observed for the three traits evaluated, so we could also identify the GxE effect on EBV of the top 1% bulls for the adjustments of SC for two traits simultaneously.

Rank correlation among the best 1% bulls according to the EBV for each trait is presented at Table 3. Spearman's correlation among high, medium, and low environments varied from 0.30 to 0.86. The lower correlations were observed between the extreme environments for all traits evaluated. The correlation between medium and high environment were higher than 0.80, except for SC_{WH}, while the correlation between medium and the worst environments were higher than 0.80 only for SC_A, SC_{AW}, and SC_{AH}.

DISCUSSION

The increase of BW from the lowest to the highest EG was expected, since the solutions of the GC for BW were used to determine the EG. So, it is possible to assume that the lowest EG represented more challenging environments, where animals tend to be lighter than those were raised in highest EG. Considering the existence of favorable genetic correlation between BW and SC (Pires et al., 2017; Schmidt et al., 2019), it is also expected that animals raised in better environments present larger SC than those raised in worst environments.

The decrease in additive genetic variance in the worse environments observed for SC_A, SC_H, and SC_{AH} indicate that, when evaluated for those traits, the animals had difficult in express their genetic potential when raised in challenging environments. On the other hand, for SC_W, SC_{AW}, and SC_{WH}, the additive genetic variance decreased with the improvement of the environment. However, it is important to notice that different groups of genes may be acting on these traits depending on the raising environment, since there is a change in genetic variance with the change of environment. Thus, improvement in environment may not guarantee better performance. Moreover, some animals may perform better in less favorable environments. So, choosing sire according to the environment may be more interesting as a way to increase genetic gain. In the literature, studies about GxE effect on SC in Montana cattle (Santana Jr. et al., 2013) and Nellore cattle (Chiaia et al., 2015; Lemos et al., 2015) reported increase in variances with the improvement of the environment, indicating that additive genetic differences among animals are more evident in better environments. The use of BW in the description of the environment and later in the adjustment of SC may be the main contributing factor in the difference on the additive genetic variance curves behavior observed between adjustments. According to Chung et al. (2020), there may be a correlation between the trait and the environmental modulator, that is, a genotype x environment correlation. This is not normally taken into account in the estimates of (co)variance and genetic parameters, which may cause spurious GxE. When the adjustment of the SC considered the effect of the BW, the trait chosen to determine the EG, the correlation between them was computed in the analysis, eliminating the bias of the estimate and, consequently, avoiding spurious GxE. Thus, it is likely that these adjustments truly represent the GxE between sexual precocity and environmental gradient.

The medium to high magnitude of h^2 observed for all traits over the environment gradient indicated that SC could respond to direct selection in each environment. The h^2 vary through the environments, being higher in better EG for all traits evaluated. So, better conditions in the environment can increase the chance to express genetic potential, but, even in bad environments, animals with superior breeding values will be distinguishable from those

with worst breeding values (Legates, 1962). It is important to point out that just providing a better environment for the animal is not interesting from the point of view of genetic improvement. This is because the gain in performance coming from environmental factors will not be inherited by the following generations. So again, choosing the most suitable sire for the breeding environment can lead to greater genetic gains over time. Similar trend was observed in studies of GxE for SC in Nellore cattle (Chiaia et al., 2015; Lemos et al., 2015; Raidan et al., 2015). However, the estimates of h^2 from those studies vary from 0.32 to 0.74, which was similar or higher than presented in ours (0.33 – 0.48), probably by the absence of adjustment in SC, which may overestimate the h^2.

For all the adjustments evaluated, the variability for the slope was close to zero. This is an indicative of the existence of GxE by scaling effect (Kolmodin et al., 2002). The negative correlation between intercept and slope observed in SC_W and SC_{WH} imply in decrease of the EBV from the worst to the best environment. The effect of direction and magnitude of the $r_{a,b}$ presented above reflects on the positioning of the reaction norms. As expected, upward lines were observed for SC_A, SC_H, and SC_{AH}, while for SC_{AW}, the reaction norms were almost parallels. For all the adjustments, the reaction norms presented some degree of changing in variance over the extreme environments, but few crosses are notable. This was expected since studies of reaction norms for SC found that this trait usually have more parallel reaction norms (Santana Jr et al., 2013; Santana Jr et al., 2015) in comparison to the reaction norms for growth traits such as body weight. However, sensibility to changes in environment is observed, as there is variation on slopes (Falconer, 1990).

When the rank correlation is lower than 0.80 there is an indicative of existence of GxE (Robertson, 1959). Lower correlation between extreme environments is expected, since the differences in management and nutrition tend to be higher between better and worse environments. In our study, for all traits, extreme environments presented correlations lower than 0.80. These results indicated that when an animal is selected for one environment probably it will be not the best for the other one. However, it is important to consider that those results are related to a sample of the top 1% bulls from our dataset. As animals with higher EBV tend to be more sensitive to changes in environment (Ribeiro et al., 2015; Carvalheiro et al., 2019), re-ranking can be expected in this sample of bulls. This result is corroborated by Kolmodin et al. (2002), evaluating ranking correlation for Dutch dairy cattle. The authors observed lower correlations when the best 100 bulls were evaluated, in comparison to the correlation for all dataset.

312 The existence of GxE in SC adjusted for growth traits was evident. The use of RN
313 model to consider the effect of GxE on the genetic evaluation for SC in beef cattle breeding
314 programs is advantageous, since allows to rank bulls according to the environment where their
315 offspring will be raised, choosing the best one to each reality. Considering the differences in
316 nutrition, management, climate, and other environmental factors that affect livestock
317 production in Brazil, choosing the best bull for each environment is advantageous, since their
318 progeny can better express their genetic potential when raised in favorable environments,
319 consequently, increasing profit. However, their performance will not be the same as if they
320 were raised in good environments. So, it is interesting for producers in low EG to choose sires
321 more adapted to challenging environments, because the progeny of those animals can perform
322 relatively well even in unfavorable conditions. On the other hand, farmers in good
323 environments will notice good performance in bulls with high or low breeding values.
324 Nevertheless, the offspring of those sires with low breeding values will not inherit this
325 performance, since it is due to the environment. So, choosing bulls with good breeding values
326 evaluated in environments similar to those where their progeny will be raised is of paramount
327 importance to achieve genetic progress, regardless of the quality of the environment.
328
329 **LITERATURE CITED**
330
331 Ambrosini, D. P., C. H. M. Malhado, R. Martins Filho, and P. L. S. Carneiro. 2016. Interação
332 genótipo x ambiente via modelos de normas de reação para características de crescimento
333 em bovinos Nelore. Pesq. Agropec. Bras. 51:177-186. doi: 10.1590/S0100-
334 204X2016000200010.
335 Auguie, B. 2017. gridExtra: Miscellaneous function for “grid” graphics. R Package Version
336 2.3. Avaliable at: <https://CRAN.R-project.org/package=gridExtra>.
337 Boligon, A. A., J. A. V. Silva, R. C. Sesana, J. C. Sesana, J. B. Junqueira, and L. G.
338 Albuquerque. 2010. Estimation of genetic parameters for body weights, scrotal
339 circumference, and testicular volume measured at different ages in Nellore cattle. J.
340 Anim. Sci. 88:1215–1219. doi:10.2527/jas.2008-1719.
341 Boligon, A. A., P. P. Farias, V. M. Roso, M. L. Santana Jr, A. B. Bignardi, and F. R. P. Souza.
342 2017. Genetic relations and indirect response to selection based on indices for scrotal
343 circumference, visual scores and weight gain in beef cattle. Anim. Prod. Sci. 58:1210-
344 1217. doi: 10.1071/AN16365.

345 Calus, M. P. L., A. F. Groen, and G. de Jong. 2002. Genotype × environment interaction for
346 protein yield in Dutch dairy cattle as quantified by different models. J. Dairy Sci.
347 85:3115–3123. doi: 10.3168/jds.S0022-0302(02)74399-3.
348 Carvalheiro, R., R. Costilla, H. H. R. Neves, L. G. Albuquerque, S. Moore, and B. J. Hayes.
349 2019. Unraveling genetic sensitivity of beef cattle to environmental variation under
350 tropical conditions. Genet. Sel. Evol. 51:29. doi: 10.1186/s12711-019-0470-x.
351 Chiaia, H. L. J., M. V. A. de Lemos, G. C. Venturini, C. Aboujaoude, M. P. Berton, F. B.
352 Feitosa, R. Carvalheiro, L. G. Albuquerque, H. N. de Oliveira, and F. Baldi. 2015.
353 Genotype × environment interaction for age at first calving, scrotal circumference, and
354 yearling weight in Nellore cattle using reaction norms in multitrait random regression
355 models J. Anim. Sci. 93:1503–1510. doi:10.2527/jas2014-8217.
356 Chung, Y. S., S. H. Lee, H-K. Lee, D. Lim, J. van der Werf, and S. H. Lee SH. 2020. THI
357 modulation of genetic and non-genetic variance cComponents for carcass traits in
358 Hanwoo cattle. Front. Genet. 11:576377. doi: 10.3389/fgene.2020.576377.
359 Falconer, D. S. 1990. Selection in different environments: effects on environmental sensitivity
360 (reaction norm) and on mean performance. Genet. Res. 56:57-70.
361 doi:10.1017/S0016672300028883.
362 Falconer, D. S. and T. F. C. Mackay. 1996. Introduction to quantitative genetics. 4th. ed.
363 Pearson, United Kingdom.
364 Grolemund, G. and H. Wickham. 2011. Dates and times made easy with lubridate. J. Stat. Soft.
365 40:1:25. doi: 10.18637/jss.v040.i03.
366 Kolmodin, R., E. Strandberg, P. Madsen, J. Jensen, and H. Jorjani. 2002. Genotype by
367 environment interaction in Nordic dairy cattle studied using reaction norms. Acta Agric.
368 Scand. A. 52:11-24. doi: 10.1080/09064700252806380.
369 Legates, J. E. 1962. Heritability of fat yields in herds with different production levels. J. Anim.
370 Sci. 45:990-993. doi: 10.3168/jds.S0022-0302(62)89540-X.
371 Lemos, M. V. A., H. L. J. Chiaia, M. P. Berton, F. L. B. Feitosa, C. Aboujaoude, G. C.
372 Venturini, H. N. Oliveira, L. G. Albuquerque, and F. Baldi. 2015. Reaction norms for
373 the study of genotype-environment interaction for growth and indicator traits of sexual
374 precocity in Nellore cattle. Genet. Mol. Res. 14:7151-7162. doi:
375 10.4238/2015.June.29.9.
376 Misztal, I., S. Tsuruta, D. Lourenço, Y. Masuda, I. Aguilar, A. Legarra, Z. Vitezica. 2018.
377 Manual for BLUPF90 family of programs. University of Georgia, Athens, GA. Avaliable
378 at: < http://nce.ads.uga.edu/wiki/lib/exe/fetch.php?media=blupf90_all7.pdf>.

379 Nascimento, B. M., G. F. Silva, R. Carvalheiro, R. A Teixeira, and L. T Dias. 2020. Alternative
380 adjustment of scrotal circumference for growth traits in Nellore cattle. R. Bras. Zootec.,
381 49:e20190183. doi: 10.37496/rbz4920190183.
382 Ortiz Peña, C. D., S. A. Queiroz, and L. A. Fries. 2000. Estimação de fatores de correção do
383 perímetro escrotal para idade e peso corporal em touros jovens da raça Nelore. Rev. Bras.
384 Zootec. 29:1667-1675. doi: 10.1590/S1516-35982000000600011.
385 Pégolo, N. T., H. N. Oliveira, L. G. Albuquerque, L. A. F. Bezerra, and R. B. Lôbo. 2009.
386 Genotype by environment interaction for 450-day weight of Nelore cattle analyzed by
387 reaction norm models. Genet. Mol. Biol. 32:281-287. doi: 10.1590/S1415-
388 47572009005000027.
389 Pires, B. C., P. Tholon, M. E. Buzanskas, A. P. Sbardella, J. O. Rosa, L. O. C. Silva, R. A. A.
390 Torres Júnior, D. P. Munari, and M. M. Alencar. 2017. Genetic analyses on bodyweight,
391 reproductive, and carcass traits in composite beef cattle. Anim. Prod. Sci. 57:415–421.
392 doi: 10.1071/AN15458.
393 R Core Team. 2020. R: A language and environment for statistical computing. R Foundation
394 for Statistical Computing, Vienna, Austria.
395 Raidan, F. S. S., J. S. A. Tineo, M. M. Moraes, T. C. Escarce, A. E. M. Araújo, M. M. C.
396 Gomes, H. T. Ventura, and F. L. B. Toral. 2017. Associations among growth, scrotal
397 circumference, and visual score of beef cattle in performance tests on pasture or in
398 feedlots. R. Bras. Zootec. 46:309-316. doi: 10.1590/S1806-92902017000400006.
399 Raidan, F. S. S., T. L. Passafaro, B. O. Fragomeni, L. A. Josahkian, I. G. Pereira, and F. L. B.
400 Toral. 2015. Genotype × environment interaction in individual performance and progeny
401 tests in beef cattle. J. Anim. Sci. 93:920–933. doi:10.2527/jas2014-7983.
402 Ribeiro, S., J. P. Eler, V. B. Pedrosa, G. J. M. Rosa, J. B. S. Ferraz, and J. C. C. Balieiro. 2015.
403 Genotype x environment interaction for weaning weight in Nellore cattle using reaction
404 norm analysis. Liv. Sci. 176:40-46. doi: 10.1016/j.livsci.2015.03.032.
405 Robertson, A. 1959. The sampling variance of the genetic correlation coefficient. Biometrics.
406 15:469-485. doi: 10.2307/2527750.
407 Roso, V. M. and F. S. Schenkel. 2006. AMC – A computer program to assess the degree of
408 connectedness among contemporary groups. Proc. World Cong. Gen. App. Liv. Prod.
409 8:13-18.
410 Santana Jr, M. L., J. P. Eler, F. F. Cardoso, L. G. Albuquerque, and J. B. S. Ferraz. 2013.
411 Phenotypic plasticity of composite beef cattle performance using reaction norms model
412 with unknown covariate. Animal. 7:202-210. doi:10.1017/S1751731112001711.

413 Santana Jr., M. L., J. P. Eler, A. B. Bignardi, A. Menéndez-Buxadera, F. F. Cardoso, and J. B.
414 S. Ferraz. 2015. Multi-trait linear reaction norm model to describe the pattern of
415 phenotypic expression of some economic traits in beef cattle across a range of
416 environments. J. Appl. Genetics. 56:219–229. doi: 10.1007/s13353-014-0242-9.
417 Savick, P. 2014. pspearman: Spearman's rank correlation test. R Package Version 0.3.0.
418 Avaliable at: <https://CRAN.R-project.org/package=pspearman>.
419 Schmidt, P. I., G. S. Campos, V. M. Roso, F. R. P. Souza, and A. A. Boligon. 2019. Genetic
420 analysis of female reproductive efficiency, scrotal circumference and growth traits in
421 Nelore cattle. Theriogenology 128:47-53. doi: 10.1016/j.theriogenology.2019.01.032.
422 Silva, M. R., V. B. Pedrosa, J. C. B. Silva, J. P. Eler, J. D. Guimarães, and L. G. Albuquerque.
423 2011. Testicular traits as selection criteria for young Nellore bulls. J. Anim. Sci.
424 89:2061–2067. doi:10.2527/jas.2010-3525.
425 Terakado, A. P. N., A. A. Boligon, F. Baldi, J. A. II V. Silva, and L. G. Albuquerque. 2015.
426 Genetic associations between scrotal circumference and female reproductive traits in
427 Nelore cattle. J. Anim. Sci. 93:2706–2713. doi:10.2527/jas2014-8817.
428 Tierney, N., D. Cook, M. McBain, and C. Fay. naniar: Data structure, summaries, and
429 visualisation for missing data. R Package Version 0.6.0. Avaliable at:
430 <https://CRAN.R-project.org/package=naniar>.
431 Wickham, H. 2016. ggplot2: Elegant graphics for data analysis. Springer-Verlag, New York,
432 NY.
433 Wickham, H., R. François, L. Henry, and K. Müller. 2021. dplyr: A grammar of data
434 manipulation. R. Package Version 1.0.4. Avaliable at: <https://CRAN.R-
435 project.org/package=dplyr>.

436 Table 1 - Means, standard deviation, minimum, and maximum of the heritability coefficient
437 estimates for scrotal circumference adjusted for age (SC_A), body weight (SC_W), hip height
438 (SC_H), age and body weight (SC_{AW}), age and hip height (SC_{AH}), body weight and hip height
439 (SC_{WH}), in Nellore cattle

Trait	Mean ± Standard deviation	Minimum	Maximum
SC_A	0.34 ± 0.01	0.33	0.39
SC_W	0.36 ± 0.02	0.34	0.48
SC_H	0.36 ± 0.01	0.35	0.42
SC_{AW}	0.36 ± 0.01	0.35	0.45
SC_{AH}	0.35 ± 0.01	0.34	0.38
SC_{WH}	0.36 ± 0.02	0.34	0.48

440

441 Table 2 - Estimates of variance (diagonal), covariance (above diagonal), and correlation (below
442 diagonal) between intercept and slope of Reaction Norm Models for additive effect for the
443 scrotal circumference adjusted for age (SC_A), body weight (SC_W), hip height (SC_H), age and
444 body weight (SC_{AW}), age and hip height (SC_{AH}), body weight and hip height (SC_{WH}) in Nellore
445 cattle

Trait	Coefficient	b0	b1
SC_A	b0 (intercept)	2.39	0.17
	b1 (slope)	0.50	0.05
SC_W	b0 (intercept)	2.15	-0.02
	b1 (slope)	-0.10	0.02
SC_H	b0 (intercept)	2.47	0.08
	b1 (slope)	0.30	0.03
SC_{AW}	b0 (intercept)	2.17	0.01
	b1 (slope)	0.05	0.02
SC_{AH}	b0 (intercept)	2.42	0.15
	b1 (slope)	0.50	0.04
SC_{WH}	b0 (intercept)	2.13	-0.03
	b1 (slope)	-0.11	0.02

446

447 Table 3 - Spearman's correlation among estimated breeding values for scrotal circumference
448 adjusted for age (SC_A), body weight (SC_W), hip height (SC_H), age and body weight (SC_{AW}),
449 age and hip height (SC_{AH}), body weight and hip height (SC_{WH}), across environmental gradients
450 of the top 1% Nellore bulls

Trait	Level of environmental gradient		
		Medium[3]	Low[4]
SC_A	High[2]	0.81[1]	0.36[1]
	Medium[3]	-	0.82[1]
SC_W	High[2]	0.80[1]	0.34[1]
	Medium[3]	-	0.78[1]
SC_H	High[2]	0.81[1]	0.35[1]
	Medium[3]	-	0.79[1]
SC_{AW}	High[2]	0.83[1]	0.45[1]
	Medium[3]	-	0.82[1]
SC_{AH}	High[2]	0.86[1]	0.51[1]
	Medium[3]	-	0.86[1]
SC_{WH}	High[2]	0.79[1]	0.30[1]
	Medium[3]	-	0.76[1]

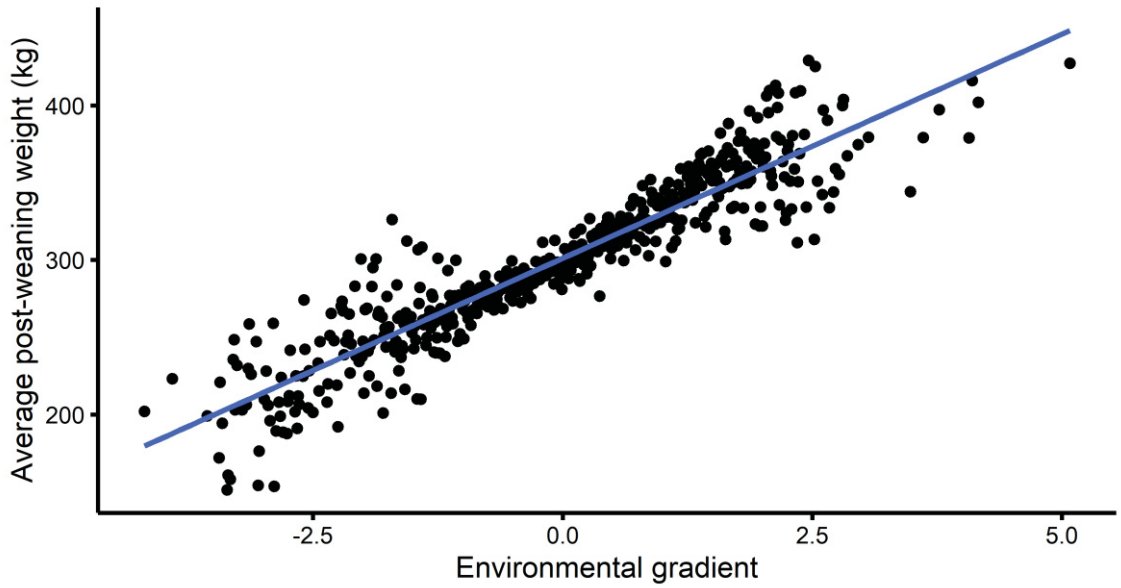
[1]$p<2.2e-16$, Ho: $\rho \neq 0$.

[2]High environmental gradient: EG = -4

[3]Medium environmental gradient: EG = 0

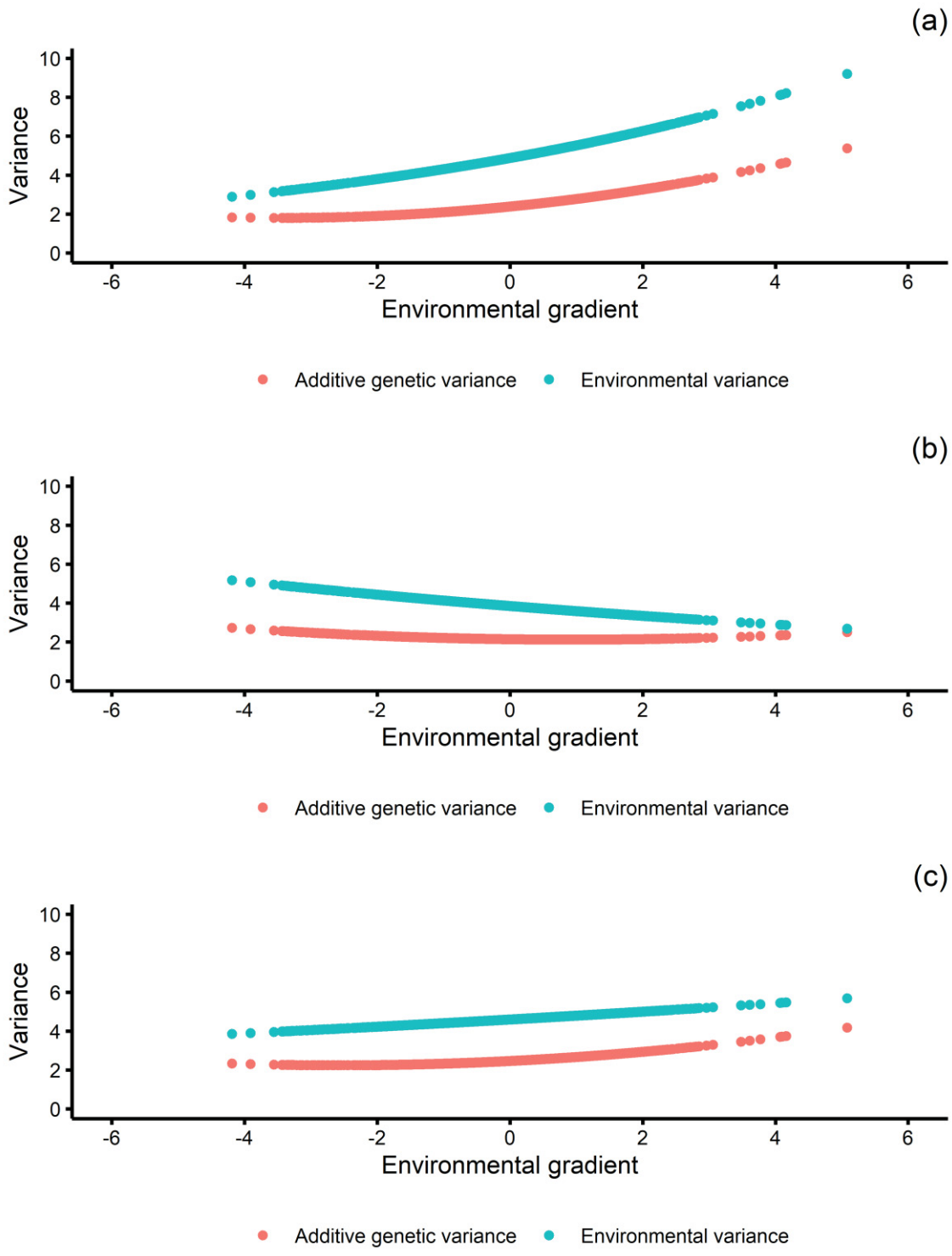
[4]Low environmental gradient: EG = +5

451



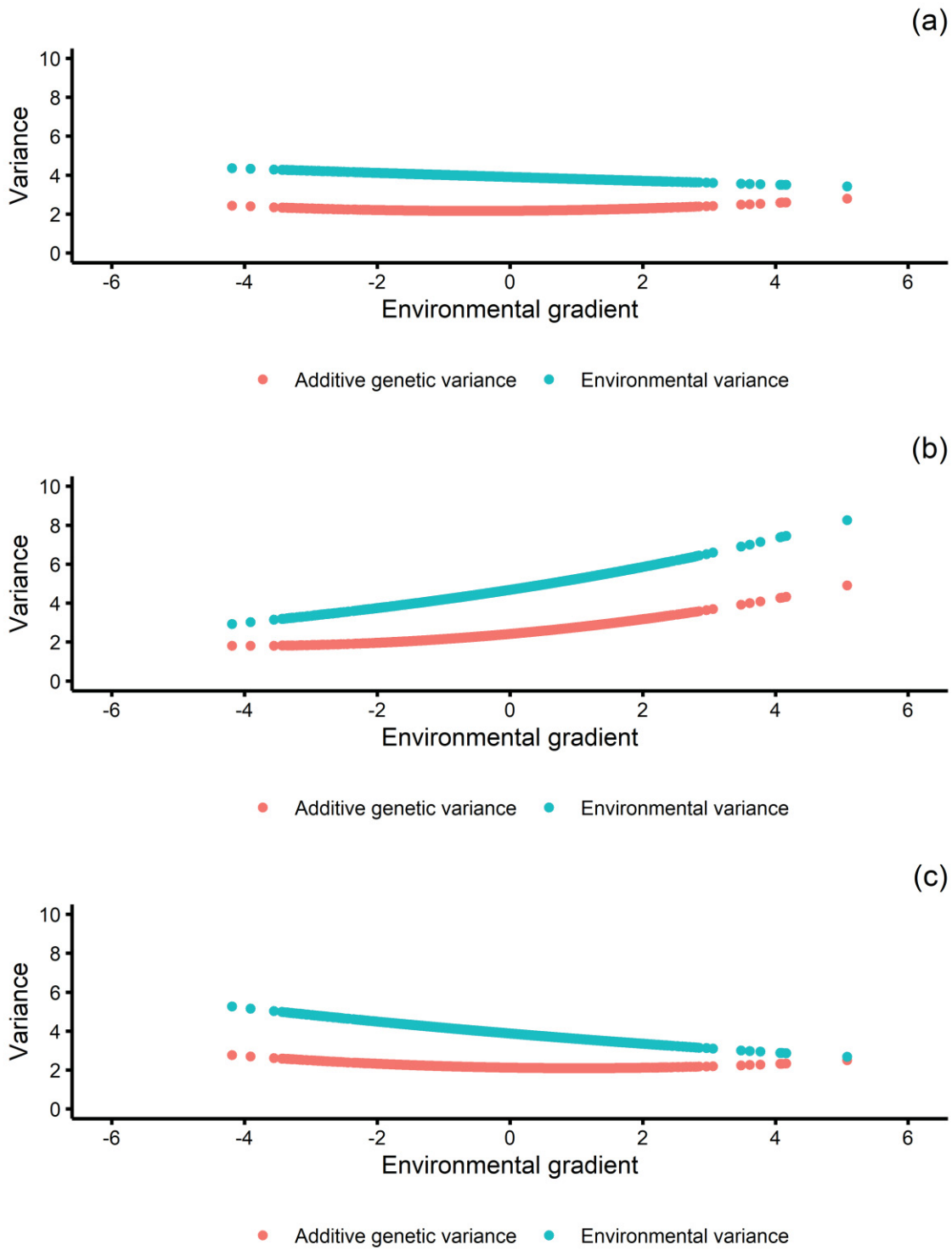
452

453 Figure 9 - Average body weight at post-yearling in Nellore cattle by environmental gradients



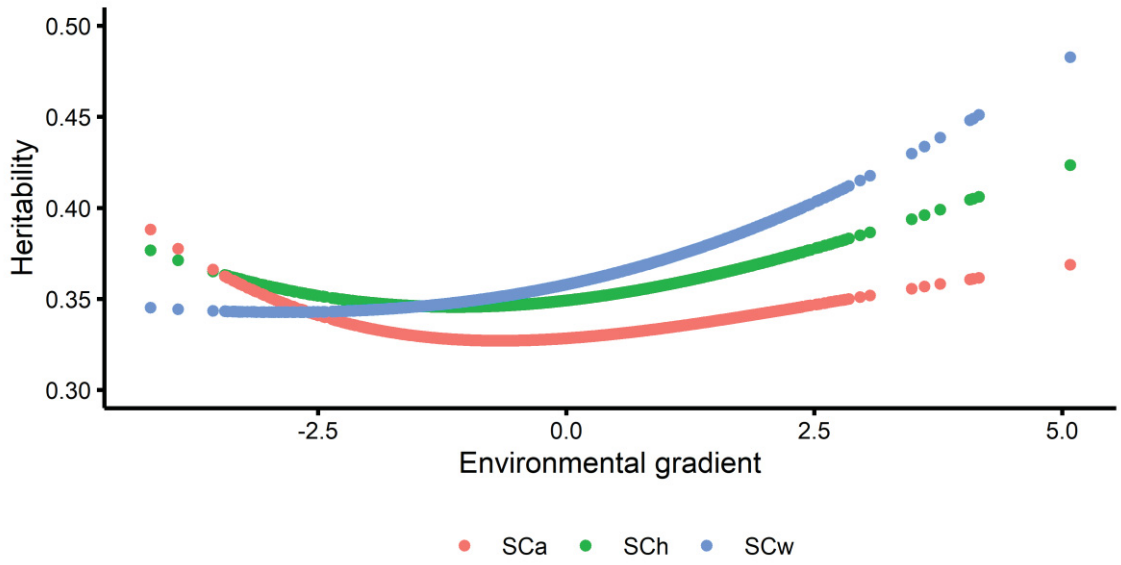
454

455 Figure 10 - Additive genetic and environmental variance estimates over the environmental
456 gradients for scrotal circumference adjusted for age at post-yearling (a), body weight at post-
457 yearling (b), and hip height (c) in Nellore cattle.



458

459 Figure 11 - Additive genetic and environmental variance estimates over the environmental
460 gradients for scrotal circumference adjusted for age and body weight at post-yearling (a), age
461 and hip height at post-yearling (b), and body weight and hip height at post-yearling (c) in
462 Nellore cattle.



463

464 Figure 12 - Heritability coefficient estimates over the environmental gradient for scrotal
465 circumference adjusted for age at post-yearling (SCa), body weight at post-yearling (SCw),
466 and hip height (SCh) in Nellore cattle.

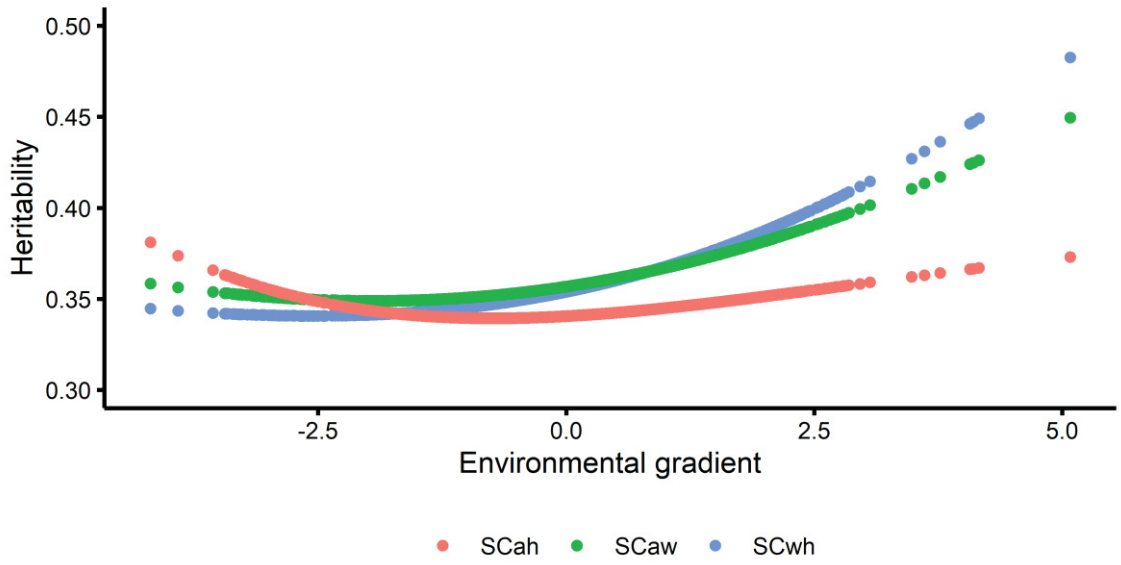


Figure 13 - Heritability coefficient estimates over the environmental gradient for scrotal circumference adjusted for age and hip height at post-yearling (SCah), age and body weight at post-yearling (SCaw), and body weight and hip height at post-yearling (SCwh) in Nellore cattle.

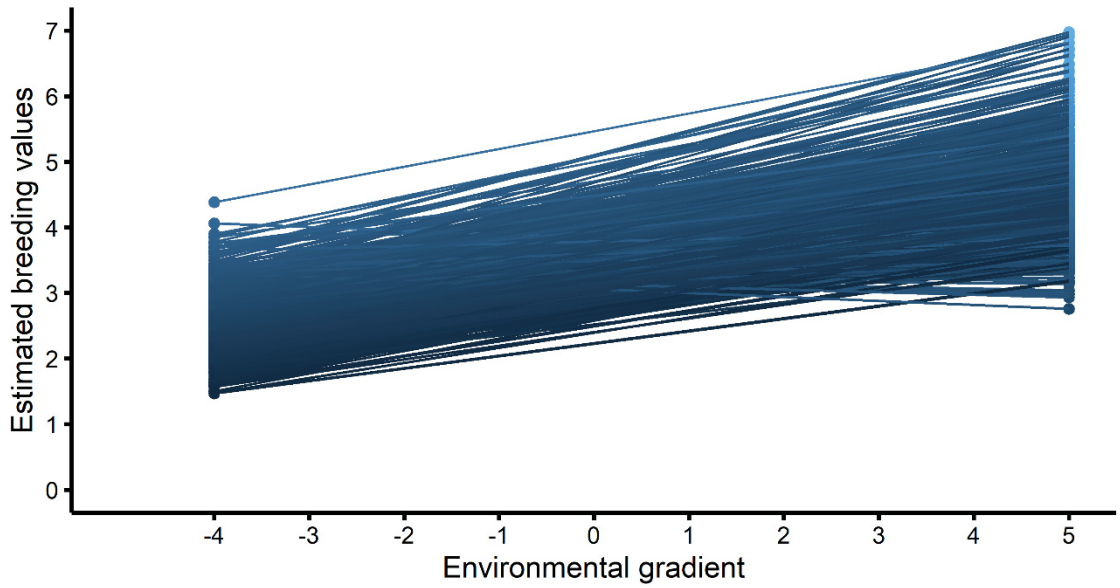
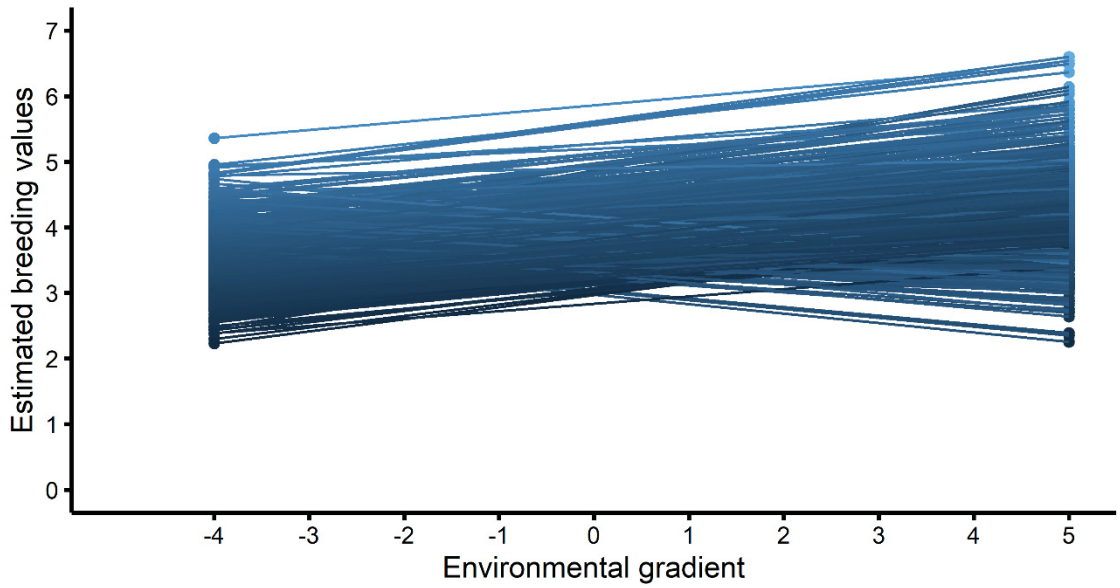


Figure 14 - Reaction norms for the top 1% bulls for scrotal circumference adjusted for age at post-yearling in Nellore cattle.



475

476 Figure 15 - Reaction norms for the top 1% bulls for scrotal circumference adjusted for hip
477 height in Nellore cattle.

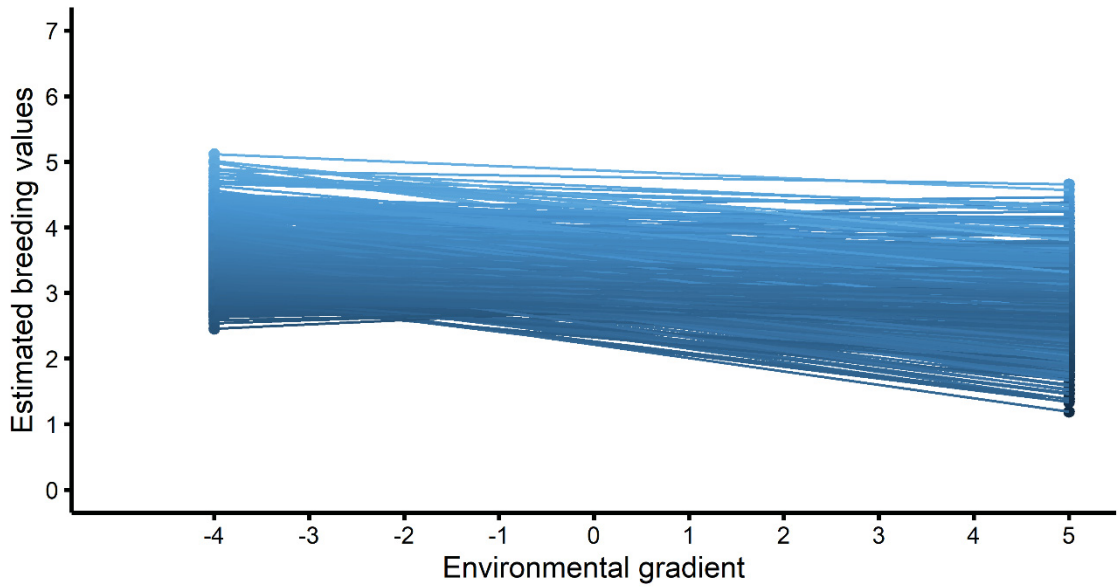
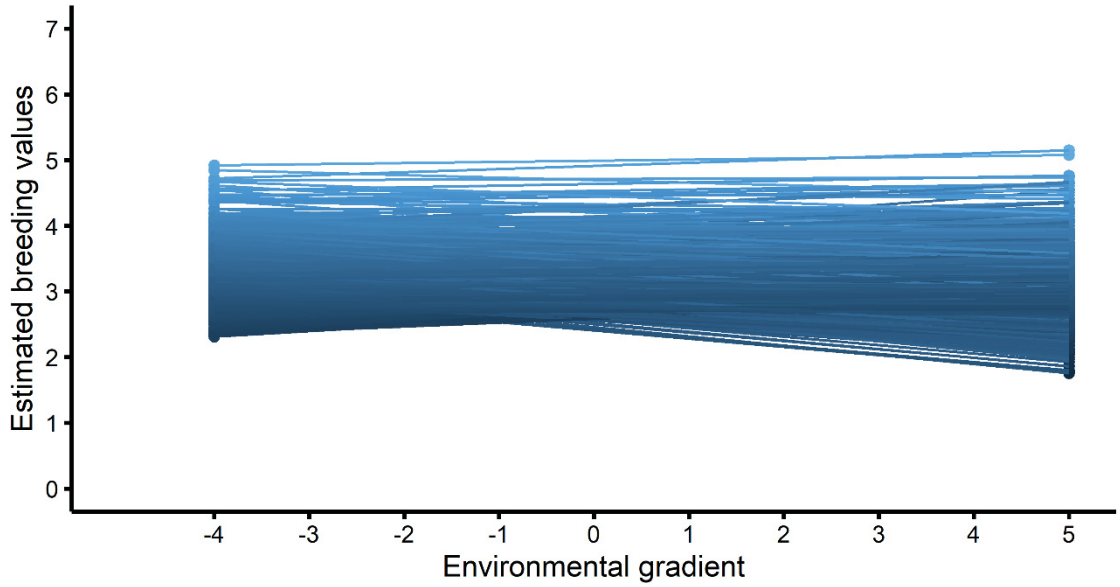


Figure 16 - Reaction norms for the top 1% bulls for scrotal circumference adjusted for body weight at post-yearling in Nellore cattle.

481



482

483 Figure 17 - Reaction norms for the top 1% bulls for scrotal circumference adjusted for age and
484 body weight at post-yearling in Nellore cattle.

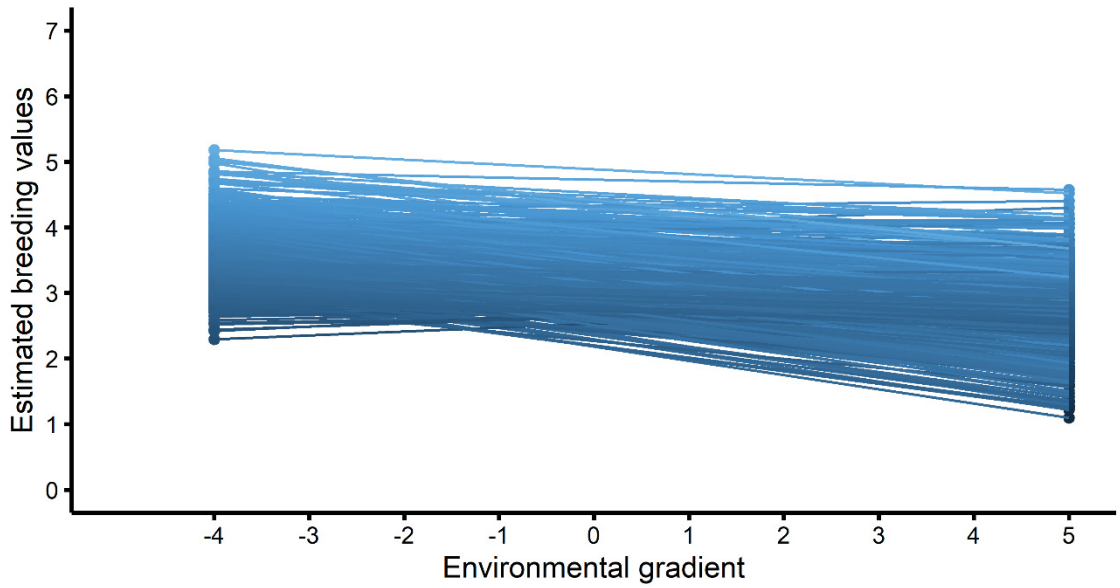


Figure 18 - Reaction norms for the top 1% bulls for scrotal circumference adjusted for body weight and hip height at post-yearling in Nellore cattle.

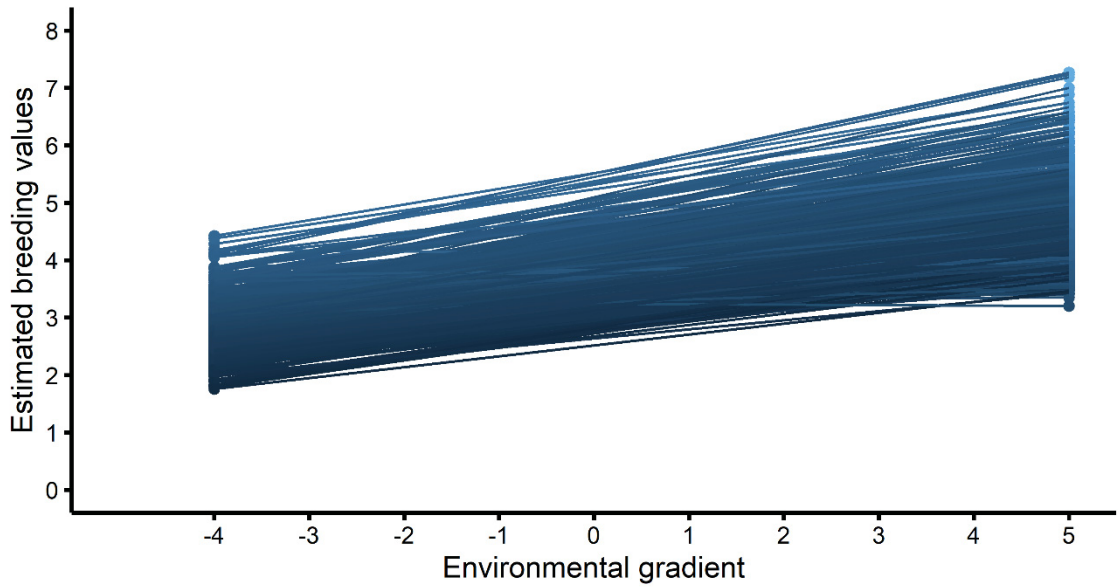


Figure 19 - Reaction norms for the top 1% bulls for scrotal circumference adjusted for age and hip height at post-yearling in Nellore cattle.

4 GENOTYPE X ENVIRONMENT INTERACTION FOR SCROTAL CIRCUMFERENCE ADJUSTED FOR VISUAL SCORES IN NELLORE CATTLE USING REACTION NORMS[4]

Running title: Reaction norms for scrotal circumference

Genotype x environment interaction for scrotal circumference adjusted for visual scores in Nellore cattle using reaction norms [5]

Bárbara M. Nascimento*[6], Roberto Carvalheiro†, Rodrigo de A. Teixeira*, Laila T. Dias*

*Department of Animal Science, Federal University of Paraná, Curitiba, Paraná, Brazil, 80035-060.

†Department of Animal Science, Paulista State University, FCAV, Jaboticabal, São Paulo, Brazil, 14884-900.

[4] This chapter was written according to the Authors Guideline from Journal of Animal Science (Appendix).

[5] The authors acknowledge CAPES for the scholarship granted and Gensys Consultores Associados for the data concession.

[6] Corresponding author.

ABSTRACT

The scrotal circumference (SC) is usually adjusted to age and body weight (BW) to better represent sexual precocity. However, BW may not be adequate to distinguish different biotypes, therefore the use of visual scores evaluation is important to identify morphologically more efficient animals. So, the aim of this study was to evaluate the genotype x environment interaction (GxE) using reaction norms for the SC adjusted for visual scores in Nellore cattle. We analyzed post-yearling measurements of SC adjusted for conformation (SC_C), precocity (SC_P), musculature (SC_M), and double adjusted for conformation and precocity (SC_{CP}), conformation and musculature (SC_{CM}), precocity and musculature (SC_{PM}), age and conformation (SC_{AC}), age and precocity (SC_{AP}), and age and musculature (SC_{AM}) from 170,198 Nellore bulls. The environmental gradient (EG) was obtained by standardizing the solutions of the contemporary groups obtained by Animal Model with BW as the dependent variable. Then, the reaction norms (RN) were determined through a linear random regression model considering the environmental variance as heterogeneous. In addition, the genetic correlation ($r_{a,b}$) between the intercept and the slope of the RN and the Spearman's correlation between the ranking of bulls according to the estimated breeding value (EBV) were estimated. The increase of genetic additive and environmental variances as the EG become more favorable for all traits evaluated indicates that, in those environments, animals have more chance in express their genetic potential. The heritability (h^2) coefficients were moderated and similar for all adjusted SC. For the adjustment of SC for two visual scores the h^2 was practically the same for SC_{CP}, SC_{CM}, and SC_{PM}. The differences in h^2 were more evident for SC_{AC}, SC_A), and SC_{AM}, especially in lower EG. Also, the h^2 increased with the increase at the EG for all traits. Visual scores can be used instead of BW for properly distinguish biotypes, but age seems to be necessary to better adjust SC for growth. The $r_{a,b}$ presented high magnitude for all traits, varying from 0.55 (SC_M) to 0.72 (SC_{AC}). So, in higher EG, the environmental sensitivity increases especially for animals with higher EBV. These results influence on the placement of RN, indicating GxE by changes in variance over the environments. Upward RN were observed for all traits evaluated, with increase in differences among animals as the environment became more favorable. Low Spearman's correlations were estimated between the extreme environments, which indicate existence of GxE and re-ranking. Thereby, GxE is expected for SC adjusted to visual scores only, or together with age, which seems to be more accurate. So, this effect should be included in beef cattle breeding programs. Rank bulls according to their EBV estimated in each possible raising environment is advantageous, since allows to choose the more adequate sire.

Keywords: beef cattle, *Bos indicus*, environmental gradient, estimated breeding values, reproductive traits

INTRODUCTION

In Brazil, beef cattle are raised in a huge variety of production systems that are adapted to local realities of climate, geography, and quality of pasture, among other environmental factors. Thus, the offspring of a sire raised in different environments may not express their genetic potential in the same way. It happens because the phenotype is basically composed by the genotype and environment, but also by the interaction of these two components, known as genotype x environment interaction (GxE). However, usually most of the beef cattle breeding programs does not considered the genotype x environment interaction effect in the estimates of the bull's breeding value.

Reproductive traits tend to be highly influenced by the environment, since their heritability is usually low. However, the most used trait that indicates sexual precocity in beef cattle is the scrotal circumference (SC), which presents moderate estimates of heritability ranging from 0.33 to 0.40 (Terakado et al., 2015; Boligon et al., 2017; Schmidt et al., 2019; Brunes et al., 2020). Moreover, SC is also an extremely important trait because it is genetically correlated with sperm quality (Silva et al., 2013; Carvalho Filho et al., 2020) and growth (Boligon et al., 2017; Raidan et al., 2017).

Because SC is influenced by body development, this trait is usually adjusted, simultaneously, for age and weight so this measure could be an accurate indicator of sexual precocity. However, the body weight may not adequately distinguish different biotypes and therefore, the use of visual scores evaluation is important to identify morphologically more efficient animals, avoiding the selection of late, extreme, or compact cattle (Koury Filho et al., 2010; Vargas et al., 2018).

There are few studies in the literature that evaluate the influence of GxE on the estimates of genetic parameters for SC (Santana Jr. et al., 2013; Chiaia et al., 2015; Lemos et al., 2015; Mota et al., 2020), but none of them consider the adjustment of this measure for any growth trait. Nevertheless, this factor should be taken into account, since this measure is an important trait to identify reproductive efficiency in bulls, especially after the adjustment to remove the effect of growth. Thus, the aim of this study was to evaluate the GxE effect using reaction norms for the SC adjusted for visual scores in Nellore cattle.

MATERIAL AND METHODS

Dataset

Data from 490,324 Nellore males, born between 1984 and 2019 from 10,228 sires and 284,803 dams were used in this paper. Those animals belonged to the historical dataset from "Aliança Nelore" beef cattle breeding program, whose calves were born in the North Region (States of Pará and Tocantins), Northeast Region (State of Bahia), Central-West Region (States of Goiás, Mato Grosso, and Mato Grosso do Sul), Southeast Region (States of Minas Gerais and São Paulo, and South Region (State of Paraná), in Brazil. In this study, were analyzed post-yearling (506.08 ± 53.39 days) measurements of SC after single adjustment for conformation (SC_C), precocity (SC_P), musculature (SC_M), and double adjustment for conformation and precocity (SC_{CP}), conformation and musculature (SC_{CM}), precocity and musculature (SC_{PM}), age and conformation (SC_{AC}), age and precocity (SC_{AP}), and age and musculature (SC_{AM}). The definition of the visual scores were described by Vargas et al. (2018). Three trained evaluators assign scores from 1 to 5 for each trait within the management groups. An intermediate animal is chosen to receive score 3 for all the three traits. Then, the other individuals are evaluated in comparison to this animal. In every group should be identified the good (score 5), the intermediate (score 3), and the worst (score 1) animal, even in homogeneous groups.

The adjustments were performed according to Nascimento et al. (2020). However, in the present study, the linear and the quadratic effects of all traits used in the adjustment were significant. The contemporary groups (CG) were formed by: farm of birth, weaning, and post-yearling, year and season of birth, management group and julian date at weaning and post-yearling.

Data edition

Animals without information for the traits evaluated, or used to create the CG, or with measurements above or below three standard deviations from the average for the evaluated traits were removed from the dataset. CG with less than 15 animals, or less than 10 genetic links among them, verified by the AMC software (Roso and Schenkel, 2006), or with sons of only one sire were also deleted. After edition, the final dataset was comprised of 170,198 males in 4,949 CG.

Environmental descriptor

The study of GxE were performed using the Reaction Norm (RN) model, where the first step was to describe the breeding environments by the estimate of best linear unbiased estimate (BLUE) of the CG through the Animal Model presented as following:

$$Y = X\beta + Za + e$$

where Y is the body weight at post-yearling (BW), β is the vector of fixed effects (CG and linear and quadratic effects of age at post-yearling as covariate), a is the vector of additive genetic effect, represented by animal, X and Z are the incidence matrices of fixed and random effects, respectively, and e is the vector of residual effects.

To determine the environmental gradients (EG), the solutions of the GC obtained in the Animal Model, described previously, were standardized according to the equation:

$$EG = \frac{GC_{sol} - GC_{mean}}{GC_{sd}}$$

where EG is the environmental gradient, GC_{sol} is the solution for each CG obtained by Animal Model, GC_{mean} is the average of the solutions from the CGs, and GC_{sd} is the standard deviation of the solutions from the CG.

Since BW was used to estimate the EG, is expected that higher EG (+4,79) correspond to less challenging environments, i.e. environments where the animals have better conditions to growth. On the other hand, environments with lower EG (-3,66) are more challenging for the animals, so individuals raised in those places tend to be lighter, as presented at Figure 20.

Reaction Norm Model

The second step was to determine the RN model using a Random Regression Model to study GxE. Because animals present only one observation for each adjustment of SC, based on the results reported by Chiaia et al. (2015), we considered a linear model as presented below:

$$Y_{ij} = F_{ij} + \sum_{m=0}^{kb-1} \beta_m \varphi_m(t_{ij}) + \sum_{m=0}^{ka-1} \alpha_{im} \varphi_m(t_{ij}) + e_{ij}$$

where Y_{ij} is the observation of SC adjusted of the sons of the i-th animal in the j-th environment; F_{ij} is the vector of fixed effects (CG); β_m is the average trajectory of the population; t_{ij} is the levels of standardized environments (EG); φ_m is the linear Legendre polynomial; α_{im} is the individual random regression coefficient of direct genetic effect; kb and ka are the order of the correspondent polynomials, fixed in 2 (linear); and e_{ij} is the random residual effect.

The additive genetic variance was obtained using the follow equation:

$$(\mathrm{Var(a)|EG}) = \mathrm{Var}(a_i + b_i \cdot EG) = \sigma_a^2 + \sigma_b^2 \cdot \mathrm{EG}^2 + 2 \cdot \mathrm{EG} \cdot \sigma_{\mathrm{a,b}}$$

where $(\mathrm{Var(a)|EG})$ is the additive genetic variance by EG, $\mathrm{a_i}$ and $\mathrm{b_i}$ are the intercept and slope of the RN model, respectively, σ_a^2 is the additive genetic variance for the intercept, σ_b^2 is the additive genetic variance for the slope, EG is the environmental gradient, as defined before, and $\sigma_{a,b}$ is the covariance between intercept and slope.

Considering that heteroscedastic RN model performs better than homoscedastic model (Carvalheiro et al., 2019), the environmental variance was considered as heterogeneous in this analysis, and was obtained using the following equation:

$$(\mathrm{Var(e)|EG}) = \exp(\mathrm{z_0} + \mathrm{z_1} \cdot \mathrm{EG})$$

where $(\mathrm{Var(e)|EG})$ is the residual variance by EG, exp is the exponential function to transform the values of the residual coefficients, obtained by logarithmic function, $\mathrm{z_0}$ is the intercept of the residual function for SC, $\mathrm{z_1}$ is the slope of the residual function for SC in the RN model, considering heterogeneous residual variance, and EG is the environmental gradient.

The heritability ($\mathrm{h^2}$) estimates were given by the following equation:

$$(\mathrm{h^2|EG}) = \frac{(\mathrm{Var(a)|EG})}{(\mathrm{Var(a)|EG}) + (\mathrm{Var(e)|EG})}$$

where $\mathrm{h^2|EG}$ is the heritability by EG, $\mathrm{Var(a)|EG}$ is the additive genetic variance by EG, and $\mathrm{Var(e)|EG}$ is the residual variance by EG.

The genetic correlation between intercept and slope ($\mathrm{r_{a,b}}$) was given by:

$$r_{a,b} = \frac{\sigma_{a,b}}{\sqrt{\sigma_a^2 \sigma_b^2}}$$

where $r_{a,b}$ is the genetic correlation between intercept and slope, $\sigma_{a,b}$ is the covariance between intercept and slope, σ_a^2 is the additive genetic variance for the intercept, and σ_b^2 is the additive genetic variance for the slope.

The breeding values (EBV) for the bulls in each environment were predicted by following equation:

$$EBV_{i|EG} = b_{0_i} + b_{1_i} \cdot EG$$

where$EBV_{i|EG}$ is the estimated breeding value of the i-th bull in each EG, b_{0_i} is the intercept of the RN for the i-th bull, b_{1_i} is the slope of the RN for the i-th bull, and EG is the environmental gradient. To represent the RN, the top 1% bulls according to general EBV where selected.

Ranking correlation

The Spearman's correlation among EBV for the top 1% bulls previously selected for each trait evaluated was performed to evaluate changes in ranking in high, medium, and low environmental gradients. This analysis was performed using the pspearman function from software R (Savicky, 2014).

Data edition, statistics, and additional analysis were performed using software R (R Core Team, 2020) and the following packages: lubridate (Grolemund and Wickham, 2011), naniar (Tierney et al., 2020), and dplyr (Wickham et al., 2021). Also, the figures presented were developed and constructed through ggplot2 (Wickham, 2016) and gridExtra packages (Auguie, 2017) from the same software. The analysis from Animal Model and Random Regression Model were performed by AIREMLF90 software (Misztal et al., 2018).

RESULTS

Figure 20 presents an increase in the average BW in each EG. At the lowest EG (-3.66), animals presented, on average, 202.04 kg, at the EG equal to zero, the average BW was 295.97 kg, and in the higher EG (+4.79), the average BW was 427.30 kg.

The additive genetic and environmental variances estimates increased as the environment becomes better SC adjusted for one visual score (Figure 21a-c). Also, the magnitude of both additive genetic and environmental variances was similar for the three traits evaluated.

When the simultaneous adjustments for two visual scores (SC_{CP}, SC_{CM}, and SC_{PM}) were proceeded, the estimates also increased as the environment becomes more favorable (Figure 22a-c). The same behavior was observed on the additive genetic and environmental variances for SC adjusted for age and visual scores simultaneously (Figure 23a-c).

The h^2 coefficients were moderated and similar for all SC adjusted for a single trait, as presented at Table 4. The highest h^2 was estimated for SC_C, while the lowest was for SC_P. As presented at Figure 24, the behavior of the h^2 over the EG was almost the same for SC_C, SC_P, and SC_M. The lowest h^2 was estimated at the worst environment (-3.66), while the best environment (4.79) had the highest, and almost the same, h^2 for all traits.

Similar h^2 coefficients were observed for SC adjusted for two traits, independently if we considered only visual scores, or age and visual score together (Table 4). There was no difference among the curves of h^2 over the environments for the SC_{CM}, SC_{CP}, and SC_{PM} (Figure 25). Differences in the estimate of h^2 were more evident among SC_{AC}, SC_{AM}, and SC_{AP} (Figure 26), with higher h^2 for SC_{AM}. As the environment became more favorable, differences among h^2 decreased, being nearly the same for SC_{AM} and SC_{AP}.

The $r_{a,b}$ presented high magnitude for all traits, varying from 0.55 to 0.72 (Table 5). Lower estimates were observed for single adjustment of SC for visual scores. When the adjustment was performed using age and visual scores, genetic correlation varies from 0.68 to 0.72.

The reaction norms according to the EBV of bulls for SC_C showed an increase in the variance from the worst to the best environment, indicating existence of GxE (Figure 27). The same trend was observed for SC_P (Figure 28) and SC_M (Figure 29), where it was also visible changing in rank when some animals were evaluated in extreme environments. So, it is possible to affirm the existence of GxE for all those traits.

For double adjustments, the reaction norms model showed for SC_{CP} (Figure 30), SC_{CM} (Figure 31), and SC_{PM} (Figure 32) an increase of variance in the EBV from the worst to the best environment. When the SC was adjusted for age and visual scores, the difference between the EBV in the worst and the best environments were more evident (Figures 33 to 35). The change in ranking of classification could be observed when those animals were evaluated in a bad and in a good environment for SC_{CM} and SC_{PM} (Figures 31 and 32, respectively). Those are evidences of existence of GxE when SC adjusted to visual scores is considered as selection criteria.

Rank correlation among the best 1% bulls according to the EBV for each trait is presented at Table 6. Spearman correlation among high, medium, and low environments vary from 0.14 to 0.90. The lower correlations were observed, in general, between the extreme environments, but, for SC_P, SC_M, SC_{CM}, and, SC_{PM}, correlation between high and medium environments and low and medium environments were smaller than 0.80.

DISCUSSION

The increase of BW from the lowest to the highest EG was expected, since the solutions of the GC for BW were used to determine the EG. So, it is possible to assume that the lowest EG represented more challenging environments, where animals tend to be lighter than those were raised in highest EG. Considering the existence of favorable genetic correlation between BW and SC (Pires et al., 2017; Schmidt et al., 2019), it is also expected that animals raised in better environments present larger SC than those raised in worst environments.

In Brazil, beef cattle breeding programs consider the additive genetic variance as being the same for all environments (Lemos et al., 2015). However, our study demonstrated changes in those parameters according to the environment where the animals are raised. This change in variances over the EG indicates the existence of GxE. The increase of genetic additive and environmental variances as the EG become more favorable indicates that, in those environments, animals have more chance in express their genetic potential. This trend is observed in studies with growth traits (Pégolo et al., 2009; Oliveira et al., 2018; Carvalheiro et al., 2019) and SC (Chiaia et al., 2015, Lemos et al., 2015) in Nellore cattle. However, it is important to notice that different groups of genes may be acting on these traits depending on the raising environment, since there is a change in genetic variance with the change of environment. Thus, improvements in environment may not guarantee better performance. Moreover, some animals may perform better in more favorable environment while others perform better in less favorable environments. So, choosing sire according to the environment may be more interesting as a way to increase genetic gain.

The estimate h^2 indicates that all traits will respond to direct selection. For the double adjustment of SC using only visual scores (SC_{CP}, SC_{CM}, SC_{PM}) the h^2 was practically the same for all traits. The differences in the estimates were more evident when the adjustment considers visual score and age simultaneously (SC_{AC}, SC_{AP}, SC_{AM}), especially in lower EG. However, all traits presented an increase of h^2 as the environment become more favorable. Literature report that animals raised in better environments have more opportunity to express their genetic potential because of higher h^2 (Lemos et al., 2015; Ambrosini et al., 2016). It is important to point out that just providing a better environment for the animal is not interesting from the point of view of genetic improvement because the gain in performance coming from environmental factors will not be inherited by the following generations. So again, choosing the most suitable sire for the breeding environment can lead to greater genetic gains over time. Chiaia et al. (2015), evaluating GxE in Nellore cattle, found that h^2 vary from 0.51 to 0.67 for unadjusted SC over the EG. The absence of adjustment can overestimate the estimative of h^2,

since growth effects may act on the measure, causing a bias in the phenotype, which will reflect in phenotypic variance and, consequently, in h^2. The adjustment of SC for age and visual scores presented lower h^2 in comparison to the adjustment using one or two visual scores. Visual scores can be used instead of body weight for better distinguish biotypes, but it seems that age should be considered in the adjustment in order to better adjust SC for growth.

For all the adjustments evaluated, the variability for the slope was close to zero. This is an indicative of the existence of GxE by scaling effect (Kolmodin et al., 2002). According to Ribeiro et al. (2015), high $r_{a,b}$ is advantageous since indicates increase in production in better environments. However, any decrease in quality of the environment will directly impact on performance of selected bulls, i.e., those with higher EBV. Considering Brazilian conditions, where most of the beef cattle farms are located in regions with well-defined rainy and dry seasons, lack of forage during the dry season will impact the performance of bulls with higher EBV more severely than bulls with lower EBV. Therefore, the farmer should, for example, provide supplementation for those animals to reduce production losses. Probably select a bull with higher EBV when evaluated in more challenging environments, but with slightly lower EBV in better environments is more adequate in these situations, since it will perform well in good environment, but less impact on its performance will be observed in low environment.

The high $r_{a,b}$ influences on the placement of reaction norms because it indicates GxE by changes in variance over the environments, but with little changes in ranking (Kolmodin et al., 2002; Santana Jr et al., 2015). Upward reaction norms were observed for all traits evaluated, with increase in differences among animals as the environment became more favorable, corroborating the results obtained in $r_{a,b}$. Santana Jr et al. (2013) and Santana Jr et al. (2015), in studies with GxE with Montana and Nellore cattle, respectively, observed more parallel reaction norms for SC, similar to the trend observed in our study. The slope of reaction norm is related to the sensibility of a genotype to changes in environment (Falconer, 1990). As our results showed low slope for all traits evaluated, reaction norms close to parallelism were expected.

However, when rank correlation between the worst and the best environments were evaluated, values less than 0.80 were observed for all traits, which is an indicative of GxE and re-ranking (Robertson, 1959). This result may be due to selection of top 1% bulls for this evaluation. As animals with higher EBV tend to be more sensitive to changes in environment (Ribeiro et al., 2015; Carvalheiro et al., 2019), re-ranking can be expected in this sample of bulls. This result is corroborated by Kolmodin et al. (2002), evaluating ranking correlation for

Dutch dairy cattle. The authors observed smaller correlations when the best 100 bulls were evaluated, in comparison to the correlation for all dataset.

Thereby, GxE is expected for SC adjusted to visual scores, especially when the age is also considered. So, this effect should be included in beef cattle breeding programs. Rank bulls according to their EBV estimated in each possible raising environment would be ideal to farmers to choose the more adequate sires to their reality. However, considering the difficulty of creating a rank for the huge possibility of environments in countries with large territorial extension, grouping the farms in high, medium, and low environmental level would allow choosing of sires more suitable to each reality.

LITERATURE CITED

Ambrosini, D. P., C. H. M. Malhado, R. Martins Filho, and P. L. S. Carneiro. 2016. Interação genótipo x ambiente via modelos de normas de reação para características de crescimento em bovinos Nelore. Pesq. Agropec. Bras. 51:177-186. doi: 10.1590/S0100-204X2016000200010.

Auguie, B. 2017. gridExtra: Miscellaneous function for "grid" graphics. R Package Version 2.3. Avaliable at: <https://CRAN.R-project.org/package=gridExtra>.

Boligon, A. A., P. P. Farias, V. M. Roso, M. L. Santana Jr, A. B. Bignardi, and F. R. P. Souza. 2017. Genetic relations and indirect response to selection based on indices for scrotal circumference, visual scores and weight gain in beef cattle. Anim. Prod. Sci. 58:1210-1217. doi: 10.1071/AN16365.

Brunes, L. C., F. Baldi, M. F.O. e Costa, R. B. Lobo, F. B. Lopes, and C. U. Magnabosco. 2020. Genetic-quantitative analysis for reproductive traits in Nellor cattle selected for sexual precocity. Anim. Prod. Sci. 60:896–902. doi: 10.1071/AN19120.

Carvalheiro, R., R. Costilla, H. H. R. Neves, L. G. Albuquerque, S. Moore, and B. J. Hayes. 2019. Unraveling genetic sensitivity of beef cattle to environmental variation under tropical conditions. Genet. Sel. Evol. 51:29. doi: 10.1186/s12711-019-0470-x.

Carvalho Filho, I., D. B. D. Marques, C. F. Campos, J. D. Guimarães, S. E. F. Guimarães, P. S. Lopes, F. Fonseca e Silva, R. Veroneze. 2020. Genetic parameters for fertility traits in Nellore bulls. Reprod. Dom. Anim. 55:38–43. doi: 10.1111/rda.13578.

Chiaia, H. L. J., M. V. A. de Lemos, G. C. Venturini, C. Aboujaoude, M. P. Berton, F. B. Feitosa, R. Carvalheiro, L. G. Albuquerque, H. N. de Oliveira, and F. Baldi. 2015. Genotype × environment interaction for age at first calving, scrotal circumference, and

348 yearling weight in Nellore cattle using reaction norms in multitrait random regression
349 models J. Anim. Sci. 93:1503–1510. doi:10.2527/jas2014-8217.
350 Falconer, D. S. 1990. Selection in different environments: effects on environmental sensitivity
351 (reaction norm) and on mean performance. Genet. Res. 56:57-70.
352 doi:10.1017/S0016672300028883.
353 Grolemund, G. and H. Wickham. 2011. Dates and times made easy with lubridate. J. Stat. Soft.
354 40:1:25. doi: 10.18637/jss.v040.i03.
355 Kolmodin, R., E. Strandberg, P. Madsen, J. Jensen, and H. Jorjani. 2002. Genotype by
356 environment interaction in Nordic dairy cattle studied using reaction norms. Acta Agric.
357 Scand. A. 52:11-24. doi: 10.1080/09064700252806380.
358 Koury Filho, W., L. G. Albuquerque, S. Forni, J. A. II V. Silva, M. J. Yokoo, and M. M.
359 Alencar. 2010. Estimativas de parâmetros genéticos para os escores visuais e suas
360 associações com peso corporal em bovinos de corte. R. Bras. Zootec. 39:1015-1022. doi:
361 10.1590/S1516-35982010000500011.
362 Lemos, M. V. A., H. L. J. Chiaia, M. P. Berton, F. L. B. Feitosa, C. Aboujaoude, G. C.
363 Venturini, H. N. Oliveira, L. G. Albuquerque, and F. Baldi. 2015. Reaction norms for
364 the study of genotype-environment interaction for growth and indicator traits of sexual
365 precocity in Nellore cattle. Genet. Mol. Res. 14:7151-7162. doi:
366 10.4238/2015.June.29.9.
367 Misztal, I., S. Tsuruta, D. Lourenço, Y. Masuda, I. Aguilar, A. Legarra, Z. Vitezica. 2018.
368 Manual for BLUPF90 family of programs. University of Georgia, Athens, GA. Avaliable
369 at: < http://nce.ads.uga.edu/wiki/lib/exe/fetch.php?media=blupf90_all7.pdf>.
370 Mota, L. F. M., G. A. Fernandes Jr, A. C. Herrera, D. C. B. Scalez, R. Espigolan, A. F. B.
371 Magalhães, R. Carvalheiro, F. Baldi, and L. G. Albuquerque. 2020. Genomic reaction
372 norm models exploiting genotype x environment interaction on sexual precocity
373 indicator traits in Nellore cattle. Anim. Gen. 51:210-223. doi: 10.1111/age.12902.
374 Nascimento, B. M., G. F. Silva, R. Carvalheiro, R. A Teixeira, and L. T Dias. 2020. Alternative
375 adjustment of scrotal circumference for growth traits in Nellore cattle. R. Bras. Zootec.,
376 49:e20190183. doi: 10.37496/rbz4920190183.
377 Oliveira, D. P., D. A. L. Lourenco, S. Tsuruta, I. Misztal, D. J. A. Santos, F. R. de Araújo Neto,
378 R. R. Aspilcueta-Borquis, F. Baldi, R. Carvalheiro, G. M. F. de Camargo, L. G.
379 Albuquerque, and H. Tonhati. 2018. Reaction norm for yearling weight in beef cattle
380 using single-step genomic evaluation. J. Anim. Sci. 96:27–34. doi: 10.1093/jas/skx006.

Pégolo, N. T., H. N. Oliveira, L. G. Albuquerque, L. A. F. Bezerra, and R. B. Lôbo. 2009. Genotype by environment interaction for 450-day weight of Nelore cattle analyzed by reaction norm models. Genet. Mol. Biol. 32:281-287. doi: 10.1590/S1415-47572009005000027.

Pires, B. C., P. Tholon, M. E. Buzanskas, A. P. Sbardella, J. O. Rosa, L. O. C. Silva, R. A. A. Torres Júnior, D. P. Munari, and M. M. Alencar. 2017. Genetic analyses on bodyweight, reproductive, and carcass traits in composite beef cattle. Anim. Prod. Sci. 57:415–421. doi: 10.1071/AN15458.

R Core Team. 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Raidan, F. S. S., J. S. A. Tineo, M. M. Moraes, T. C. Escarce, A. E. M. Araújo, M. M. C. Gomes, H. T. Ventura, and F. L. B. Toral. 2017. Associations among growth, scrotal circumference, and visual score of beef cattle in performance tests on pasture or in feedlots. R. Bras. Zootec. 46:309-316. doi: 10.1590/S1806-92902017000400006.

Ribeiro, S., J. P. Eler, V. B. Pedrosa, G. J. M. Rosa, J. B. S. Ferraz, and J. C. C. Balieiro. 2015. Genotype x environment interaction for weaning weight in Nellore cattle using reaction norm analysis. Liv. Sci. 176:40-46. doi: 10.1016/j.livsci.2015.03.032.

Robertson, A. 1959. The sampling variance of the genetic correlation coefficient. Biometrics. 15:469-485. doi: 10.2307/2527750.

Roso, V. M. and F. S. Schenkel. 2006. AMC – A computer program to assess the degree of connectedness among contemporary groups. Proc. World Cong. Gen. App. Liv. Prod. 8:13-18.

Santana Jr, M. L., J. P. Eler, F. F. Cardoso, L. G. Albuquerque, and J. B. S. Ferraz. 2013. Phenotypic plasticity of composite beef cattle performance using reaction norms model with unknown covariate. Animal. 7:202-210. doi:10.1017/S1751731112001711.

Santana Jr., M. L., J. P. Eler, A. B. Bignardi, A. Menéndez-Buxadera, F. F. Cardoso, and J. B. S. Ferraz. 2015. Multi-trait linear reaction norm model to describe the pattern of phenotypic expression of some economic traits in beef cattle across a range of environments. J. Appl. Genetics. 56:219–229. doi: 10.1007/s13353-014-0242-9.

Savick, P. 2014. pspearman: Spearman's rank correlation test. R Package Version 0.3.0. Avaliable at: <https://CRAN.R-project.org/package=pspearman>.

Schmidt, P. I., G. S. Campos, V. M. Roso, F. R. P. Souza, and A. A. Boligon. 2019. Genetic analysis of female reproductive efficiency, scrotal circumference and growth traits in Nelore cattle. Theriogenology 128:47-53. doi: 10.1016/j.theriogenology.2019.01.032.

415 Silva, M. R., V. B. Pedrosa, J. C. Borges-Silva, J. P. Eler, J. D. Guimarães, and L. G.
416 Albuquerque. 2013. Genetic parameters for scrotal circumference, breeding soundness
417 examination and sperm defects in young Nellore bulls. J. Anim. Sci. 91:4611–4616.
418 doi:10.2527/jas2012-6067.
419 Terakado, A. P. N., A. A. Boligon, F. Baldi, J. A. II V. Silva, and L. G. Albuquerque. 2015.
420 Genetic associations between scrotal circumference and female reproductive traits in
421 Nelore cattle. J. Anim. Sci. 93:2706–2713. doi:10.2527/jas2014-8817.
422 Tierney, N., D. Cook, M. McBain, and C. Fay. naniar: Data structure, summaries, and
423 visualisation for missing data. R Package Version 0.6.0. Avaliable at:
424 <https://CRAN.R-project.org/package=naniar>.
425 Vargas, G., F. S. Schenkel, L. F. Brito, H. H. R. Neves, D. P. Munari, A. A. Boligon, R.
426 Carvalheiro. 2018. Unravelling biological biotypes for growth, visual score and
427 reproductive traits in Nellore cattle via principal component analysis. Livest. Sci. 217:37-
428 43. doi: 10.1016/j.livsci.2018.09.010.
429 Wickham, H. 2016. ggplot2: Elegant graphics for data analysis. Springer-Verlag, New York,
430 NY.
431 Wickham, H., R. François, L. Henry, and K. Müller. 2021. dplyr: A grammar of data
432 manipulation. R. Package Version 1.0.4. Avaliable at: <https://CRAN.R-
433 project.org/package=dplyr>.

Table 4 - Means, standard deviation, minimum, and maximum of the heritability coefficient estimates for scrotal circumference adjusted for conformation (SC_C), precocity (SC_P), musculature (SC_M), conformation and precocity (SC_{CP}), conformation and musculature (SC_{CM}), precocity and musculature (SC_{PM}), age and conformation (SC_{AC}), age and precocity (SC_{AP}), age and musculature (SC_{AM}) in Nellore cattle

Trait	Mean ± Standard deviation	Minimum	Maximum
SC_C	0.37 ± 0.02	0.33	0.44
SC_P	0.35 ± 0.03	0.31	0.45
SC_M	0.36 ± 0.03	0.32	0.45
SC_{CP}	0.36 ± 0.03	0.31	0.45
SC_{CM}	0.36 ± 0.03	0.32	0.45
SC_{PM}	0.36 ± 0.03	0.31	0.45
SC_{AC}	0.36 ± 0.02	0.32	0.43
SC_{AP}	0.35 ± 0.03	0.30	0.42
SC_{AM}	0.35 ± 0.02	0.32	0.42

Table 5 - Estimates of variance (diagonal), covariance (above diagonal), and correlation (below diagonal) between intercept and slop of reaction norm models for additive effect for scrotal circumference adjusted for conformation (SC_C), precocity (SC_P), musculature (SC_M), conformation and precocity (SC_{CP}), conformation and musculature (SC_{CM}), precocity and musculature (SC_{PM}), age and conformation (SC_{AC}), age and precocity (SC_{AP}), age and musculature (SC_{AM}) in Nellore cattle

Trait	Coefficient	b0	b1
SC_C	b0 (intercept)	2.28	0.13
	b1 (slope)	0.59	0.02
SC_P	b0 (intercept)	2.25	0.14
	b1 (slope)	0.58	0.02
SC_M	b0 (intercept)	2.30	0.14
	b1 (slope)	0.55	0.03
SC_{CP}	b0 (intercept)	2.34	0.14
	b1 (slope)	0.62	0.02
SC_{CM}	b0 (intercept)	2.36	0.14
	b1 (slope)	0.60	0.02
SC_{PM}	b0 (intercept)	2.26	0.14
	b1 (slope)	0.58	0.03
SC_{AC}	b0 (intercept)	2.24	0.17
	b1 (slope)	0.72	0.03
SC_{AP}	b0 (intercept)	2.17	0.18
	b1 (slope)	0.71	0.03
SC_{AM}	b0 (intercept)	2.22	0.19
	b1 (slope)	0.68	0.03

447 Table 6 - Spearman's correlation among estimated breeding values for scrotal circumference
448 adjusted for conformation (SC_C), precocity (SC_P), musculature (SC_M), conformation and
449 precocity (SC_{CP}), conformation and musculature (SC_{CM}), precocity and musculature (SC_{PM}),
450 age and conformation (SC_{AC}), age and precocity (SC_{AP}), age and musculature (SC_{AM}), across
451 environmental gradients of the top 1% Nellore bulls

Trait	Level of environmental gradient		
		Medium[3]	Low[4]
SC_C	High[2]	0.80[1]	0.37[1]
	Medium[3]	-	0.83[1]
SC_P	High[2]	0.73[1]	0.15[1]
	Medium[3]	-	0.75[1]
SC_M	High[2]	0.74[1]	0.14[1]
	Medium[3]	-	0.73[1]
SC_{CP}	High[2]	0.78[1]	0.29[1]
	Medium[3]	-	0.80[1]
SC_{CM}	High[2]	0.76[1]	0.26[1]
	Medium[3]	-	0.79[1]
SC_{PM}	High[2]	0.77[1]	0.27[1]
	Medium[3]	-	0.82[1]
SC_{AC}	High[2]	0.74[1]	0.16[1]
	Medium[3]	-	0.75[1]
SC_{AP}	High[2]	0.84[1]	0.54[1]
	Medium[3]	-	0.90[1]
SC_{AM}	High[2]	0.78[1]	0.35[1]
	Medium[3]	-	0.84[1]

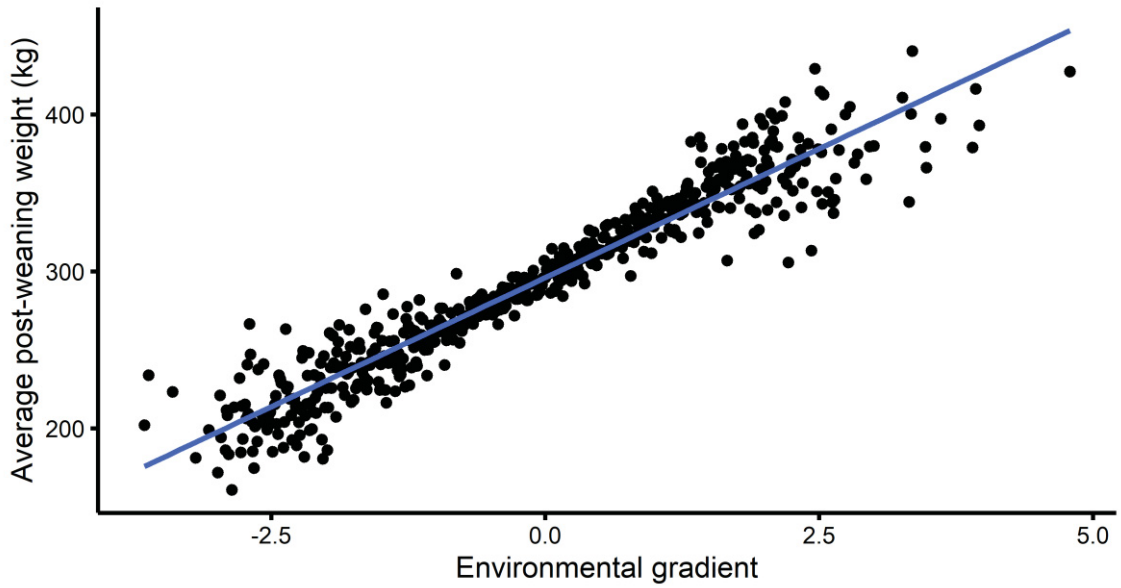
[1]$p<2.2e\text{-}16$, Ho: $\rho \neq 0$.

[2]High environmental gradient: EG = -4

[3]Medium environmental gradient: EG = 0

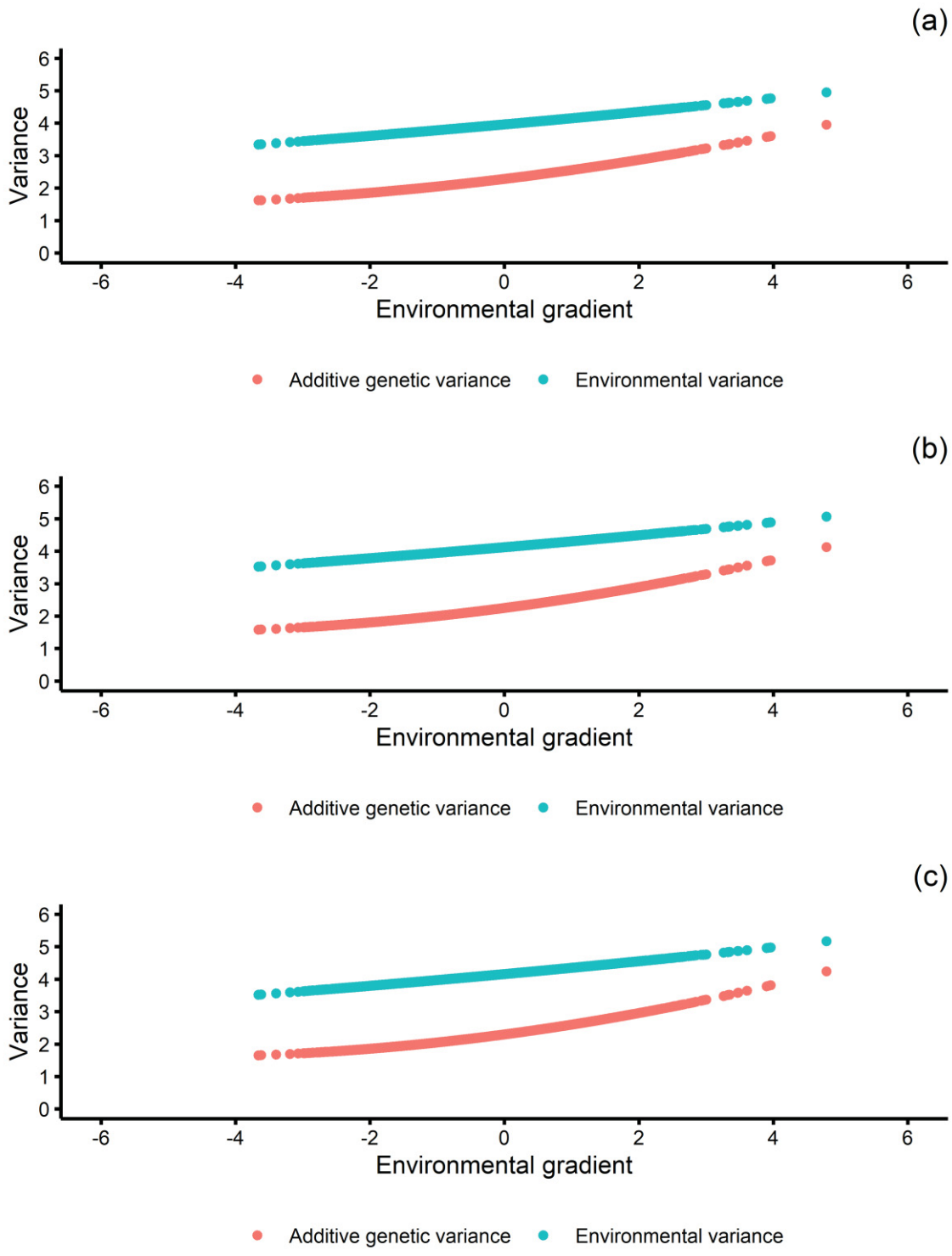
[4]Low environmental gradient: EG = +5

452



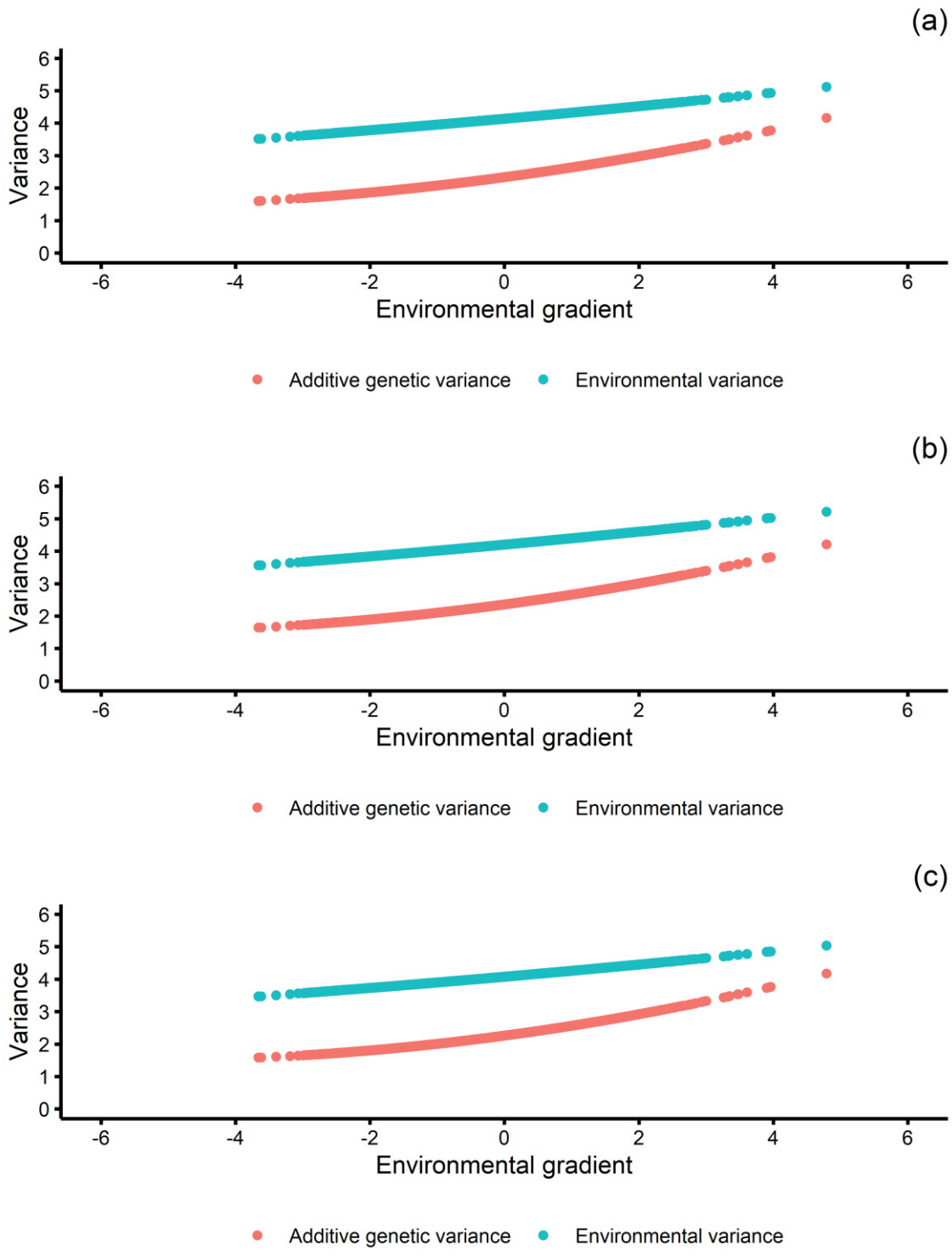
453

454 Figure 20 - Average body weight at post-yearling in Nellore cattle by environmental gradients



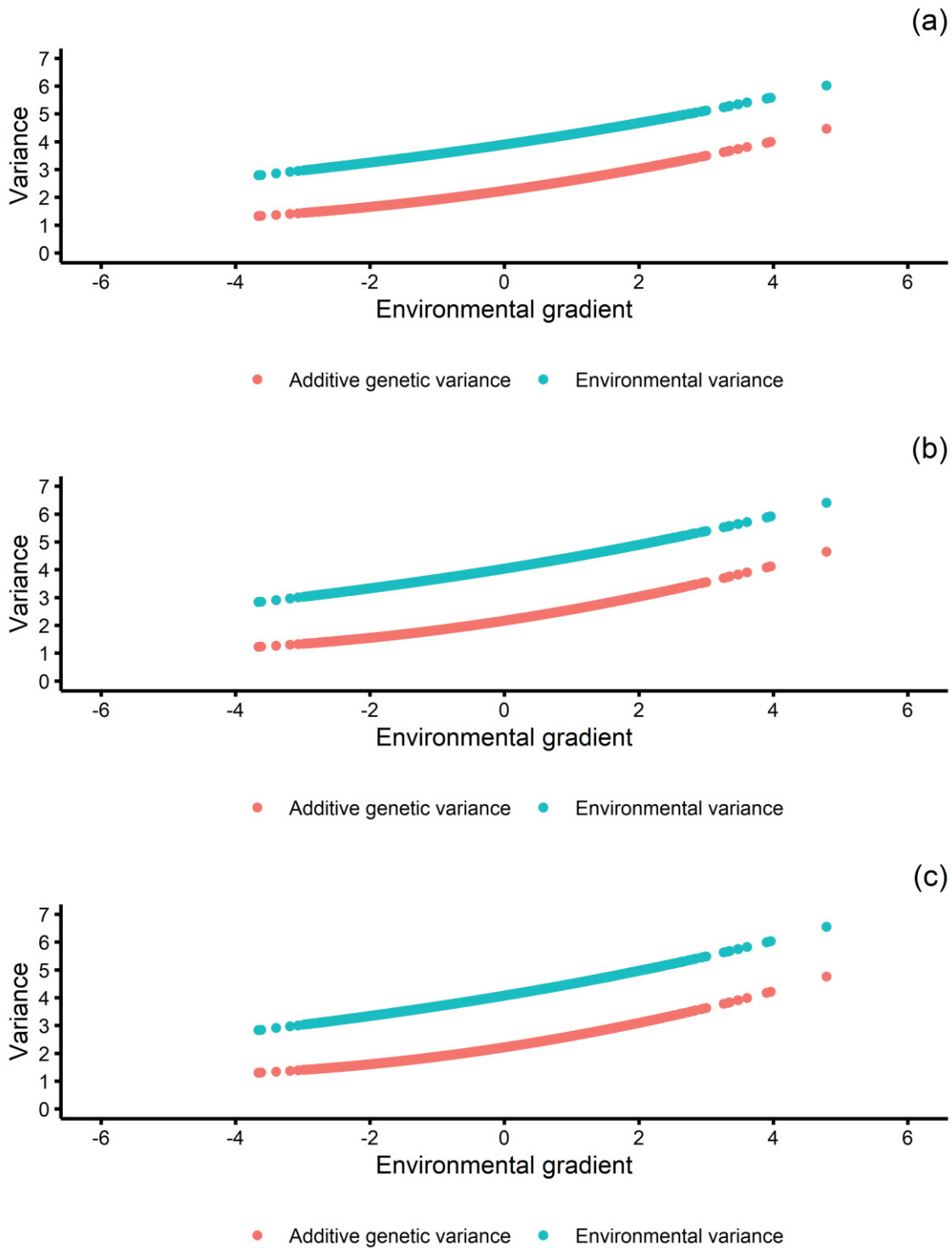
455

456 Figure 21 - Additive genetic and environmental variance estimates over the environmental
457 gradients for scrotal circumference adjusted for conformation (a), precocity (b), and
458 musculature (c) in Nellore cattle.



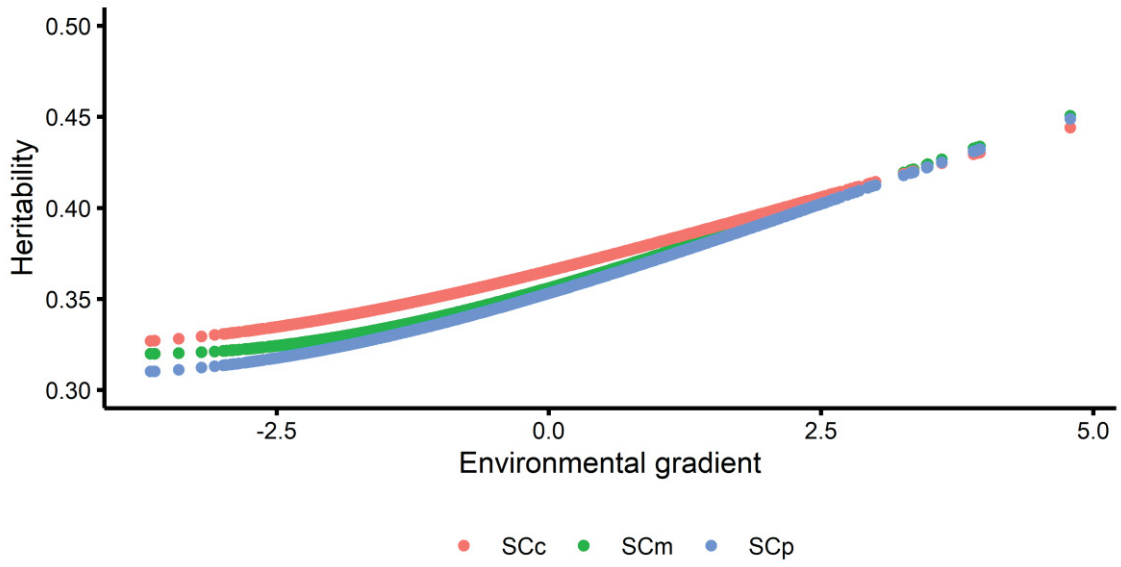
459

460 Figure 22 - Additive genetic and environmental variance estimate over the environmental
461 gradients for scrotal circumference adjusted for conformation and precocity (a), conformation
462 and musculature (b), and precocity and musculature (c) in Nellore cattle.



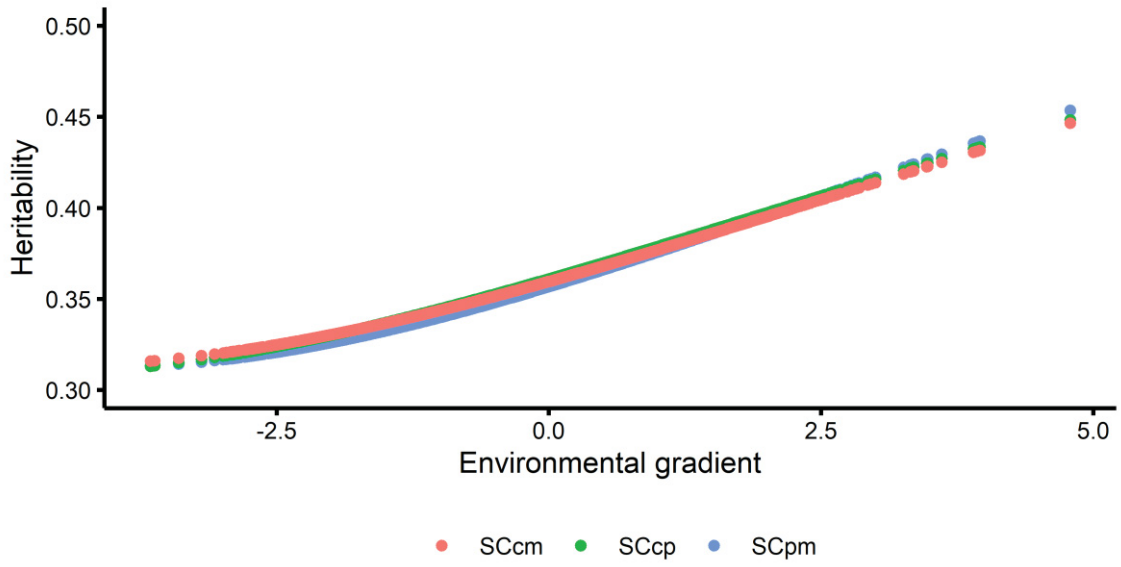
463

464 Figure 23 - Additive genetic and environmental variance estimate over the environmental
465 gradients for scrotal circumference adjusted for age and conformation (a), age and precocity
466 (b), and age and musculature (c) in Nellore cattle.



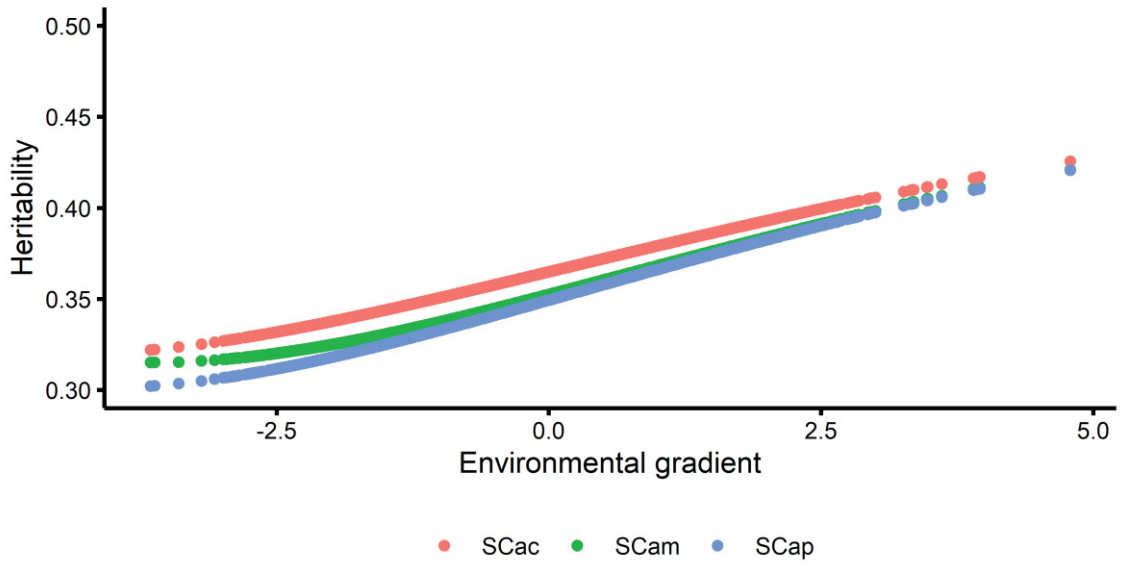
467

468 Figure 24 - Heritability coefficient estimates over the environmental gradient for scrotal
469 circumference adjusted for conformation (SCc), precocity (SCp), and musculature (SCm) in
470 Nellore cattle.



471

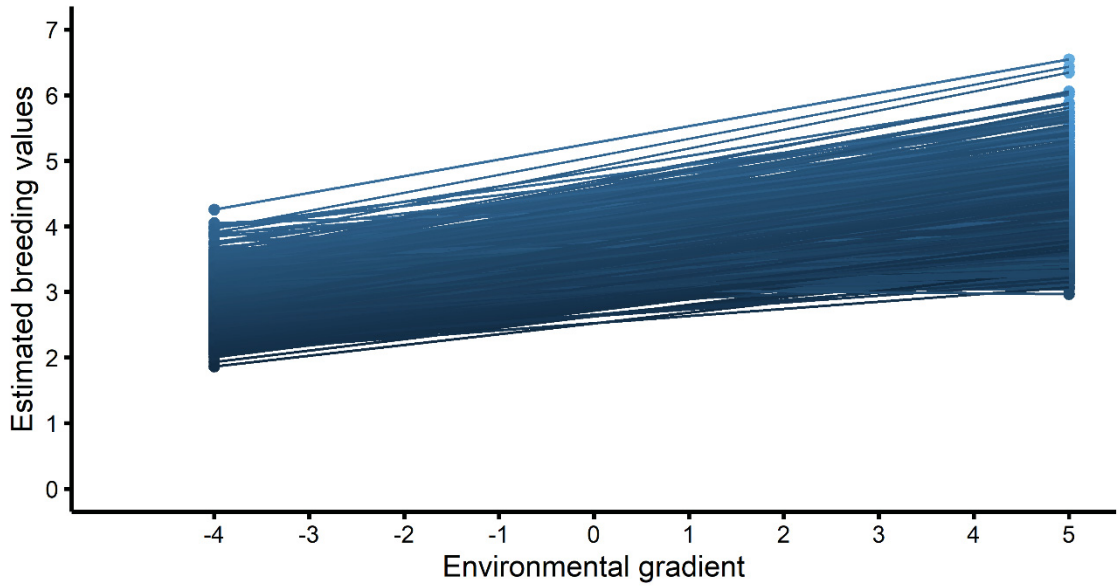
472 Figure 25 - Heritability coefficient estimates over the environmental gradient for scrotal
473 circumference adjusted for conformation and precocity (SCcp), conformation and musculature
474 (SCcm), and precocity and musculature (SCpm) in Nellore cattle.



475

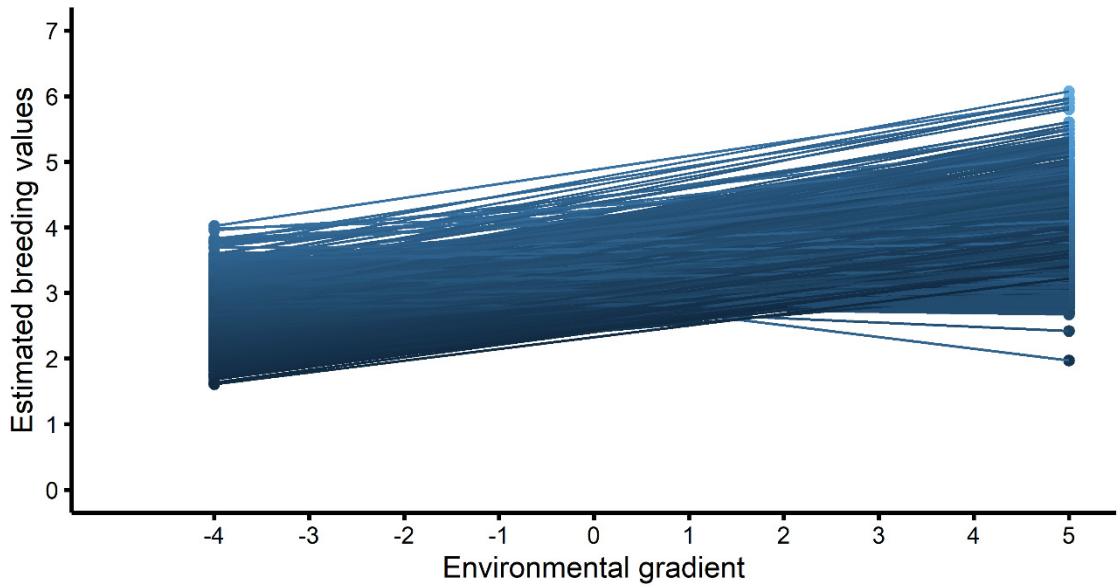
476 Figure 26 - Heritability coefficient estimates over the environmental gradient for scrotal
477 circumference adjusted for age and conformation (SCac), age and precocity (SCap), and age
478 and musculature (SCam) in Nellore cattle.

479



480

481 Figure 27 - Reaction norms for the top 1% bulls for scrotal circumference adjusted for
482 conformation in Nellore cattle.



483

484 Figure 28 - Reaction norms for the top 1% bulls for scrotal circumference adjusted for precocity
485 in Nellore cattle.

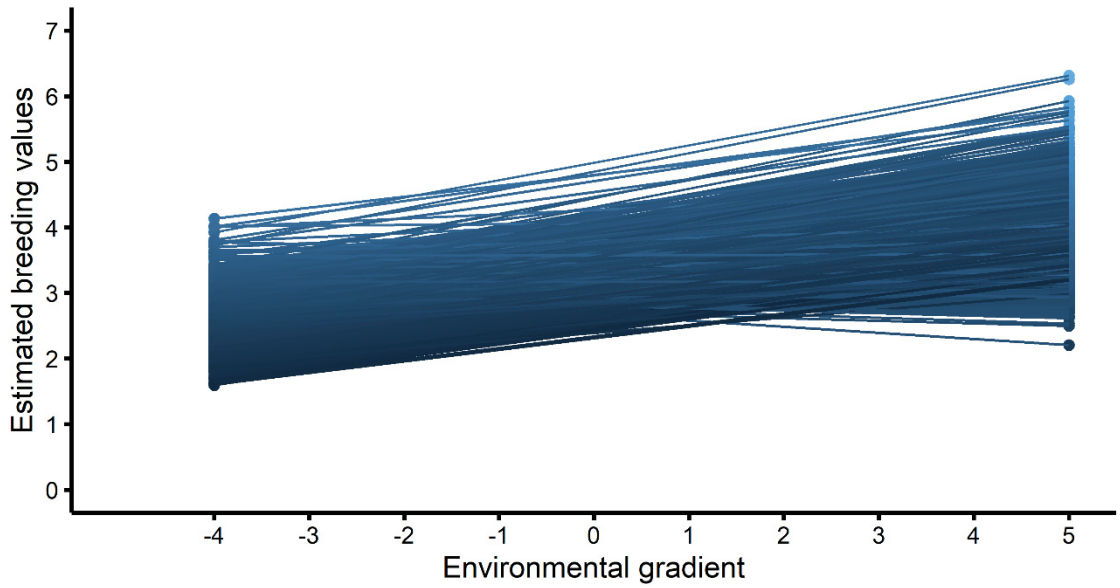
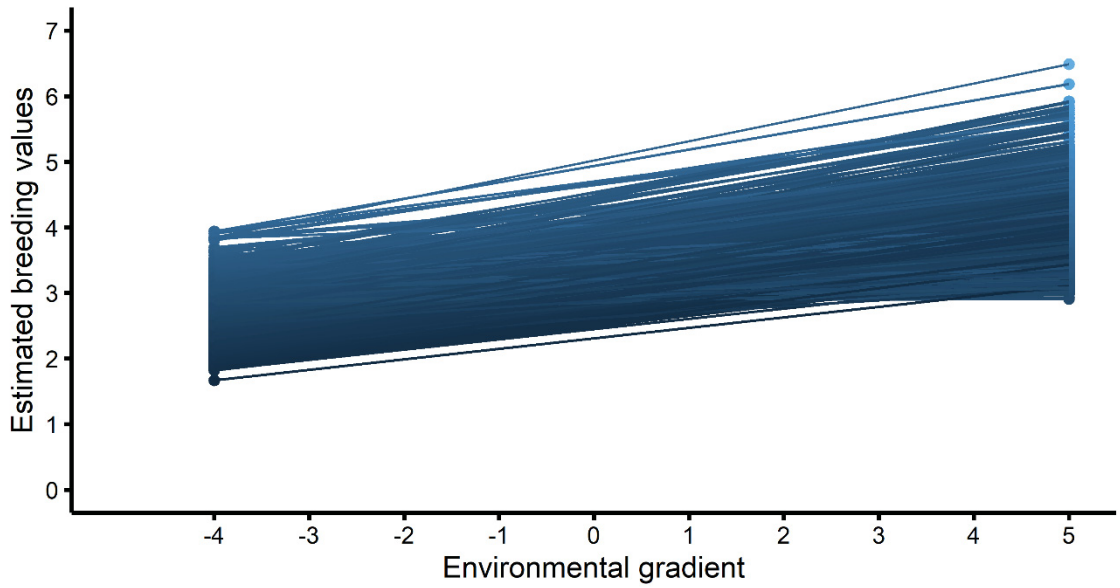
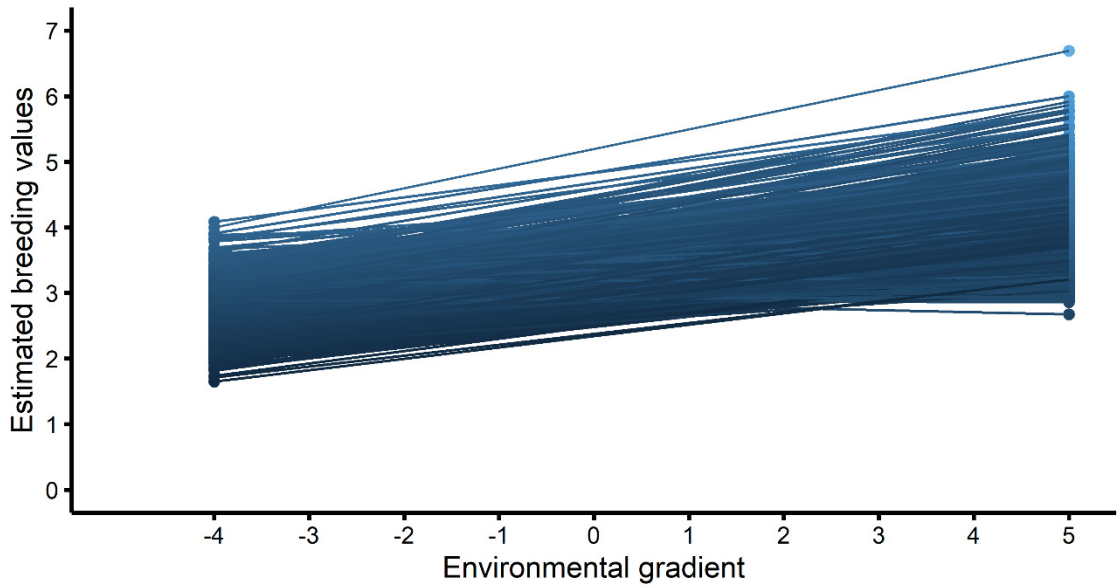


Figure 29 - Reaction norms for the top 1% bulls for scrotal circumference adjusted for musculature in Nellore cattle.



489

490 Figure 30 - Reaction norms for the top 1% bulls for scrotal circumference adjusted for
491 conformation and precocity in Nellore cattle.



492

493 Figure 31 - Reaction norms for the top 1% bulls for scrotal circumference adjusted for
494 conformation and musculature in Nellore cattle.

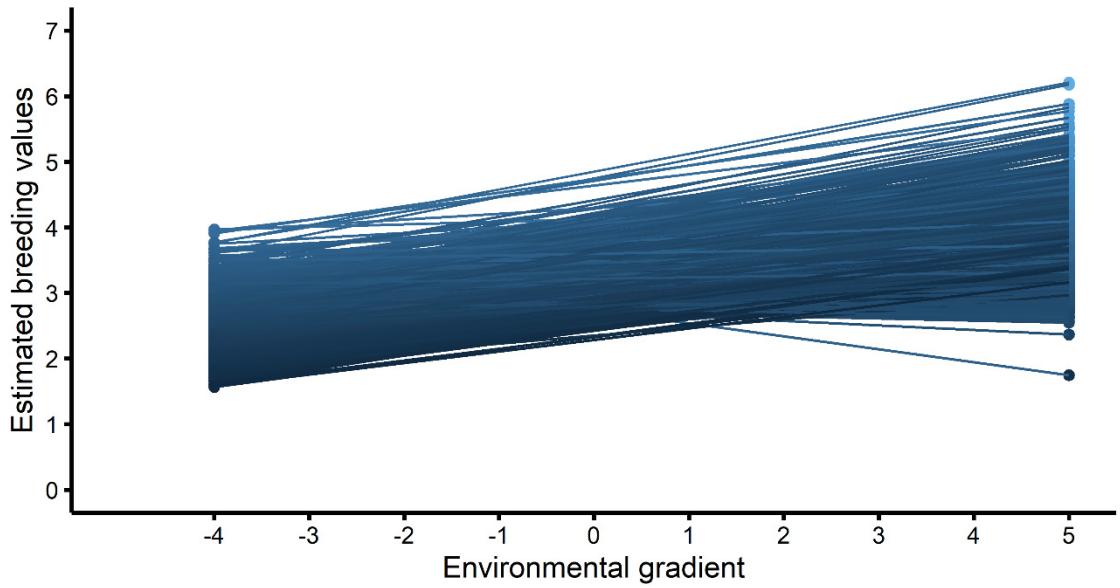
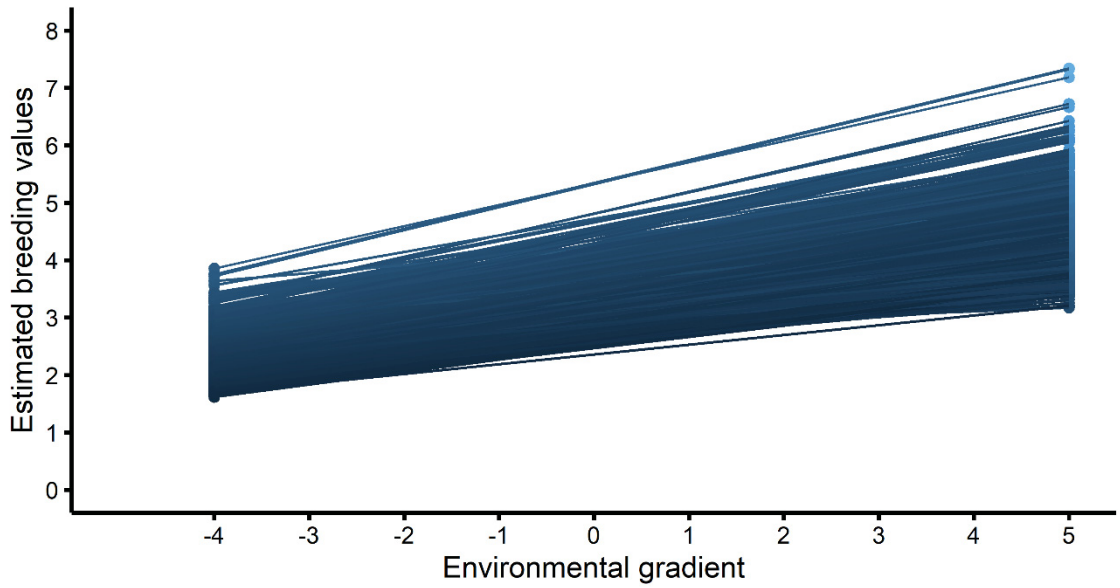
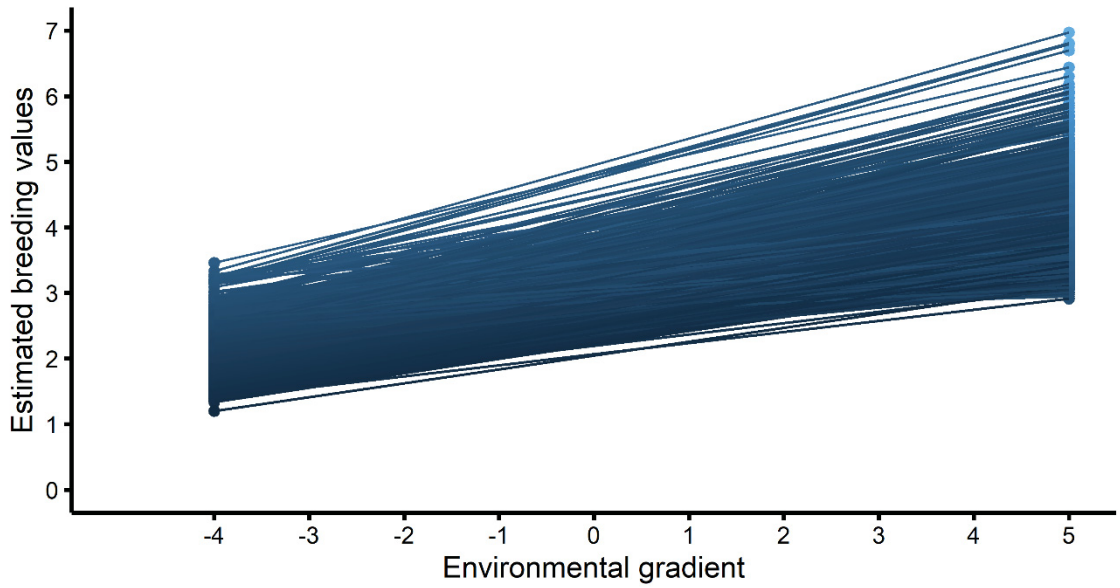


Figure 32 - Reaction norms for the top 1% bulls for scrotal circumference adjusted for precocity and musculature in Nellore cattle.



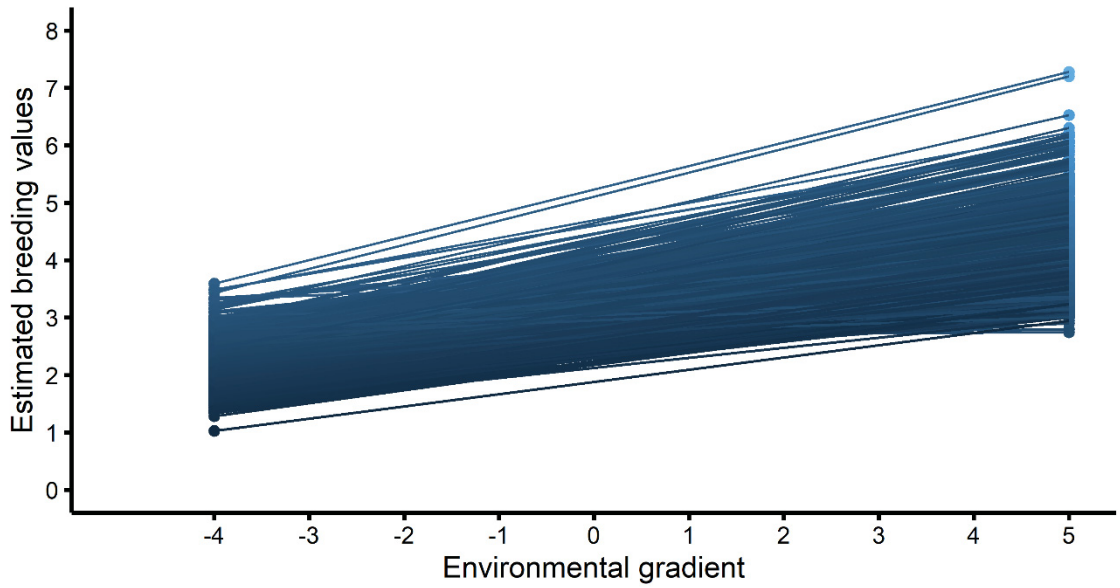
498

499 Figure 33 - Reaction norms for the top 1% bulls for scrotal circumference adjusted for age and
500 conformation in Nellore cattle.



501

502 Figure 34 - Reaction norms for the top 1% bulls for scrotal circumference adjusted for age and
503 precocity in Nellore cattle.



504

505 Figure 35 - Reaction norms for the top 1% bulls for scrotal circumference adjusted for age and
506 musculature in Nellore cattle.

507

5 GENOTYPE X ENVIRONMENT INTERACTION FOR SCROTAL CIRCUMFERENCE USING GENOMIC REACTION NORM MODEL IN BRAHMAN CATTLE[7]

Running head: Genotype x environment interaction in Brahman

Genotype x environment interaction for scrotal circumference using genomic reaction norm model in Brahman cattle[8]

Bárbara M. Nascimento*, Roberto Carvalheiro†, Rodrigo de A. Teixeira*, Laila T. Dias*, Marina R. S. Fortes‡[9]

*Department of Animal Science, Federal University of Paraná, Curitiba, Paraná, Brazil, 80035-060.

†Department of Animal Science, Paulista State University, FCAV, Jaboticabal, São Paulo, Brazil, 14884-900.

‡School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, Queensland, Australia, 4072.

[7] This chapter was written according to the Authors Guideline from Journal of Animal Science (Appendix).

[8] The authors acknowledge CAPES for the scholarship, and The University of Queensland and Beef CRC for the concession of the dataset.

[9] Corresponding author.

ABSTRACT

The aim of this study was to evaluate the genotype x environment interaction (GxE) for scrotal circumference (SC) measured at different ages using pedigree-based and pedigree and genomic-based relationship matrices in Brahman cattle. Data from 1,515 Brahman bulls, from the Cooperative Research Centre for Beef Genetic Technologies (Beef CRC) experimental dataset were used in this study. SC was adjusted to age and body weight measured at 6 months (SC6), 12 months (SC12), 18 months (SC18) and 24 months of age (SC24). Body weight (BW) measured at 6 months (BW6), 12 months (BW12), 18 months (BW18) and 24 months of age (BW24) were used as criteria to describe the environment for SC in each age. All the animals measured were genotyped using medium-density SNP chips ("50k" or "70k" SNP). High-density genotyping with the "770K" chip was performed for another 1,698 animals creating a reference panel with seven breeds that was used for imputation. The environment gradient (EG) was obtained by standardizing the solutions of the contemporary groups obtained by Animal Model with BW as the dependent variable. Then, the reaction norms (RN) were determined through a Random Regression Model. The breeding values (EBV) were estimated using either the inverse of the A matrix (A-1), which considers only pedigree information, or the H matrix (H-1), that combines the pedigree with genetic markers to generate the relationship matrix. The rank correlation was obtained using Spearman's correlation among the EBV estimated for the traits in analysis. For SC6 and SC24, higher estimates of heritability (h^2) were obtained using the A-1, when compared to the estimates observed with the H-1. In those ages, the improvement of the environment decreases the h^2 coefficient. On the other hand, the h^2 for SC12 and SC18 increased as the environment became more favorable, regardless of the matrix used. So, higher h^2 was observed in the best environment at those ages. The RN for SC6 and SC24 estimated using A-1 and H-1 showed a decrease of variance from the worst to the best environment, an indication of existence of GxE. On the other hand, for SC12 and SC18, there were no significant differences between the EBV estimated in the lower and in the higher environments, regardless of the relationship matrix used. These results suggested the absence of GxE on those ages. Spearman's correlation among EBV estimated using A-1 and H-1 in different EG were practically equal to unit for all traits evaluated. In our study, there was weak evidence of GxE effect on SC in ages suitable for selection for sexual precocity. Thereby, is important to consider the age when selecting for SC, because evaluate this trait in too young or too old animals may not be adequate to selection objective of sexual precocity. So, consider selection in ages near to puberty is important, thus this trait could be an accurate selection criterion for sexual precocity.

Keywords: *Bos indicus*, cattle breeding, environmental gradient, estimated breeding values, high-density genotypes, reproductive traits

INTRODUCTION

Genotype x environment interaction (GxE) effect is especially important to consider mainly when the animals are raised in countries with a huge environmental diversity like Australia, United States, or Brazil, where the selection candidates are under different managements, pastures, temperatures, humidity. However, the beef cattle genetic evaluations programs usually do not consider the GxE effect to predict the breeding values.

One way to evaluate GxE effect is by Reaction Norm Models (RNM), which describe the environmental sensitivity of a genotype (Falconer and Mackay, 1996; Kolmodin et al., 2002). In this methodology, it is possible to quantify the environments to determine the environmental value and to estimate the breeding value by environmental gradient (EG). So, the mean performance of the animals is used as a proxy for characterizing their environment (Falconer and Mackay, 1996). The mean performance for body weight (BW) is a useful indicator of the environment, as this trait is largely influenced by the quality and quantity of feed available (i.e. the quality of pasture in grazing systems). Many studies estimated GxE for productive traits in beef cattle, such as BW and weight gain (Mattar et al., 2011; Pegolo et al., 2011; Oliveira et al., 2018; Carvalheiro et al., 2019). However, studies that estimate GxE effect for reproductive traits are less common in comparison to the studies evaluating productive traits, despite the importance of those characteristics to the improvement of beef cattle

Scrotal circumference (SC) is widely used as reproductive trait because it is favorable and genetically correlated to spermatic traits (Boligon et al., 2010; Silva et al., 2011) and female reproductive efficiency (Terakado et al., 2015; Pires et al., 2017). SC is also highly heritable comparing to other reproductive traits (Lemos et al., 2015; Pires et al., 2017; Schmidt et al., 2019). In commercial herds, SC is usually evaluated around 18 months of age in Zebu cattle, because it is routine to weigh animals around this age, so this measurement is easily introduced at the farm's routine. Also, puberty in Zebu cattle occurs between 9 and 18 months (Lunstra and Cundiff, 2003; Fortes et al., 2012b; Lima et al., 2013; Menezes et al., 2014; Stafuzza et al., 2020). However, at very young ages, these animals have a large amount of skin in the scrotal region, which makes it difficult to take accurate measurements of scrotal circumference.

Studies using experimental herds could help to determine the most suitable age for using SC as a selection criterion, and also verify the existence of GxE effect over SC. With the increase of the availability of genomic information, it is expected greater accuracy in predicting the breeding values of reproductive traits such as SC. So, study the influence of the use of genomic matrices in GxE may also improve the identification of this effect in different raising environments. Thus, the aim of this study was to evaluate the GxE interaction effect for SC

measured at four different ages using pedigree-based and pedigree and genomic-based relationship matrices in experimental Brahman cattle herd.

MATERIAL AND METHODS

Dataset

Data from 1,515 Brahman bulls born between 2004 and 2010, progeny of 63 sires and 795 dams, belonging to the Cooperative Research Centre for Beef Genetic Technologies (Beef CRC) experimental dataset were used in this study. The animals were raised in the following research stations located in the state of Queensland, Australia: Swans Lagoon Beef Cattle Research Station (SL), latitude 19.62°S, longitude 147.38°E; Toorak Research Station (TK), latitude 21.03°S, longitude 141.80°E; CSIRO Belmont Research Station (BEL), latitude 23.22°S, longitude 150.38°E; Brigalow Research Station (BRG), latitude 24.84°S, longitude 149.80°E. For a full description of animal management and data collection see Burns et al. (2013).

The traits studied were: SC measured at 6 months (SC6), 12 months (SC12), 18 months (SC18) and 24 months (SC24) of age. BW measured at 6 months (BW6), 12 months (BW12), 18 months (BW18) and 24 months (BW24) of age were used as criteria to describe the environment for SC in each age. A full description of these measurements can be found in Burns et al. (2013). In order to better represent sexual precocity, the SC was adjusted simultaneously for each age and body weight, according to the methodology presented by Nascimento et al. (2020).

The contemporary group (CG) was formed by year and month of birth, pre- and post-weaning location, age of dam, and dam's cohort, being cohort the year and pre-weaning location combined. The CG with less than 5 animals and records with 3 standard deviations under or above the mean of the traits evaluated were removed from the dataset. The connectedness among CG was verified by the software AMC (Roso and Schenkel, 2006), and only the CG with at least 10 genetic links among them were considered. Data edition and previous statistics were performed using R software (R Core Team, 2020) and its packages: naniar (Tierney et al., 2020) and dplyr (Wickham et al., 2021), and summary of the final dataset for each trait is presented at Table 7.

Genotypes

1,098 Brahman bulls initially measured were genotyped using medium-density SNP chips. The Animal Genetics Laboratory of the University of Queensland Gatton provided

genotyping services with Illumina Infinium chemistry using the bovine SNP chips with 54,000 ("50K") SNP for most of the bulls. Additional bulls from the same population of Brahman were genotyped with the GeneSeek Genomic Profiler chip (also Illumina Infinium chemistry), which features approximately 78,000 SNP. Duplicated samples were included in both chip assays for quality control. Quality control (QC) was performed within a chip and only SNP with an Illumina GenCall higher than 0.6 were considered for analyses (Bolormaa et al., 2013). The SNPs that mapped to more than one position in the genome or had a call rate lower than 90% or with minor allele frequency smaller than 0.01 were discarded. If a SNP presented no heterozygous bull, in the presence of both homozygous, the SNP was discarded (except for chromosome X). For the genotyping results for the "50K" chip, 50,353 SNP passed the QC presented above in Brahman (Fortes et al., 2012a). In the additional genotyping with the "70K" chip, 68,406 SNP were available after QC (MAF > 0.01, call rate > 90%, and genotype call > 0.60).

High-density genotyping with the "770K" chip was performed for 1,698 animals from seven breed to create a reference panel to be used in accurate genotype imputation (R2 > 0.90) as described by Bolormaa et al. (2013). After QC using the same criteria described above, genotypes for 729,068 SNP were available for 302 Brahman cattle belonging to the reference population.

Genotype imputation

Missing genotypes were resolved for each SNP chip using Beagle (Browning and Browning, 2010) so the complete genotype sets were available for analyses. All 729,068 SNP from the reference panel were used as reference for imputation from either of the medium-density panels to the HD chip. The 302 Brahman animals genotyped with HD were the reference and imputation used 30 iterations of Beagle (Bolormaa et al., 2013). After imputation, allelic frequencies were compared between the "50K" and the "70K" data and SNP that had very different frequencies, for example, which changed from minor alleles to major alleles, were removed from the dataset. Imputed genotypes on all 729,068 SNP were further filtered to exclude sex-chromosome SNPs and exclude SNPs that had a minor allele frequency (MAF) lower than 0.01. After this final filtering, 436,539 SNP were used to inform the genetic relationship matrix H^{-1} as described below.

Environmental descriptor

The best linear unbiased estimates (BLUE) of the CG effects were used to describe the environment. The solutions to the Animal Models were estimated using the software

AIREMLF90 (Misztal et al., 2018). It was considered as fixed the effect of CG and as covariate the linear effect of age when BW was measured, as follows:

$$Y = X\beta + Za + e$$

where Y is the vector of observations (BW6, BW12, BW18, BW24), β is the vector of fixed effects (CG and covariate, respectively for each measure), a is the vector of additive direct genetic coefficients, X and Z are the incidence matrix of the fixed and additive direct genetic effects, respectively, and e is the random residual vector.

To determine the environmental gradient (EG), the solutions for CG were standardized using the equation below:

$$\mathrm{EG} = \frac{\mathrm{CG_{sol}} - \mathrm{CG_{mean}}}{\mathrm{CG_{SD}}}$$

where EG is the environmental gradient; $\mathrm{CG_{sol}}$ is the solution for each CG; $\mathrm{CG_{mean}}$ is the mean of the solutions for all CG; and $\mathrm{CG_{SD}}$ is the standard deviation of the solutions for all CG.

After standardization, the minimum, maximum, and average EG corresponded to the low, high, and medium environment, respectively. Because BW was used as criterion to determine the EG, is expected that animals in the lowest EG are lighter than those raised at the high EG (Figure 36). In short, the low environment tends to be more challenging than the high environment.

Reaction Norm Model

The Reaction Norm (RN) model were obtained by the software AIREMLF90 (Misztal et al, 2018). Linear model was considered based on the study of Chiaia et al. (2015) for SC6, SC12, SC18, and SC24 and is presented below:

$$\mathrm{Y_{ij}} = \mathrm{F_{ij}} + \sum_{\mathrm{m=0}}^{\mathrm{kb-1}} \beta_{\mathrm{m}} \varphi_{\mathrm{m}}(\mathrm{t_{ij}}) + \sum_{\mathrm{m=0}}^{\mathrm{ka-1}} \alpha_{\mathrm{im}} \varphi_{\mathrm{m}}(\mathrm{t_{ij}}) + \mathrm{e_{ij}}$$

where $\mathrm{Y_{ij}}$ is the observation of progeny of the i-th animal in the j-th environment; $\mathrm{F_{ij}}$ is the vector of fixed effects (year of birth and pre- and post-weaning location combined, month

of birth, age of dam, and dam's cohort); β_m is the model of the mean trajectory of the population; t_{ij} are the levels of EG; φ_m is the linear Legendre polynomial; α_{im} is the random regression coefficient for each animal i of the direct additive genetic effect; kb and ka are the order of the correspondent polynomials, fixed in 2 (linear); e_{ij} is the random error effect.

The breeding values were estimated using mixed model equations that consider the inverse of two different relationship matrices: A^{-1} and H^{-1}. The A matrix (A^{-1}) considers only the information of the pedigree, while the H matrix (H^{-1}) combines the pedigree with genetic markers to generate the relationship matrix. H^{-1} can be written as:

$$H^{-1} = A^{-1} + \begin{bmatrix} 0 & 0 \\ 0 & G^{-1} - A_{22}^{-1} \end{bmatrix}$$

where A is the pedigree relationship matrix, A_{22} is the pedigree relationship matrix for the genotyped animals, and G is the genomic relationship matrix.

The additive genetic variance was obtained using the follow equation:

$$(\mathrm{Var(a)|EG}) = \sigma^2_{a\,b_0} + \sigma^2_{a\,b_1} \cdot \mathrm{EG}^2 + 2 \cdot \mathrm{EG} \cdot \sigma_{b_0,b_1}$$

where $(\mathrm{Var(a)|EG})$ are the genetic additive variances given the EG; b_0 and b_1 are the intercept and the slope of the reaction norm, respectively; $\sigma^2_{a\,b_0}$ is the genetic variance component of the intercept; $\sigma^2_{a\,b_1}$ is the genetic variance component of the slope; EG is the environmental gradient; and σ_{b_0,b_1} is the covariance component between the intercept and the slope.

Considering that heteroscedastic reaction normal model performs better than homoscedastic model (Carvalheiro et al., 2019), the environmental variance was considered as heterogeneous in this analysis, and was obtained using the following equation:

$$(\mathrm{Var(e)|EG}) = \exp(z_0 + z_1 \cdot \mathrm{EG})$$

where $(\mathrm{Var(e)|EG})$ are the residual variances given the EG; exp is the exponential function to transform back the residual coefficients, that were obtained using logarithmic function; z_0 is the intercept of the residual function for SC at different ages; z_1 is the slope of

the residual function for SC at different ages in the reaction norm model, considering heterogeneous residual variance; EG is the environmental gradient.

The heritability (h^2) for SC6, SC12, SC18, and SC24 in each environment $(h^2|EG)$ were calculated using the equation:

$$(h^2|EG) = \frac{(Var(a)|EG)}{(Var(a)|EG) + (Var(e)|EG)}$$

where $h^2|EG$ is the heritability by EG, $Var(a)|EG$ is the additive genetic variance by EG, and $Var(e)|EG$ is the residual variance by EG.

The estimated breeding values (EBV) for the bulls in each EG were obtained as follows:

$$EBV_{i|EG} = b_{0_i} + b_{1_i} \cdot EG$$

where $EBV_{i|EG}$ are the estimated breeding values of bull i in each EG; b_{0_i} is the intercept of the RN for bull i; b_{1_i} is the slope of the EN for bull i; EG is the environmental gradient.

Rank correlation

The Spearman's correlation among the EBV estimated by A matrix and H matrix for SC6, SC12, SC18, and SC24 for each EG was used to compare the ranking of the bulls. This analysis was performed by corrplot function (Wei and Simko, 2017) from software R (R Core Team, 2020). Also, the figures presented were developed and constructed through ggplot2 (Wickham, 2016) and gridExtra packages (Auguie, 2017) from the same software.

RESULTS

The lowest mean of BW for each age evaluated was observed for the worst EG, while the highest mean of BW was observed for the best EG, as expected (Figure 36). At Table 8 is presented the average BW for the lowest, the intermediate, and the highest EG. The difference in the BW between the lowest and the highest EG was around 66 kg, 111 kg, 131 kg, and 132 kg for 6 months, 12 months, 18 months, and 24 months, respectively.

For SC6, the additive genetic variance estimates using the A matrix were lower than the values obtained by H matrix (Figure 37a). Using both matrices, we observed that the

estimates of additive variance decreased over the environments for this trait. For the environmental variance (Figure 38a), higher estimates were observed when A matrix was used, in comparison with the values obtained using the H matrix. The estimates of environmental variance increase through the environments, except when using H matrix, where the estimate remains practically the same.

For SC12 (Figure 37b), the additive variance obtained with the A matrix was higher than with H matrix and it increased as the environment improved. The opposite trend was observed for environmental variance (Figure 38b), when the estimate using H matrix was higher than when A matrix was used. For this trait, as the environment becomes more favorable, the environmental variance decreases.

The additive variance obtained for SC18 (Figure 37c) using A matrix was higher than with H matrix and increased as the environment improved. However, the environmental variance estimated using H matrix was higher than when A matrix was used for SC18 (Figure 38c) and decreases as the environment becomes more favorable.

For SC24 (Figure 37d), the additive variance obtained with the A matrix was higher than with H matrix. The values of additive variance decreased over the environments. The estimate of environmental variance using H matrix was higher than that obtained using A matrix (Figure 38d). For SC24, the environmental variance increases through the environments.

As presented in Table 9, for SC6 and SC24, higher estimates of h^2 were obtained using A matrix, comparing to those observed when the H matrix was used. In those ages, the improvement of the environment decreases the h^2 coefficient (Figure 39a and 39d, respectively). On the other hand, the h^2 for SC12 and SC18 increased through the environments, regardless of the matrix used (Figure 39b and 39c, respectively). Because of this, higher h^2 was observed in the best environment at those ages, as shown in Table 9.

Table 10 shows the (co)variance components and genetic correlation between intercept and slope ($r_{a,b}$) for all the analyses performed. The reaction norms for SC6 estimated using A matrix (Figure 40a) and H matrix (Figure 40b) showed a decrease of variance from the worst to the best environment, evidenced by the negative $r_{a,b}$ (Table 10). The negative correlation is an indication of existence of GxE interaction by changing in variance. Similar results were observed for SC24 (Figure 43a-b), also for both relationship matrices. On the other hand, for SC12 (Figure 41a-b) and SC18 (Figure 42a-b) there were no difference between the EBV estimated in the lower and in the higher environments, regardless of the relationship

matrix used. For those adjustment, in both matrices, the variance for the slope was close to zero (Table 10), which suggested the absence of GxE interaction at those ages.

Spearman correlation among EBV estimated using A matrix and H matrix in different EG were practically equal to unit for all traits evaluated (Tables 11 and 12).

DISCUSSION

The increase of BW from the lowest to the highest EG was expected, since the solutions of the GC for BW were used to determine the EG. So, it is possible to assume that the lowest EG represented the harsh environments, where animals tend to be lighter than those were raised in highest EG. Considering the existence of favorable genetic correlation between BW and SC (Pires et al., 2017; Schmidt et al., 2019), it is expected that those animals from good environment present higher SC than those were raised in unfavorable environment.

The changes in the additive genetic variance over the EG indicated the existence of GxE effect, as defined by Bowman (1972). It means that, in the best environment, the additive genetic variances were greater than that one on others EG, so the animals were able to express their genetic potential (Lemos et al., 2015). However, it is important to notice that different groups of genes may be acting on these traits depending on the raising environment, since there is a change in genetic variance with the change of environment. Thus, improvements in environment may not guarantee better performance. Moreover, some animals may perform better in less favorable environments.

In our study, the optimal genetic expression could occur in the most challenging (SC6 and SC24) or even in the less challenging environment (SC12 and SC18), depending on when the SC was measured. Those results may indicate that, depending on the environment where the selection will be made, the selection criterion for sexual precocity will not be the same, i.e. according to the environment of selection the SC could be measured in different ages. However, is important to take care with measures at 6 months and 24 months. At the first one, difficulties to precisely measure SC due to little development of scrotum can lead to high error levels. Also, both measurements will not reflect sexual precocity, since they are made out of the range of the age of puberty, which occurs between 9 and 18 months for Zebu cattle (Lunstra and Cundiff, 2003; Fortes et al., 2012b; Lima et al., 2013; Menezes et al., 2014; Stafuzza et al., 2020), and therefore may not be an interesting value when the aim is to increase the Zebu sexual precocity.

The slightly higher estimates of h^2 coefficients obtained when A matrix was used, in comparison to H matrix were also reported by de los Campos et al. (2015), in study with simulated human genotypes. The authors verified lower h^2 obtained using genetic markers-based relationship matrix, comparing to the h^2 of the trait, i.e., without computing genomic relationship matrix. This result may occur because genomic heritability (obtained using genomic information) consider only causal variants that are in linkage disequilibrium with SNP markers, while the usual heritability considers any cause of variation in the estimate, which may overestimate it. Oliveira et al. (2018) did not notice significant differences between estimates of h^2 using A matrix or H matrix for yearling weight in beef cattle, since the estimates overlapped considering their standard deviation. However, Mota et al. (2020), in study of GxE for SC in Nellore cattle observed estimates of h^2 8,14% higher when considering H matrix in comparison to those estimated obtained using A matrix. The authors related the difference in the estimates due to the increase of connectedness among herds with the inclusion of genomic information, which influence on the prediction of genetic relationships and, consequently, on the estimate of h^2.

The direct selection for SC will lead to genetic gain, regardless of the age when the animals were selected. The low environment presented higher h^2 for SC6 and SC24, irrespective of the matrix used in the estimative. However, as those traits are not good indicators of sexual precocity as explained above, caution should be taken when evaluating their use as selection criteria. For SC12 and SC18, with the improvement of the environment, the estimate of h^2 increased. Similar results were observed by Chiaia et al., (2015) evaluating genotype x environment interaction for SC. The authors noticed increase in the h^2 estimates for that trait in Nellore cattle with the improvement of the environment. Thus, better environments allowed the animals to express their genetic potential, increasing the h^2. It is important to point out that just providing a better environment for the animal is not interesting from the point of view of genetic improvement because the gain in performance coming from environmental factors will not be inherited by the following generations. So again, choosing the most suitable sire for the breeding environment can lead to greater genetic gains over time.

In the present study, an indicative of GxE effect by changing in variance was observed for all traits evaluated, being more evident for SC6 and SC24. When extreme environments were compared, it was possible to observe the differences on the variance of the EBV. So, according to Bowman (1972), it was possible to noticed the GxE effect. Santana Jr et al., (2013) and Chiaia et al., (2015) observed the existence of GxE for SC measured at yearling age in beef cattle. The authors expected higher response to selection in environments that were less

restricted. However, in our study, the presence of GxE in SC6 may be due to the fact that the measurement of the SC at 6 months is not precise in Zebu cattle, since excess of skin folds in the scrotal region will influence on the measurement. For SC24, the differences in environment will not increase or decrease sexual precocity, since at that age bulls already reached sexual maturity. So, changes in environment will lead to changes in growth only.

The absence of crossing in RN for SC18 were similar to the results found in literature for SC measured at post-yearling, where studies with Montana cattle (Santana Jr. et al., 2013) and Nellore cattle (Lemos et al., 2015; Santana Jr. et al., 2015) showed almost parallels RN. As mentioned before, studies demonstrated age at puberty from 9 to 18 months for Zebu cattle (Lunstra and Cundiff, 2003; Fortes et al., 2012b; Lima et al., 2013; Menezes et al., 2014; Stafuzza et al., 2020). Those results indicated that the selection for SC around 18 months performed by breeding programs in Zebu cattle is adequate when the objective of selection is sexual precocity. At this age, Brahman cattle has shown higher genetic correlation with percent of normal sperm, progressive motility, and mass activity (Corbet et al., 2013), reinforcing this age as an important indicative of sexual precocity. Thus, the absence of GxE on SC18 is interesting since SC measured at this age is the usual selection criterion for sexual precocity in male. Then, in this case, the best sire will be the same for all environments.

The rank correlation showed that animals selected for SC in the best environment will be the same when the selection is based on the worst environment. This result was expected, since the RN already indicated the absence crossing among then. To be considered GxE, the correlation should be smaller than 0.80 (Robertson, 1959), different from what was observed in our study. Lemos et al. (2015) also noticed that the rank of Nellore cattle considering the EBV estimates for SC analyzed in different environments did not changed. According to the authors, the selected sires should be the same, regardless the environment, which seems to be the case of this study.

In our study, there was weak evidence of GxE effect on SC, regardless of the kinship matrix used. There was no significant contribution of the H matrix on the estimate of breeding values, since the values are close to those obtained using A matrix. Because only one State and four experimental farms in Australia were considered, further studies using Brahman cattle raised in other parts of the country are important to indicate if the absence of GxE is maintained. Furthermore, is important to consider the age when selecting for SC, because evaluate this trait in too young or too old animals may not indicate precisely sexual precocity. Therefore, consider select for SC in ages near to puberty is important, so this trait will be an accurate selection criterion. The absence of GxE in those ages are important considering selection for sexual

382 precocity, since no changes in classification will be observed when the sires are evaluated in
383 different environments.
384
385 **LITERATURE CITED**

386 Auguie, B. 2017. gridExtra: Miscellaneous function for “grid” graphics. R Package Version
387 2.3. Avaliable at: <https://CRAN.R-project.org/package=gridExtra>.
388 Boligon, A. A., J. A. V. Silva, R. C. Sesana, J. C. Sesana, J. B. Junqueira, and L. G.
389 Albuquerque. 2010. Estimation of genetic parameters for body weights, scrotal
390 circumference, and testicular volume measured at different ages in Nellore cattle. J.
391 Anim. Sci. 88:1215–1219. doi:10.2527/jas.2008-1719.
392 Bolormaa, S., J. E. Pryce, K. Kemper, K. Savin, B. J. Hayes, W. Barendse, Y. Zhang, C. M.
393 Reich, B. A. Mason, R. J. Bunch, B. E. Harrison, A. Reverter, R. M. Herd, B. Tier, H.-
394 U. Graser, and M. E. Goddard. 2013. Accuracy of prediction of genomic breeding
395 values for residual feed intake and carcass and meat quality traits in *Bos taurus*, *Bos*
396 *indicus*, and composite beef cattle. J. Anim. Sci. 91:3088–3104. doi:10.2527/jas2012-
397 5827.
398 Bowman, J. C. 1972. Genotype x environment interactions. Ann. Génét. Sél. Anim. 4:117-123.
399 doi: 10.1186/1297-9686-4-1-117.
400 Browning, S. R., and B. L. Browning. 2010. High-resolution detection of identity by descent
401 in unrelated individuals. Am. J. Hum. Genet. 86:526–539. doi:
402 10.1016/j.ajhg.2010.02.021.
403 Burns, B. M., N. J. Corbet, D. H. Corbet, J. M. Crisp, B. K. Venus, D. J. Johnston, Y. Li, M.
404 R. McGowan, and R. G. Holroyd. 2013. Male traits and herd reproductive capability in
405 tropical beef cattle. 1. Experimental design and animal measures. Anim. Prod. Sci.
406 53:87–100. doi: 10.1071/AN12162.
407 Carvalheiro, R., R. Costilla, H. H. R. Neves, L. G. Albuquerque, S. Moore, and B. J. Hayes.
408 2019. Unraveling genetic sensitivity of beef cattle to environmental variation under
409 tropical conditions. Genet. Sel. Evol. 51:29. doi: 10.1186/s12711-019-0470-x.
410 Chiaia, H. L. J., M. V. A. de Lemos, G. C. Venturini, C. Aboujaoude, M. P. Berton, F. B.
411 Feitosa, R. Carvalheiro, L. G. Albuquerque, H. N. de Oliveira, and F. Baldi. 2015.
412 Genotype × environment interaction for age at first calving, scrotal circumference, and
413 yearling weight in Nellore cattle using reaction norms in multitrait random regression
414 models J. Anim. Sci. 93:1503–1510. doi:10.2527/jas2014-8217.

415 Corbet, N. J., B. M. Burns, D. J. Johnston, M. L. Wolcott, D. H. Corbet, B. K. Venus, Y. Li,
416 M. R. McGowan, and R. G. Holroyd. 2013. Male traits and herd reproductive capability
417 in tropical beef cattle. 2. Genetic parameters of bull traits. Anim. Prod. Sci. 53:101–
418 113. doi: 10.1071/AN12163.
419 de los Campos, G., D. Sorensen, D. Gianola. 2015. Genomic heritability: what is it? PLoS
420 Genet. 11:e1005048. doi:10.1371/journal.pgen.1005048.
421 Falconer, D. S. and T. F. C. Mackay. 1996. Introduction to quantitative genetics. 4th. ed.
422 Pearson, United Kingdom.
423 Fortes, M. R. S., A. Reverter, R. J. Hawken, S. Bolormaa, and S. A. Lehnert. 2012a. Candidate
424 genes associated with testicular development, sperm quality, and hormone levels of
425 inhibin, luteinizing hormone, and insulin-like growth factor 1 in Brahman bulls. Biol,
426 Reprod. 87:1–8. doi: 10.1095/biolreprod.112.101089.
427 Fortes, M. R. S., S. A. Lehnert, S. Bolormaa, C. Reich, G. Fordyce, N. J. Corbet, V. Whan, R.
428 J. Hawken, and A. Reverter. 2012b. Finding genes for economically important traits:
429 Brahman cattle puberty. Anim. Prod. Sci. 52:143–150. doi:10.1071/AN11165.
430 Kolmodin, R., E. Strandberg, P. Madsen, J. Jensen, and H. Jorjani. 2002. Genotype by
431 environment interaction in Nordic dairy cattle studied using reaction norms. Acta Agric.
432 Scand. A. 52:11-24. doi: 10.1080/09064700252806380.
433 Lemos, M. V. A., H. L. J. Chiaia, M. P. Berton, F. L. B. Feitosa, C. Aboujaoude, G. C.
434 Venturini, H. N. Oliveira, L. G. Albuquerque, and F. Baldi. 2015. Reaction norms for
435 the study of genotype-environment interaction for growth and indicator traits of sexual
436 precocity in Nellore cattle. Genet. Mol. Res. 14:7151-7162. doi:
437 10.4238/2015.June.29.9.
438 Lima, F. P. C., P. R. Xavier, J. A. G. Bergmann, and A. P. Marques Júnior. 2013. Perímetro
439 escrotal e características seminais de touros da raça Nelore selecionados para
440 precocidade sexual. Arq. Bras. Med. Vet. Zootec. 65:1603-1608. doi: 10.1590/S0102-
441 09352013000600003.
442 Lunstra, D. D. and Cundiff, L. V. 2003. Growth and pubertal development in Brahman-, Boran-
443 , Tuli-, Belgian Blue-, Hereford- and Angussired F1 bulls. J. Anim. Sci. 81:1414–1426.
444 doi: 10.2527/2003.8161414x.
445 Mattar, M., L. O. C. Silva, M. M. Alencar, and F. F. Cardoso. 2011. Genotype × environment
446 interaction for long-yearling weight in Canchim cattle quantified by reaction norm
447 analysis. J. Anim. Sci. 89:2349–2355. doi:10.2527/jas.2010-3770.

448 Menezes, E. B., C. R. Andrade, J. H. D. Carneiro-Júnior, G. C. Rodrigues, R. Martins Filho,
449 A. A. Araújo, and A. A. A. Moura. 2014. Testis size, peripheral concentrations of
450 testosterone, semen criteria and Sertoli and germ cell numbers in Nelore bulls. Semin.
451 Cienc. Agrar. 35:2437-2448. doi: 10.5433/1679-0359.2014v35n5p2437.
452 Misztal, I., S. Tsuruta, D. Lourenço, Y. Masuda, I. Aguilar, A. Legarra, Z. Vitezica. 2018.
453 Manual for BLUPF90 family of programs. University of Georgia, Athens, GA. Avaliable
454 at: < http://nce.ads.uga.edu/wiki/lib/exe/fetch.php?media=blupf90_all7.pdf>.
455 Mota, L. F. M., G. A. Fernandes Jr, A. C. Herrera, D. C. B. Scalez, R. Espigolan, A. F. B.
456 Magalhães, R. Carvalheiro, F. Baldi, and L. G. Albuquerque. 2020. Genomic reaction
457 norm models exploiting genotype x environment interaction on sexual precocity
458 indicator traits in Nellore cattle. Anim. Gen. 51:210-223. doi: 10.1111/age.12902.
459 Nascimento, B. M., G. F. Silva, R. Carvalheiro, R. A Teixeira, and L. T Dias. 2020. Alternative
460 adjustment of scrotal circumference for growth traits in Nellore cattle. R. Bras. Zootec.,
461 49:e20190183. doi: 10.37496/rbz4920190183.
462 Oliveira, D. P., D. A. L. Lourenco, S. Tsuruta, I. Misztal, D. J. A. Santos, F. R. de Araújo Neto,
463 R. R. Aspilcueta-Borquis, F. Baldi, R. Carvalheiro, G. M. F. de Camargo, L. G.
464 Albuquerque, and H. Tonhati. 2018. Reaction norm for yearling weight in beef cattle
465 using single-step genomic evaluation. J. Anim. Sci. 96:27–34. doi: 10.1093/jas/skx006.
466 Pégolo, N. T., L. G. Albuquerque, R. B. Lôbo, and H. N. de Oliveira. 2011. Effects of sex and
467 age on genotype × environment interaction for beef cattle body weight studied using
468 reaction norm models. J. Anim. Sci. 89:3410–3425. doi:10.2527/jas.2010-3520.
469 Pires, B. C., P. Tholon, M. E. Buzanskas, A. P. Sbardella, J. O. Rosa, L. O. C. Silva, R. A. A.
470 Torres Júnior, D. P. Munari, and M. M. Alencar. 2017. Genetic analyses on bodyweight,
471 reproductive, and carcass traits in composite beef cattle. Anim. Prod. Sci. 57:415–421.
472 doi: 10.1071/AN15458.
473 R Core Team. 2020. R: A language and environment for statistical computing. R Foundation
474 for Statistical Computing, Vienna, Austria.
475 Robertson, A. 1959. The sampling variance of the genetic correlation coefficient. Biometrics.
476 15:469-485. doi: 10.2307/2527750.
477 Roso, V. M. and F. S. Schenkel. 2006. AMC – A computer program to assess the degree of
478 connectedness among contemporary groups. Proc. World Cong. Gen. App. Liv. Prod.
479 8:13-18.

480 Santana Jr, M. L., J. P. Eler, F. F. Cardoso, L. G. Albuquerque, and J. B. S. Ferraz. 2013.
481 Phenotypic plasticity of composite beef cattle performance using reaction norms model
482 with unknown covariate. Animal. 7:202-210. doi:10.1017/S1751731112001711.
483 Santana Jr., M. L., J. P. Eler, A. B. Bignardi, A. Menéndez-Buxadera, F. F. Cardoso, and J. B.
484 S. Ferraz. 2015. Multi-trait linear reaction norm model to describe the pattern of
485 phenotypic expression of some economic traits in beef cattle across a range of
486 environments. J. Appl. Genetics. 56:219–229. doi: 10.1007/s13353-014-0242-9.
487 Schmidt, P. I., G. S. Campos, V. M. Roso, F. R. P. Souza, and A. A. Boligon. 2019. Genetic
488 analysis of female reproductive efficiency, scrotal circumference and growth traits in
489 Nelore cattle. Theriogenology 128:47-53. doi: 10.1016/j.theriogenology.2019.01.032.
490 Silva, M. R., V. B. Pedrosa, J. C. B. Silva, J. P. Eler, J. D. Guimarães, and L. G. Albuquerque.
491 2011. Testicular traits as selection criteria for young Nellore bulls. J. Anim. Sci.
492 89:2061–2067. doi:10.2527/jas.2010-3525.
493 Stafuzza, N. B., E. V. Costa e Silva., R. M. O. Silva., L. C. C. Costa Filho., F. B. Barbosa., G.
494 G. Macedo., R. B. Lobo, and F. Baldi. 2020. Genome-wide association study for age at
495 puberty in young Nelore bulls. J. Anim. Breed. Genet. 137:234–244. doi:
496 10.1111/jbg.12438
497 Terakado, A. P. N., A. A. Boligon, F. Baldi, J. A. II V. Silva, and L. G. Albuquerque. 2015.
498 Genetic associations between scrotal circumference and female reproductive traits in
499 Nelore cattle. J. Anim. Sci. 93:2706–2713. doi:10.2527/jas2014-8817.
500 Tierney, N., D. Cook, M. McBain, and C. Fay. naniar: Data structure, summaries, and
501 visualisation for missing data. R Package Version 0.6.0. Avaliable at:
502 <https://CRAN.R-project.org/package=naniar>.
503 Wei, T., and V. Simko. 2017. R package "corrplot": Visualization of a correlation matrix.
504 Version 0.84. Available from: from https://github.com/taiyun/corrplot.
505 Wickham, H. 2016. ggplot2: Elegant graphics for data analysis. Springer-Verlag, New York,
506 NY.
507 Wickham, H., R. François, L. Henry, and K. Müller. 2021. dplyr: A grammar of data
508 manipulation. R. Package Version 1.0.4. Avaliable at: <https://CRAN.R-
509 project.org/package=dplyr>
510

511 Table 7 - Statistics for age at 6 months (AGE6), age at 12 months (AGE12), age at 18 months
512 (AGE18), age at 24 months (AGE24), body weight at 6 months (BW6), body weight at 12
513 months (BW12), body weight at 18 months (BW18), body weight at 24 months (BW24), scrotal
514 circumference at 6 months (SC6), scrotal circumference at 12 months (SC12), scrotal
515 circumference at 18 months (SC18), and scrotal circumference at 24 months (SC24) for
516 Brahman cattle in Australia

Trait	N	Mean ± Standard deviation	Minimum	Maximum
AGE6 (days)	1,031	187.3 ± 20.63	114	242
AGE12 (days)	1,101	374.9 ± 25.84	295	445
AGE18 (days)	1,054	527.3 ± 26.04	446	597
AGE24 (days)	1,053	704.8 ± 23.42	627	758
BW6 (kg)	1,031	199.7 ± 21.16	134	266
BW12 (kg)	1,101	245.9 ± 32.40	149	334
BW18 (kg)	1,054	354.4 ± 36.37	239	457
BW24 (kg)	1,053	380.7 ± 39.74	266	506
SC6 (cm)	1,031	17.16 ± 1.53	12.80	22.06
SC12 (cm)	1,101	20.88 ± 2.23	15.96	34.39
SC18 (cm)	1,054	26.01 ± 2.54	18.61	37.38
SC24 (cm)	1,053	30.05 ± 2.69	23.27	42.56

517

Table 8 - Average body weight, in kilograms, at the minimum, intermediate, and maximum environmental gradient (EG) measured at 6 months, 12 months, 18 months, and 24 months in Brahman cattle

Age	EG		
	Minimum	Intermediate	Maximum
6 months	169.33 kg	197.33 kg	235.20 kg
12 months	182.60 kg	244,67 kg	293.71 kg
18 months	275.40 kg	362.56 kg	406.45 kg
24 months	326.00 kg	382.44 kg	457.57 kg

Table 9 - Estimates of heritability in the minimum and maximum environmental gradient (EG) for scrotal circumference at 6 months (SC6), scrotal circumference at 12 months (SC12), scrotal circumference at 18 months (SC18), and scrotal circumference at 24 months (SC24) using A matrix and H matrix in Brahman cattle

Trait	Matrix	Heritability		
		Minimum EG	Maximum EG	Difference
SC6	A	0.54	0.30	0.24
SC6	H	0.57	0.36	0.21
SC12	A	0.57	0.94	0.37
SC12	H	0.55	0.85	0.30
SC18	A	0.72	0.94	0.22
SC18	H	0.60	0.85	0.25
SC24	A	0.92	0.41	0.51
SC24	H	0.83	0.29	0.54

Table 10 - Estimates of variance (diagonal), covariance (above diagonal), and correlation (below diagonal) between intercept and slop of reaction norm models for additive effect for scrotal circumference measured at 6 months (SC6), 12 months (SC12), 18 months (SC18), and 24 months (SC24) estimated using A matrix and H matrix in Brahman cattle

Trait	Matrix	Coefficient	b0	b1
SC6	A	b0 (intercept)	0.66	-0.06
		b1 (slope)	-0.63	0.02
	H	b0 (intercept)	0.71	-0.06
		b1 (slope)	-0.53	0.02
SC12	A	b0 (intercept)	3.12	0.16
		b1 (slope)	1.00	0.01
	H	b0 (intercept)	2.70	0.13
		b1 (slope)	1.00	0.01
SC18	A	b0 (intercept)	4.90	0.18
		b1 (slope)	1.00	0.01
	H	b0 (intercept)	4.11	0.17
		b1 (slope)	1.00	0.01
SC24	A	b0 (intercept)	4.55	-0.59
		b1 (slope)	-0.99	0.08
	H	b0 (intercept)	3.61	-0.52
		b1 (slope)	-1.00	0.08

Table 11 - Rank correlation among estimated breeding values (EBV) for scrotal circumference measured at 6 months (SC6), 12 months (SC12), 18 months (SC18), and 24 months (SC24) obtained using A matrix in different environmental gradient (EG) in Brahman cattle

Trait	EG	Medium[3]	Low[4]
SC6	High[2]	0.9987	0.9903
	Medium[3]	-	0.9959
SC12	High[2]	1.0000	1.0000
	Medium[3]	-	1.0000
SC18	High[2]	1.0000	1.0000
	Medium[3]	-	1.0000
SC24	High[2]	0.9999	0.9999
	Medium[3]	-	0.9999

[1]$p<2.2e-16$, Ho: $\rho \neq 0$.

[2]High environmental gradient: EG = -3

[3]Medium environmental gradient: EG = 0

[4]Low environmental gradient: EG = +3

537 Table 12 - Rank correlation among estimated breeding values (EBV) for scrotal circumference
538 measured at 6 months (SC6), 12 months (SC12), 18 months (SC18), and 24 months (SC24)
539 obtained using H matrix in different environmental gradient (EG) in Brahman cattle

Trait	EG	Medium[3]	Low[4]
SC6	High[2]	0.9974	0.9824
	Medium[3]	-	0.9930
SC12	High[2]	1.0000	1.0000
	Medium[3]	-	1.0000
SC18	High[2]	1.0000	1.0000
	Medium[3]	-	1.0000
SC24	High[2]	0.9999	0.9999
	Medium[3]	-	0.9999

[1]p<2.2e-16, Ho: $\rho \neq 0$.

[2]High environmental gradient: EG = -3

[3]Medium environmental gradient: EG = 0

[4]Low environmental gradient: EG = +3

540

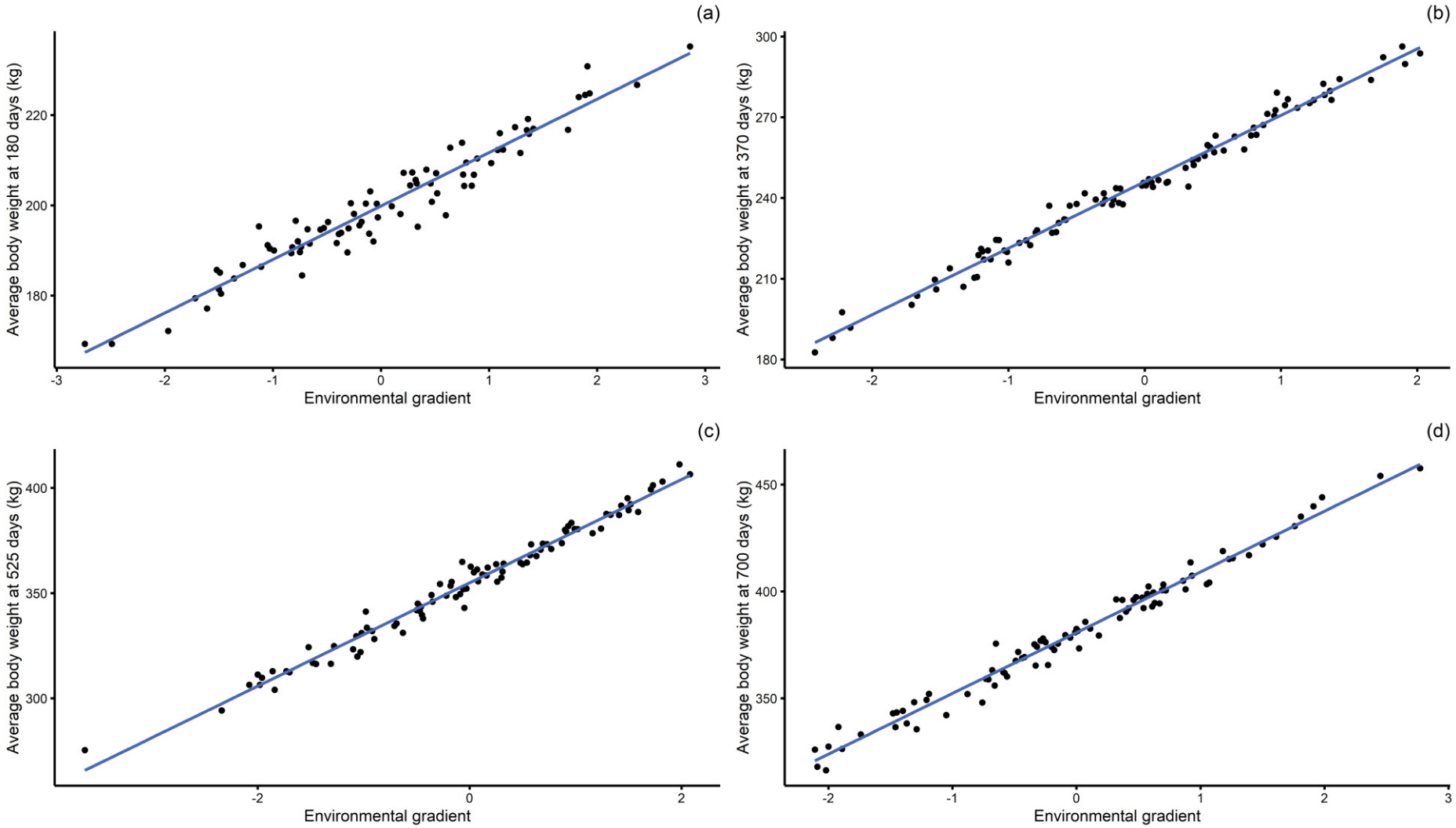


Figure 36 - Mean of scrotal circumference measured at 6 months (a), 12 months (b), 18 months (c) and 24 months (d) along the environmental gradient for Brahman cattle in Australia

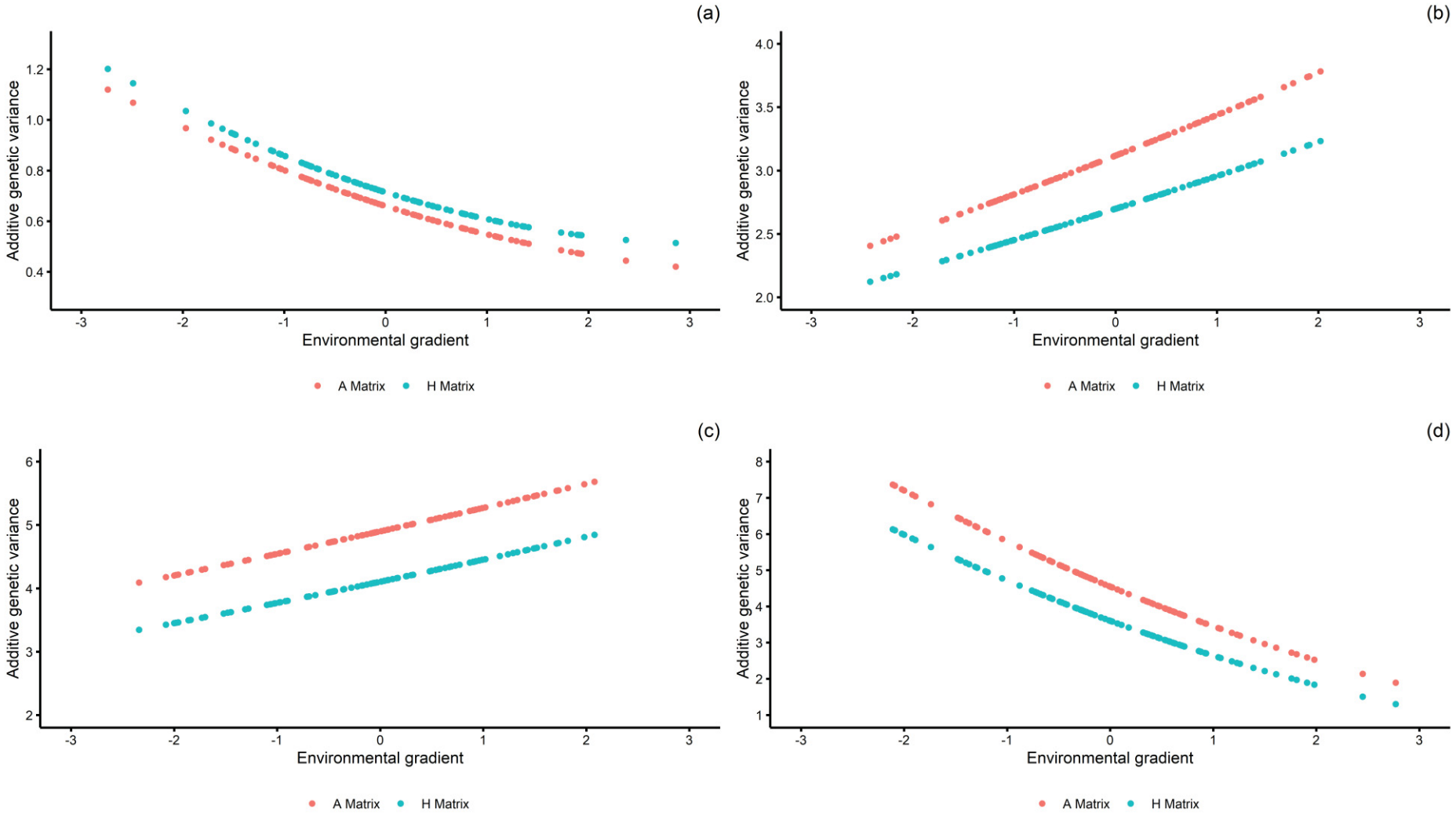


Figure 37 - Additive variances for scrotal circumference measured at 6 months (a), 12 months (b), 18 months (c) and, 24 months (d) along the environmental gradient using the A Matrix and the H Matrix for Brahman cattle in Australia

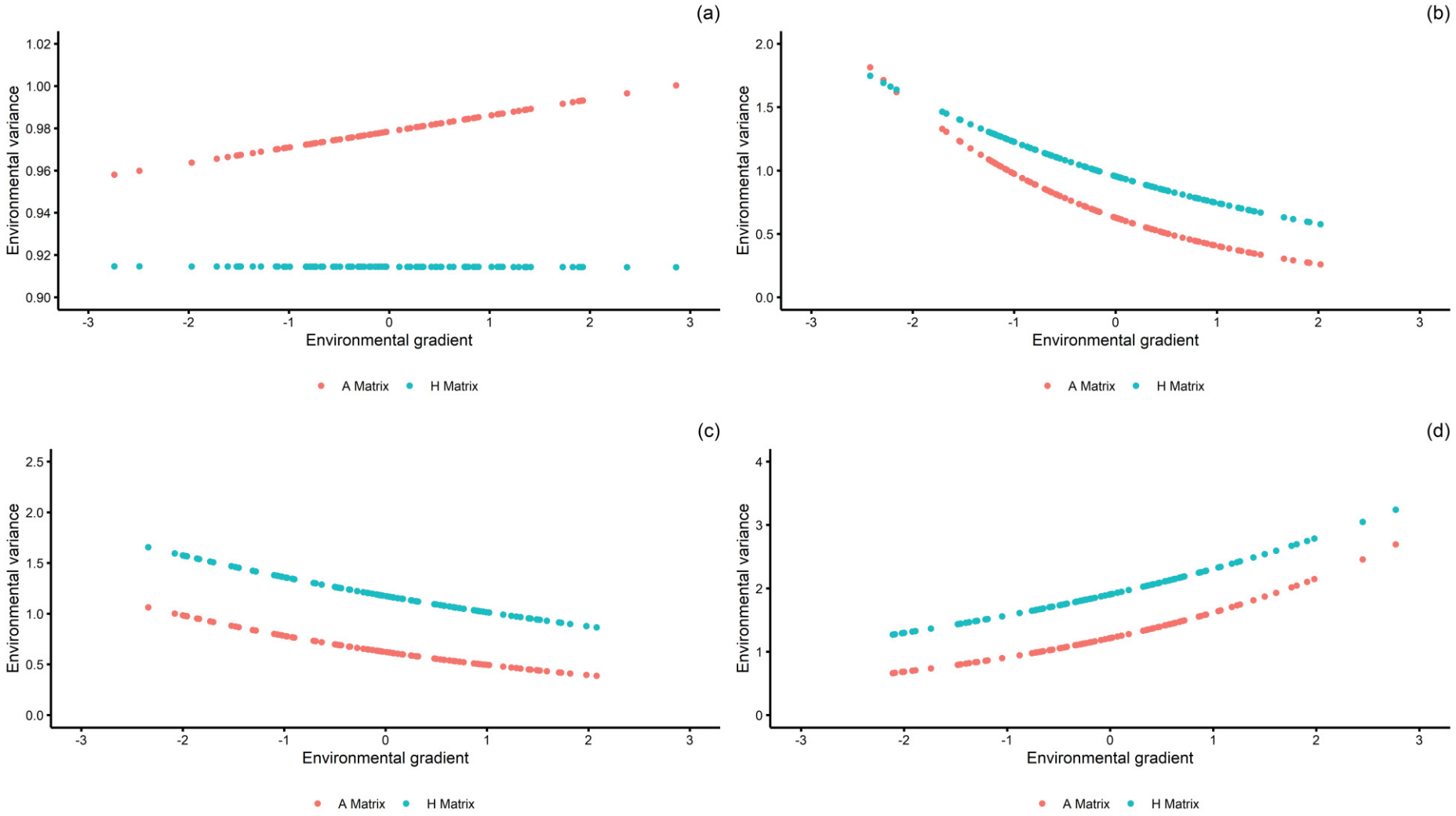
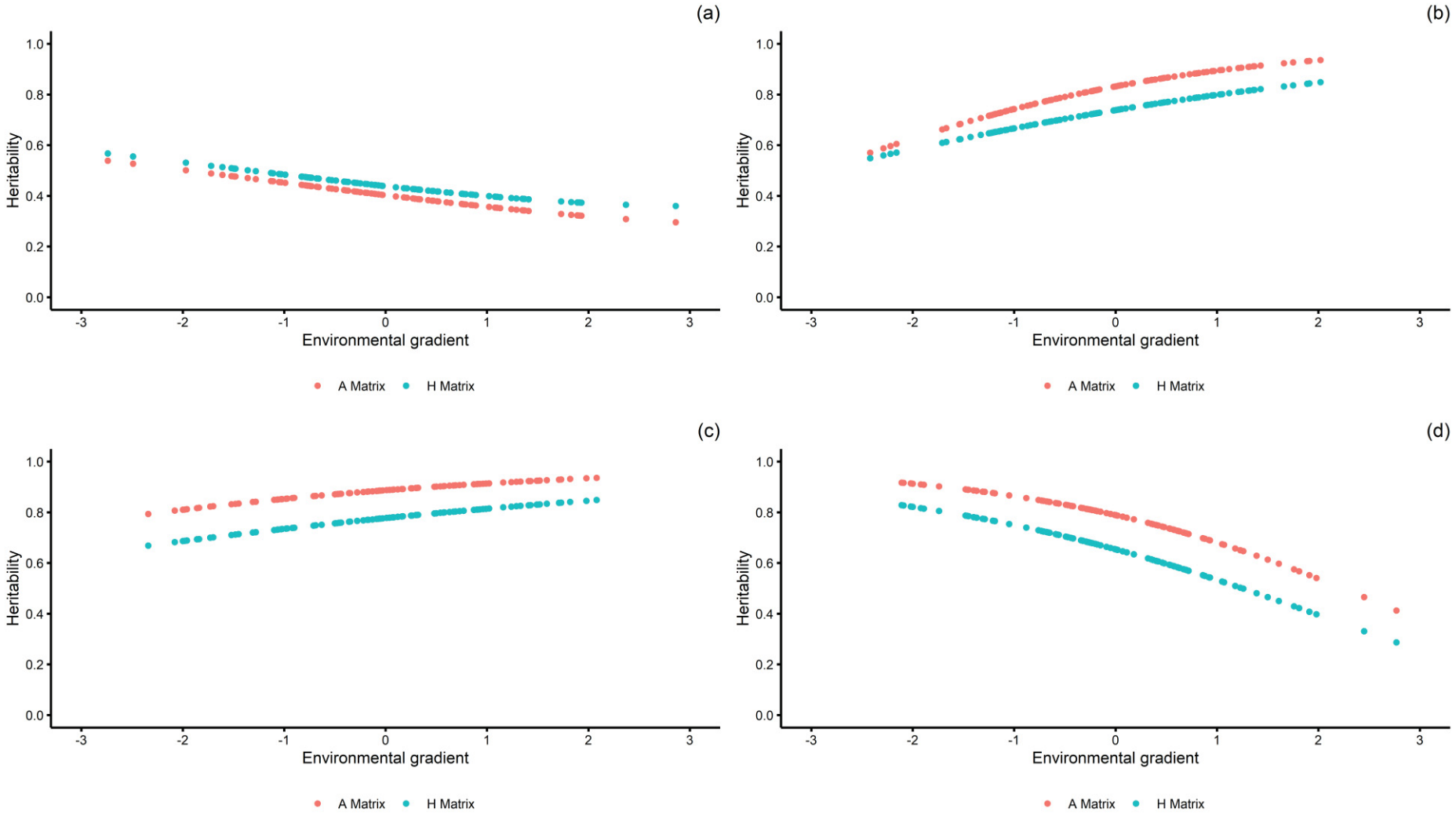
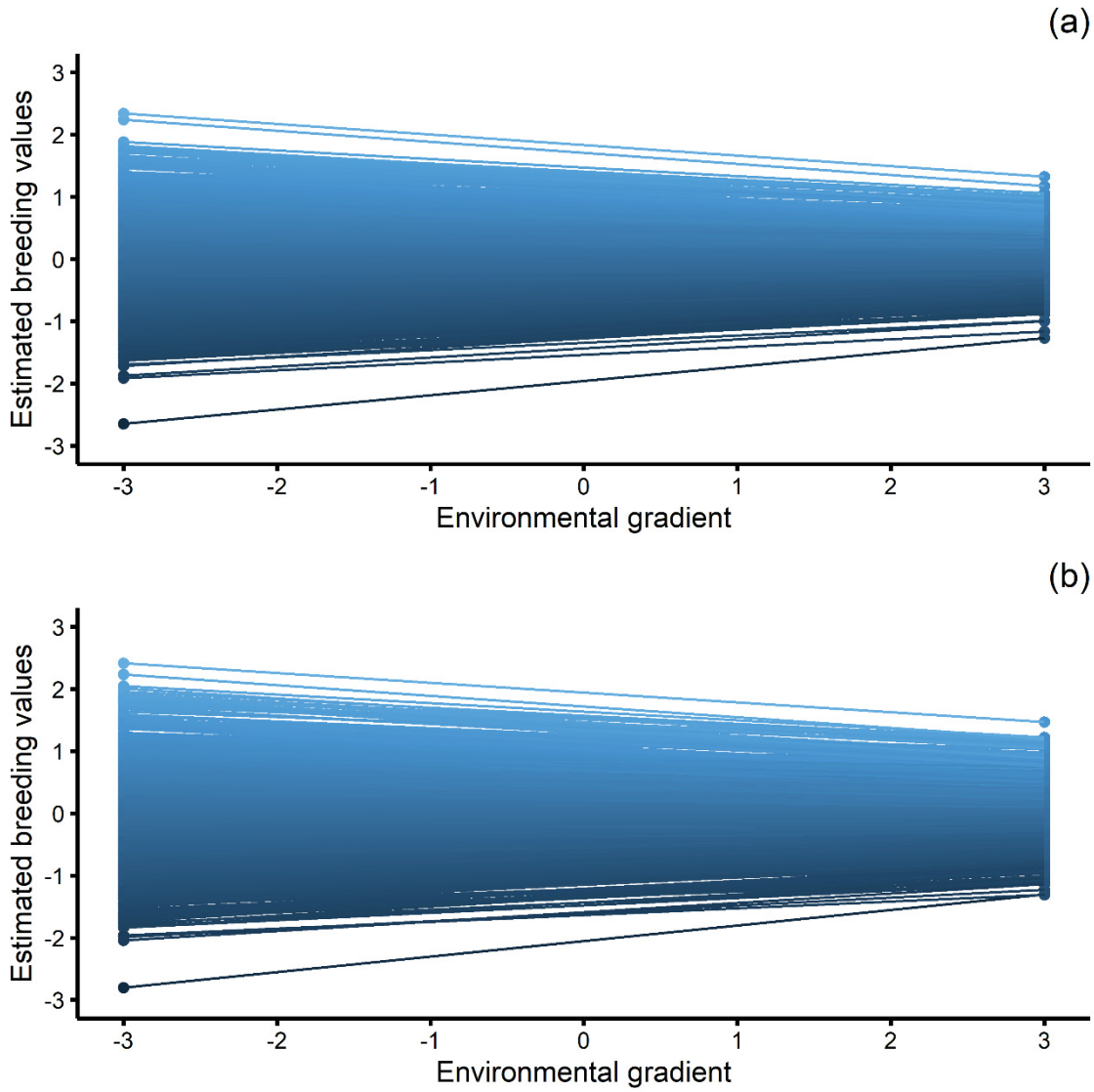


Figure 38 - Environmental variances for scrotal circumference measured at 6 months (a), 12 months (b), 18 months (c), and 24 months (d) along the environmental gradient using the A Matrix and the H Matrix for Brahman cattle in Australia



550

551 Figure 39 - Heritabilities for scrotal circumference measured at 6 months (a), 12 months (b), 18 months (c), and 24 months (d) along the
552 environmental gradient using the A Matrix and the H Matrix in Brahman cattle in Australia



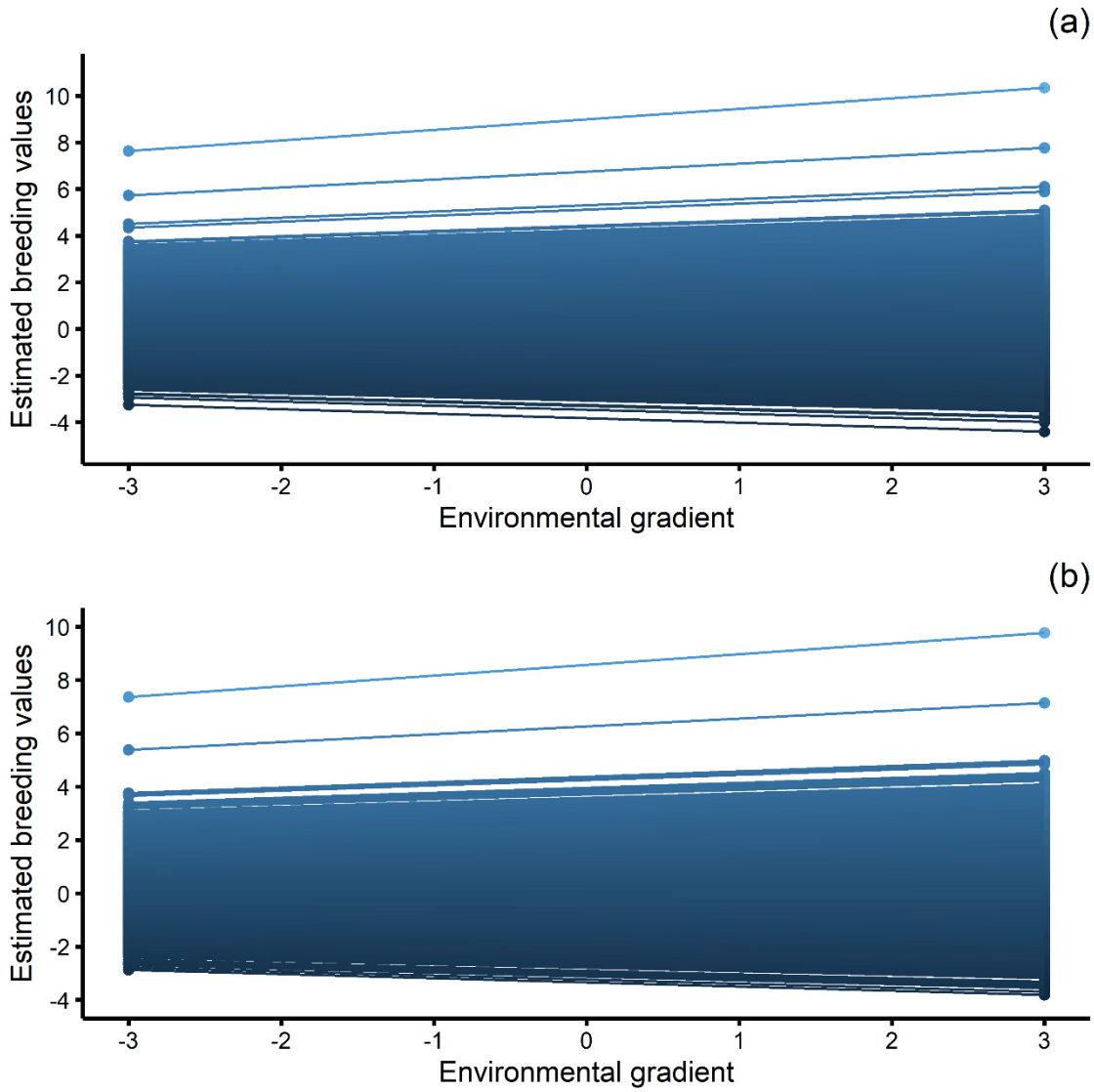
553

554 Figure 40 - Reaction norms for the estimated breeding values value using the A Matrix (a) and

555 the H Matrix (b) of scrotal circumference measured at 6 months in Brahman cattle in Australia

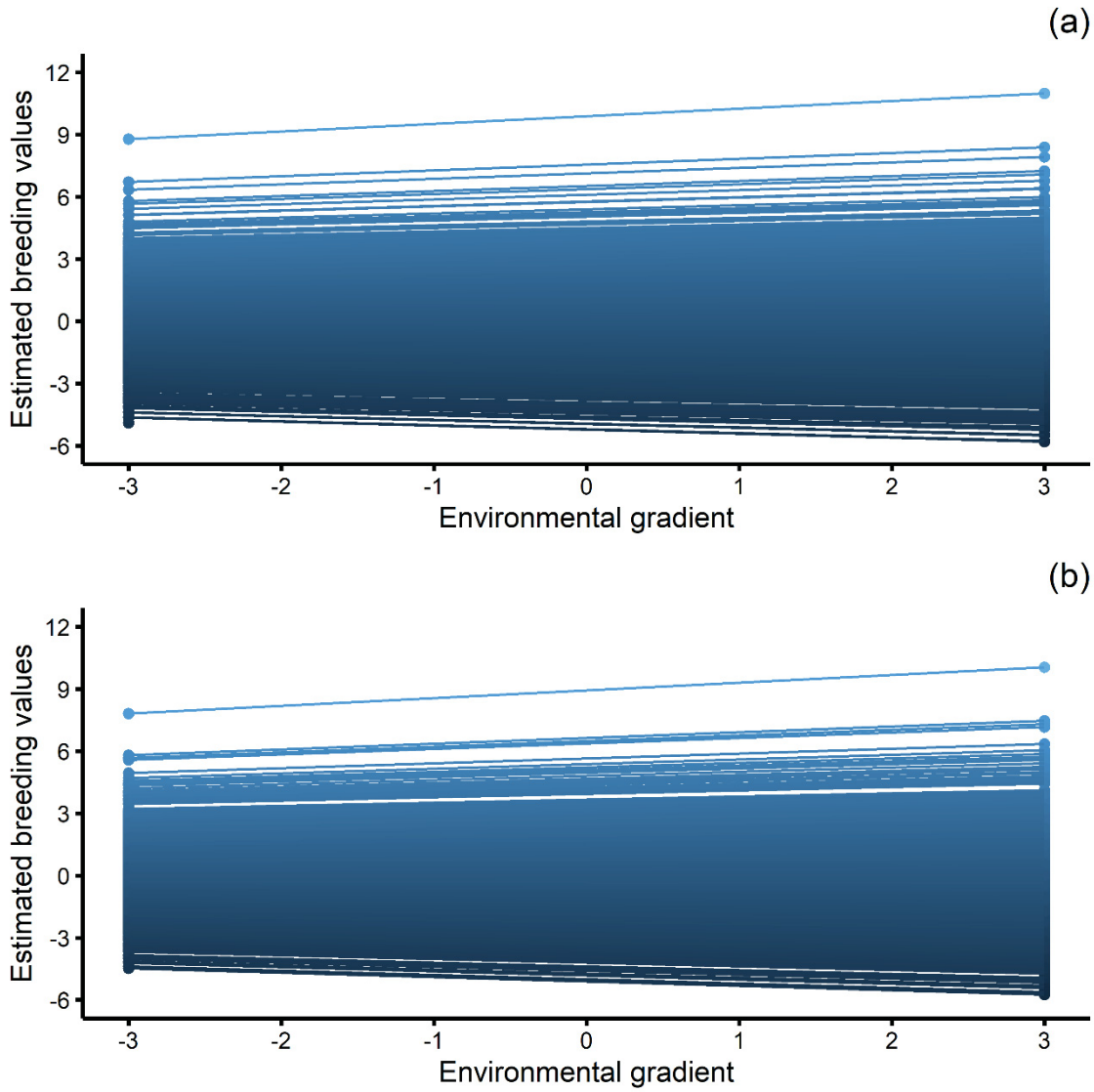
556

557



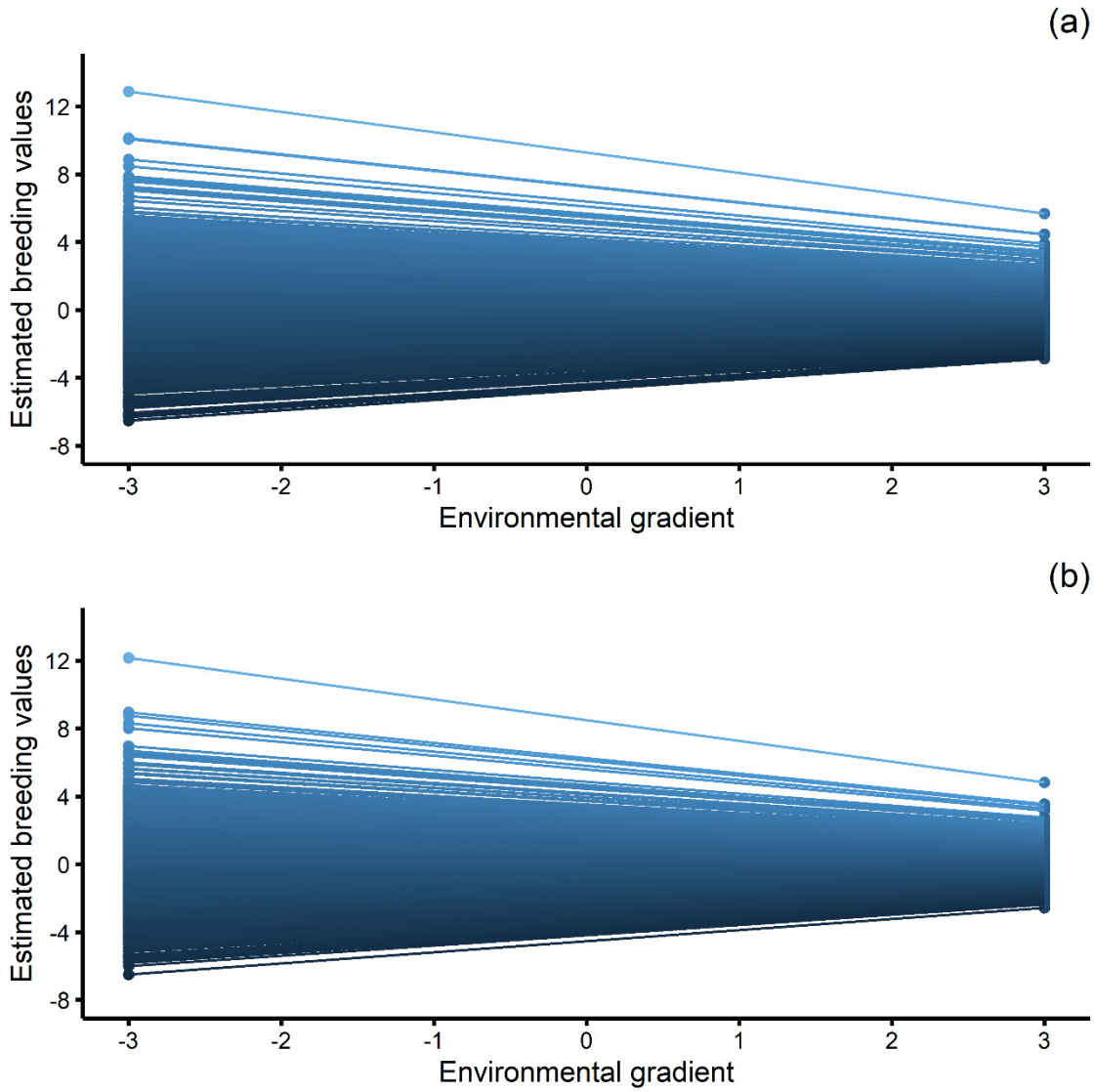
558

559 Figure 41 - Reaction norms for the estimated breeding values value using the A Matrix (a) and
560 the H Matrix (b) of scrotal circumference measured at 12 months in Brahman cattle in Australia



561

562 Figure 42 - Reaction norms for the estimated breeding values value using the A Matrix (a) and
563 the H Matrix (b) of scrotal circumference measured at 18 months in Brahman cattle in Australia



564

565 Figure 43 - Reaction norms for the estimated breeding values value using the A Matrix (a) and
566 the H Matrix (b) of scrotal circumference measured at 24 months in Brahman cattle in
567 Australia.

6 FINAL CONSIDERATIONS

In beef cattle, most of genotype x environment interaction studies are related to growth traits, such as body weight and weight gain. However, the scrotal circumference adjusted for traits related to growth should also be studied in this sense, since it has a growth component on this measure, and it is the main characteristic related to sexual precocity in genetic evaluations of beef cattle breeding programs. The results found in this thesis showed that there was genotype x environmental interaction for scrotal circumference adjusted for growth traits and visual scores. By the methodology of Reaction Norm Model (RNM), an infinite number of environmental gradients can be estimated, but classify the properties within infinite environments to subsequently choose the best bull for each of these environments is unfeasible. Thus, group the environmental gradients may help in the practical use of Reaction Norm Model (RNM). Another possibility is to create scores related to plasticity, that may facilitate the choice of the most adequate animals for each productive environment.

When the measurement of scrotal circumference at different ages was studied, no genotype x environment interaction was observed at ages close to puberty. It is important to consider the age of measurement for this trait, since its main use is as a selection criterion to identify sexual precocity, both in males and females. Therefore, evaluating this trait at ages that are not representative of puberty will not result in adequate response to the selection objective. Furthermore, the absence of genotype x environment interaction indicates that the best bulls will be superior regardless of the breeding environment, which facilitates the use of sires by breeders.

7 APPENDIX

The Chapters II, III and IV were presented according to the Author Guidelines from Journal of Animal Sciences. Those directions are attached below.

Instructions to Authors

Journal of Animal Science (*JAS*) publishes original research articles and invited review articles. The mission of the American Society of Animal Science (ASAS) is to foster communication and collaboration among individuals and organizations associated with animal science research, education, industry, or administration "To discover, disseminate, and apply knowledge for sustainable use of animals for food and other human needs". The *Journal of Animal Science* (*JAS*), which is published monthly by ASAS, accepts manuscripts presenting information for publication with this mission in mind. Its editorial policies are established by the editor-in-chief, managing editor, section editors, and editorial board, subject to review by the publications committee, board of directors, and the membership of ASAS. Views expressed in papers published in JAS represent the opinions of the author(s) and do not necessarily reflect the official policy of the institution with which the author is affiliated, ASAS, or the editor-in-chief.

The JAS is one of the most frequently cited peer-reviewed, agriculturally oriented research journals in the world, based on statistics published by ISI, Inc. (Philadelphia, PA). Its high impact factor attests to the quality standards maintained by the JAS editorial board and by authors who submit manuscripts for publication.

Manuscript Preparation (Style and Form)

General

All manuscripts submitted to the Journal must be double-spaced, 12-point Times New Roman font with 1 inch margin all around. Consecutive line and page numbers are required. Greek letters and special symbol are inserted using the symbol palette. Math equations are created with MathType or LaTex.

Title Page

Required items on the page are,

1. Running title: short, succinct title no more than 45 keystrokes (characters plus spaces) in length with first and proper nouns capitalized

2. A title with the first word and proper nouns capitalized. Species of subject is encouraged. The title should be unique. The Journal does NOT support multipart series.
3. Full names (given name, middle initial, family name) of all authors
4. Institutions of the authors with location denoted with a symbol (*, †, ‡, §, #, ||, and ¶) behind the author last name
5. Department, city, state, country, and postal code (Please note: the country must be listed for each affiliation)
6. Acknowledgements of consortia, grants, experiment station, or journal series number are given as a numerical footnote to the title

Abstract

A single paragraph of no more than 2,500 keystrokes (characters plus spaces) that summarizes the results in an understandable form using statistical evidence (P-values). Abbreviations are defined at first use in the ABSTRACT and again in the body of the manuscript.

Key words

List up to 6 words in alphabetical order and separated by a comma. Capitalize only proper nouns. Do NOT use abbreviations. Place at the end of the ABSTRACT.

List of Abbreviations

A comprehensive list of all abbreviations used in the manuscript and their definition. An example format is MRF, myogenic regulatory factor. The List should not contain standard JAS Abbreviations, diets or treatment descriptions. Abbreviations must be defined at first use in the manuscript text but not in tables and figures unless unique.

Download an MS Excel spreadsheet of JAS standard abbreviations.

Plural abbreviations do not contain a final "s" because the context of an abbreviation implies whether it is singular or plural. Use of the standard 3-letter abbreviations for amino acids (e.g., Ala) is acceptable in JAS. Use of the internationally recognized chemical symbols for chemical elements (e.g., P and S) is acceptable in JAS. Except for N (not italicized), which is the recognized abbreviation for nitrogen and newton (unit of force), chemical symbols for elements are reserved for elements (e.g., C is for carbon and never for control).

Introduction

A clear justification for conducting the research with a stated hypothesis and objective(s) is required. The rationale for the experiments should place the work into the context of existing literature. There is NO word limit on the section but brevity is encouraged.

Materials and Methods

The American Society of Animal Science (ASAS) supports rigor, reproducibility and transparency in science and seeks to ensure that publications of the society reflect these values while also minimizing the burden on authors in preparation of scientific results for publication. There are many available resources describing principles and practices to enhance rigor, reproducibility, and transparency in science. Authors considering the *Journal of Animal Science* are encouraged to consult these resources when during preparation of their submissions.

The manuscript must include a statement of institutional animal care and use committee (IACUC), or country-specific equivalent, approval of all animal procedures. The IACUC statement should appear as the first item in MATERIALS AND METHODS and should specify which publicly available animal care and use standards were followed. A clear description of all biological, analytical and statistical procedures is required with each section denoted by a short descriptive title (i.e., Animals and sampling, Western blot, Immunocytochemistry, Experimental design and analysis, etc). Materials used must include the product name and vendor at first mention. When a commercial product is used as part of an experiment, the manufacturer name and location must be given parenthetically and the generic name should be used subsequently. No ™, ®, or © symbols should be used. Sex, breed, age, species are included in the animal descriptions. Provide evidence of assay validation, or suitable published reference, as well as inter/intra-assay CV, as needed. Appropriate statistical methods should be used with experimental unit defined. Numbers of biological and experimental replicates should be stated. State the threshold for significance ($P < 0.05$) and definition of tendency if used.

Results

Experimental results are presented in tables and figures. The results should contain sufficient detail to allow the reader to interpret the data. Quantitative measures of significance (P-values) should be presented. Authors may use either absolute P-values or a defined significance level as long as usage is consistent.

Discussion

The section contains the interpretation of the results. It should be clear and concise, address the biological mechanisms and their significance, and integrate the results into existing literature. The Discussion may offer an interpretation that is consistent with the data. Do NOT include any reference to tables and figures or include P-values in the Discussion. Authors have the option to create a single RESULTS AND DISCUSSION section.

Disclosures

All JAS editors, ASAS staff, ASAS Board of Directors, and submitting authors must disclose any actual or potential conflicts of interest that may affect their ability to objectively present or review research or data. A succinct statement detailing any perceived conflict of interest is required. If none, please indicate as such.

Literature Cited

Papers in the section must be published or 'in press'. All references must include the doi, if available. Authors are encouraged to use the most recent reference style for the *Journal of Animal Science* in the reference management software of their choice. The format for references are

Journal articles

Perez, V. G., A. M. Waguespark, T. D. Bidner, L. L. Southern, T. M. Fakler, T. L. Ward, M. Steidinger, and J. E. Pettigrew. 2011. Additivity of effects from dietary copper and zinc on growth performance and fecal microbiotia of pigs after weaning. J. Anim. Sci. 89:414–425. doi:10.2527/jas.2010-2839.

Abstracts

Centon, J. R., G. E. Erickson, T. J. Klopfenstein, K. J. Vander Pol, and M. A. Greenquist. 2007. Effects of roughage source and level in finishing diets containing wet distillers grains on feedlot performance. J. Anim. Sci. 85(Suppl. 2):76. (Abstr.) doi:10.2527/jas.2006-354.

Books and chapters in books

AOAC. 1990. Official methods of analysis. 15th ed. Assoc. Off. Anal. Chem., Arlington, VA.

NRC. 2000. Nutrient requirements of beef cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.

Robinson, P. H., E. K. Okine, and J. J. Kennelly. 1992. Measurement of protein digestion in ruminants. In: S. Nissen, editor, Modern methods in protein nutrition and metabolism. Academic Press, San Diego, CA. p. 121–127.

Conference proceedings

Bailey, E. A., J. R. Jaeger, J. W. Waggoner, G. W. Preedy, L. A. Pacheco, and K. C. Olson. 2012. Effect of weaning method on welfare and performance of beef calves during receiving. Proc. West. Sec. Amer. Soc. Anim. Sci. 63:25-29.

Figure Legends

All figures must have a title and legend. The legend should be a brief description that allows the reader to interpret the results. Key elements include the level of significance, number of biological and experimental replicates, scale bar length, microscopic magnification, author defined abbreviations and other descriptors of the data.

Tables and Figures

Tables and Figures, placed at the end of the manuscript, must be prepared so they can be understood without referring to information in the body of the manuscript. Each table and figure is placed on a separate page and appropriately identified by a table/figure number. Specific details are found on-line and include,

Figures

1. Axes descriptors are separated from units (i.e., kg, mm, mL) by a comma. Do NOT place units within parentheses
2. Minimum resolution is 300 dpi for color and grayscale images and 600 dpi for line art. Color figure must be submitted in CMYK and not RGB.
3. Use Times New Roman font no smaller than 8 point following figure reduction.
4. Photomicrographs should contain a scale bar.
5. Figures should be submitted as JPEG, TIFF or EPS files but PDF and DOC are accepted.

Tables

1. All tables are created in Word using the Table function
2. Use Times New Roman font with 12 point size
3. Tables should fit on a single 8.5 X 11 inch page in either landscape or portrait view
4. Every column has a heading
5. Align column values to the decimal point whenever possible. Columns containing a mix of values, symbols and words may be aligned to the center of the heading. Columns using ± should be aligned to the symbol.
6. Units (e.g., kg) are separated from descriptor by a comma
7. Numerals are used to reference footnotes. Each footnote should begin on a new line immediately below the table.
8. Lowercase, superscript letters are used to indicate significant differences among means within a row or column and to reference footnotes explaining how to interpret the letters.
9. The order of footnotes below the table is numbers first followed by letters and special symbols.
10. If reporting significance, the column heading is P-value.

Electronic Supplements (E-Supplements)

Authors may present material in an e-supplement (e.g., detailed data sets, Excel files, and video) that is more extensive or detailed than necessary for a JAS article. A note will appear in the JAS article that more material can be found online. Material in an e-supplement must undergo peer review and, thus, should be in a format that is easily accessible (i.e., does not

require dedicated software or software that is not generally available) to most reviewers and readers.

Additional Usage Notes

Quantitative Trait Loci and DNA Markers, Microarray and RNA Sequencing Data

Authors of papers that contain original quantitative trait loci (QTL) or DNA marker association results for livestock are strongly encouraged to make their data available in an electronic form to one of the publicly available livestock QTL databases after the manuscript appears on the JAS Advance Articles website (https://academic.oup.com/jas/advance-articles). Similarly, for microarray data and RNA sequencing data, authors are encouraged to submit a complete dataset to an appropriate database.

Commercial Products

The use of names of commercial products should be minimized. When a commercial product is used as part of an experiment, the manufacturer name and location (city and state if in the US; city, administrative region or district [e.g., province], and country if outside the US) or a website address must be given parenthetically at first mention in text, tables, and figures. The generic name should be used subsequently. No ™, ®, or © symbols should be used.

Policies and Procedures of JAS

The mission of the American Society of Animal Science (ASAS) is to "foster the discovery, sharing, and application of scientific knowledge concerning the responsible use of animals to enhance human life and wellbeing" (see ASAS's History and Mission).

The *Journal of Animal Science*, which is published monthly by ASAS, accepts manuscripts presenting information for publication with this mission in mind.

The Editor-in-Chief, Managing Editor, and Section Editors establish the editorial policies of JAS, subject to review by the publications committee and ASAS Board of Directors. The views expressed in articles published in JAS represent the opinions of the author(s) and do not necessarily reflect the official policy of the institution with which an author is affiliated, the ASAS, or the JAS Editor-in-Chief. Authors are responsible for ensuring the accuracy of collection, analysis, and interpretation of data in manuscripts and ultimately for guaranteeing the veracity of the contents of articles published in JAS.

General Usage

- For general style and form, authors should follow that recommended in Scientific Style and Format: The CSE Manual for Authors, Editors, and Publishers. 7th ed. Council of Science Editors, Reston, VA.
- For American English spelling and usage, consult Merriam-Webster Online.
- For SI units, the National Institute of Standards and Technology provides a comprehensive guide.
- Abbreviations are not used to begin sentences. Words must be spelled out.
- "Sex" should be used, rather than "gender." Gender is more appropriate for describing a role in society than for describing biological sex.
- The hierarchy for brackets and parentheses is [( )]. For example, $[(2 + 3) \times (12 \div 2)] \times 2 = 60$.
- Meat shear force should be expressed in kilograms (kg), although newtons (N) may also be acceptable.
- Report time using the 24-h system (e.g., 1410 h rather than 2:10 p.m.).
- Use italics to designate genus and species.
- Names of muscles are not italicized.
- Specify the basis (i.e., as-fed or dry matter) for dietary ingredient and chemical composition data listed in text or in tables. Similarly, specify the basis for tissue composition data (e.g., wet or dry basis).
- Calculations of efficiency should be expressed as output divided by input (i.e., gain:feed, not feed:gain).
- A diet is a feedstuff or a mixture of feedstuffs; a ration is the daily allotment of the diet.
- The word "Table" is capitalized and never abbreviated.
- Except to begin a sentence, the word "Figure" should be abbreviated to "Fig."
- Except to begin a sentence, experiment and equation should be abbreviated to Exp. And Eq., respectively, when preceding a numeral (e.g., Exp. 1).
- Avoid jargon unfamiliar to scientists from other disciplines. Do not use the term "head" to refer to an animal or group of animals. Instead, use animal, sow, ewe, steer, heifer, cattle, etc.
- Avoid bi- as a prefix because of its ambiguity; biweekly means twice per week and once every 2 weeks.
- Breed and variety names should be capitalized (e.g., Landrace and Hereford).

- Trademarked or registered names should be capitalized, but no ™ or ® symbols should be used.

Contact Information

For information on the scientific content of the journal, contact the Editor-in-Chief, Dr. Sally Johnson, American Society of Animal Science, P.O. Box 7410, Champaign, Illinois 61826-7410; e-mail: sealy@vt.edu/.

For questions about submitting a manuscript and ScholarOne Manuscripts, contact Ms. Elizabeth Clark; e-mail: jas.editorialoffice@oup.com.

For assistance with author proofs, contact OUP Author Support; e-mail: jnls.author.support@oup.com.

Care and Use of Animals

All authors submitting to JAS must complete the Care and Use of Animals form certifying that any research that involves animals has followed established standards for the humane care and use of animals and must specify which standards were used. Only investigations that have followed high standards for the humane care and use of animals in research will be reported in JAS. Also, the manuscript must include a statement of institutional animal care and use committee (IACUC), or country-specific equivalent, approval of all animal procedures. The IACUC statement should appear as the first item in MATERIALS AND METHODS and should specify which publically available animal care and use standards were followed (e.g., ADSA-ASASPSA Guide for Care and Use of Agricultural Animals in Research and Teaching; Primary Industries Ministerial Council, Model code of practice for the welfare of animals: the sheep). The manuscript should describe anesthetics, analgesics, tranquilizers, and care taken to minimize pain and discomfort during preoperative, operative, and postoperative procedures. If research requires discomfort to the animals or stressful conditions, justification for these conditions must be evident in papers published in JAS.

Protection of Human Subjects

In the United States, federally funded or regulated research involving human subjects must comply with Code of Federal Regulations (CFR), Title 45 Public Welfare, Part 46 Protection of Human Subjects. However, CFR 45 Part 46.101(b) exempts some research from these regulations. For all exempted research and other details, see this page. Exempted research includes that in which the only involvement of human subjects is for “taste and food quality evaluation and consumer acceptance if 1) wholesome foods without additives are consumed or 2) a food is consumed that contains a food ingredient at or below the level and for a use found to be safe, or agricultural chemical or environmental contaminant at or below the level found

to be safe, by the Food and Drug Administration or approved by the Environmental Protection Agency or the Food Safety and Inspection Service of the U.S. Department of Agriculture." If human subjects were used in exempted research and the research was in compliance with CFR 45 Part 46, or equivalent regulations where the research was conducted, authors must state in MATERIALS AND METHODS or acknowledgements that they were in full compliance. If human subjects were used in research that was not exempted in CFR 45 Part 46, or equivalent regulations where the research was conducted, authors must certify that the research received prior approval from an appropriate Institutional Review Board.

Types of Articles

Research Articles

Results of research contained in manuscripts submitted to JAS must not have been published in or submitted to another peer reviewed scientific journal prior to receiving a decision from JAS. Previous presentation at a scientific meeting or the use of data in field-day reports or similar documents, including press publications or postings to personal or departmental websites, does not preclude the publication of such data in JAS.

Articles simultaneously posted to websites and submitted to JAS should carry a disclaimer on the website that this version of the paper has not undergone JAS peer review and is not to be considered the final published form of the article. If the article has been published in JAS, the author should include the complete JAS citation.

Because JAS holds the copyright to articles it publishes, posting altered JAS articles that are represented as exact duplicates of the published version constitutes copyright violation.

Special Topics. This Section includes Biographical or Historical Sketches and Contemporary Issues in the animal sciences. Contemporary Issues include topics such as environmental concerns, legislative proposals, systems analysis, and various "newsworthy" scientific issues. Even though Contemporary Issues manuscripts do not have to include original data, authors' assertions should be substantiated with references to established information from credible published sources. Special Topics papers will be subject to peer review in a manner similar to other JAS submissions. Because of the nature of these manuscripts, their format may vary from that of standard scientific articles, although the ABSTRACT must be consistent with keystroke (characters and spaces) limitations defined earlier in this document. Teaching articles should be submitted to Translational Animal Science.

Short Communications. JAS will consider publication of short communications that are hypothesis-driven and report novel results. Submitted papers should follow JAS guidelines for headings and format, but are restricted to 2 figures or tables or a combination of 1 figure/1 table. The words "Short Communication:" should begin the title. The final published paper will be published Open Access using the current pricing structure.

Technical Notes. A technical note is used to report a new method, technique, or procedure of interest to JAS readers. When possible, a technical note should include a comparison of results from the new method with those from previous methods, using appropriate statistical tests. The advantages and disadvantages of the new procedure should be discussed. When typeset for publication, a technical note shall not exceed 10 pages (approximately 18 Microsoft Word document pages), including tables and figures. "Technical note:" shall be the first portion of the title of such manuscripts. The review process for a technical note will be the same as that for other manuscripts. Information that is more extensive or detailed than necessary for a Technical note may be presented in an e-supplement (see E-Supplements).

Letters to the Editor. A letter judged suitable for publication will be printed in a "Letters to the Editor" section of JAS. The purpose of this section is to provide a forum for scientific exchange relating to articles published in JAS. To be acceptable for publication, a letter must adhere to the following guidelines. 1) Only a letter that addresses matters of science and relates to information published in JAS will be considered. In general, a letter should not exceed 5,000 keystrokes and should contain no more than 5 citations. 2) A letter should provide supporting evidence based on published data for the points made or must develop logical scientific hypotheses. A letter based on conjecture or unsubstantiated claims will not normally be published. No new data may be presented in a letter. 3) The Editor-in-Chief will evaluate each letter and determine whether a letter is appropriate for publication. If a letter is considered appropriate, the author(s) of original JAS article(s) will be invited to write a letter of response. Normally both letters will be published together. 4) All letters will be subject to acceptance and editing by the Editor-in-Chief and editing by a technical editor.

Review Articles

The journal publishes invited review articles only.

Submission of Manuscripts

Manuscripts should be submitted electronically through ScholarOne Manuscripts. Authors with questions about using the electronic manuscript submission system or, for technological

reasons, are unable to submit manuscripts electronically may contact Ms. Elizabeth Clark (jas.editorialoffice@oup.com).

Copyright and Permission to Publish

Authors shall complete the Manuscript Submission form for each new manuscript submission. The form is completed during the submission process through ScholarOne Manuscripts. Authors, such as United States government employees, who are unable to grant copyright to ASAS for material that was produced as an official duty of a U.S. Government employee is considered public domain. Authors of JAS manuscripts who include material (usually tables or figures) taken from other copyrighted sources must secure permission from the copyright holders and provide evidence of this permission at the time the manuscript is submitted to JAS for review. Tables or figures reproduced from the work of others, or data extracted from the work of others and used to construct summary tables (or figures) or for meta-analyses, must include an acknowledgement of the original source in a footnote or legend and, when appropriate, a complete citation in LITERATURE CITED. The ASAS and the author(s) of JAS articles agree to publish under the Creative Commons CC BY-NC-ND license; the author agrees that ASAS will manage any requests for rights not granted under this license.

Review of Manuscripts

General Procedures

The Editor-in-Chief and Section Editors determine whether manuscripts are suitable for publication in JAS. All communications about a submitted manuscript should maintain confidentiality. Each manuscript will undergo closed scientific review. Manuscripts that are not written clearly, concisely, and coherently, or they are not consistent with guidelines in the current Instructions for Authors, *Journal of Animal Science* may be rejected without review. Authors whose first language is not English are urged to have an editing service review their manuscripts before they are submitted to *JAS*. For your convenience, JASEdits is available from ASAS.

Appeals

If a manuscript is rejected, the decision may be appealed to the Editor-in- Chief if the author(s) believe(s) that the judgment was erroneous or biased. A letter presenting the reasons for the appeal should be sent to the Editor-in-Chief within 30 days of the date on the rejection notification. The Editor-in-Chief will decide whether to accept or deny the appeal.

Revisions

All revised manuscripts must be returned to Section Editors via *JAS* Scholar- One Manuscripts. Authors will be permitted 15 days to revise and return manuscripts classified as Minor Revision and permitted 35 days to revise and return manuscripts classified as Major Revision. In most cases manuscripts will not be allowed more than a single revision. Unsatisfactory or incomplete revisions will be a cause for rejection of the manuscript.

Manuscripts that exceed the revision-option deadline will be withdrawn. If withdrawn for lack of timely revision, they may be resubmitted for new review. Requests for extensions must be communicated to the Section Editor responsible for the manuscript before the revision-option expires.

Papers in Press, Author Proofs, and Publication Charges

Advanced Papers

To facilitate earlier disclosure of research results, accepted manuscripts will be assigned a digital object identifier (doi) and posted to the JAS Advance Articles site in the form in which they are accepted. The authors bear the primary responsibility for the content of manuscripts posted to the Papers in Press site. Articles posted to this site have not been professionally edited and typeset, and do not represent the final, published form of the manuscript. The date a complete monthly issue of *JAS* is posted online is the official publication date for *JAS* articles. However, the date on which a manuscript is posted to the *JAS*-Advanced Papers website may represents the official public disclosure date for the contents of the article. Authors concerned about intellectual property issues, such as patents and disclosure dates, should seek legal counsel before submitting manuscripts to a scientific journal.

Author Proofs

Proofs of all manuscripts will be provided to the corresponding author and should be read carefully and checked against the typed manuscript. Accuracy of the author proof is the sole responsibility of the author(s). Authors will receive a link to the PDF proof of their manuscript on our online system by email, and it is essential that a current email address is supplied with all manuscripts. Proofing instructions will accompany the PDF file but the proof should be checked immediately upon receipt and uploaded in accordance with covering instructions. Only essential corrections should be made at the proof stage. Excessive author changes made at the proof stage may result in a $250 surcharge for additional typesetting, and they may be deemed so excessive that the manuscript will be returned to the Section Editor for additional scientific review.

Policies Regarding Number Usage for *Journal of Animal Science*

Number usage in *JAS* is consistent with the Scientific Style and Format: The CSE Manual for Authors, Editors, and Publishers.

Additional Resources

Sample Template for Building Your JAS Manuscript

JAS Professional Writing Service

JAS Ethics Policy

Revision Checklist for Authors

Guidelines for Creating Tables in Microsoft Word

Quality Guidelines for JAS Figures

Biographical Sketches

If you have trouble viewing any of these PDF files please be sure you have the most current version of Adobe Reader.

Ethics

Authors should observe high standards with respect to publication ethics as set out by the Commission on Publication Ethics (COPE). Falsification or fabrication of data, plagiarism, including duplicate publication of the authors' own work without proper citation, and misappropriation of the work are all unacceptable practices. Any cases of ethical misconduct are treated very seriously and will be dealt with in accordance with the COPE guidelines.

Third-party copyright

In order to reproduce any third party material, including tables, figures, or images, in an article authors must obtain permission from the copyright holder and be compliant with any requirements the copyright holder may have pertaining to this reuse. When seeking to reproduce any kind of third party material authors should request the following:

- non-exclusive rights to reproduce the material in the specified article and journal;
- electronic rights, preferably for use in any form or medium;
- the right to use the material for the life of the work; and
- world-wide English-language rights.

It is particularly important to clear permission for use in the online version of the journal, and we are not able to accept permissions which carry a time limit because we retain journal articles as part of our online journal archive.

Further guidelines on clearing permissions.

Third-party content in Open Access papers

If you will be publishing your paper under an Open Access license but it contains material for which you do not have Open Access re-use permissions, please state this clearly by supplying the following credit line alongside the material:

Title of content

Author, Original publication, year of original publication, by permission of [rights holder]

Permissions regarding reuse of OUP material

Self-archiving policy

Publication Fees

The journal has two options available for publication: open access (OA) and conventional page charges. For conventional publication, the charge is $120 per printed page if at least one author is an ASAS member; the page charge is $240 when no author is a member of ASAS.

Open Access

Journal of Animal Science offers the option of publishing under either a standard licence or an open access licence. Please note that some funders require open access publication as a condition of funding. If you are unsure whether you are required to publish open access, please do clarify any such requirements with your funder or institution. You will need to pay an open access charge to publish under an open access licence.

Should you wish to publish your article open access, you should select your choice of open access licence in our online system after your article has been accepted for publication.

Details of the open access licences and open access charges.

Please note that you may be eligible for a discount to the open access charge based on society membership. Authors may be asked to prove eligibility for the member discount.

Preprint Policy

Authors retain the right to make an Author's Original Version (preprint) available through various channels, and this does not prevent submission to the journal. For further information see our Online Licensing, Copyright and Permissions policies. If accepted, the authors are required to update the status of any preprint, including your published paper's DOI, as described on our Author Self-Archiving policy page.