

**UNIVERSIDADE ESTADUAL PAULISTA – UNESP
CÂMPUS DE JABOTICABAL**

**GENETIC ANALYSIS OF FEET AND LEGS IN NELORE
CATTLE**

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Zootecnista

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CATTLE**

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Giovana Vargas, nascida em Jaboticabal – SP, no dia 27 de janeiro de 1988, filha de Cleyton Vargas e Márcia Fátima Paula Rodrigues Vargas, Zootecnista formada pela Universidade Estadual Paulista, Faculdade de Ciências Agrárias e Veterinárias, Câmpus de Jaboticabal. Durante o período de graduação foi bolsista PIBIC sob orientação do Prof. Dr. Danísio Prado Munari. Em março de 2013, ingressou no Programa de Pós-Graduação em Genética e Melhoramento Animal da mesma faculdade, inicialmente como bolsista CAPES e posteriormente FAPESP, sob orientação do Dr. Roberto Carvalheiro e coorientação do Prof. Dr. Danísio Prado Munari e Dr. Haroldo Henrique de Rezende Neves.

“Só existem dois dias no ano que nada pode ser feito. Um se chama ontem e o outro se chama amanhã, portanto hoje é o dia certo para amar, acreditar, fazer e principalmente viver.”

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Aos meus amados pais,

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GENETIC ANALYSIS OF FEET AND LEGS IN NELORE CATTLE

RESUMO - O objetivo deste estudo foi estimar os componentes de variância para a característica aprumo em bovinos da raça Nelore, e realizar estudos de associação genômica ampla para identificar possíveis regiões genômicas relacionadas com sua expressão. Os registros de aprumo foram obtidos pela atribuição de escores visuais em dois momentos diferentes: FL1) característica binária medida ao sobreano, com o objetivo de identificar se o animal apresenta (FL1=1) ou não (FL1=0) defeito de aprumo; FL2) escores de aprumo variando de 1 (menos desejável) a 5 (mais desejável), foram designados aos animais top 20% para o índice de seleção adotado pelo programa de melhoramento genético de bovinos de corte PAINT (CRV Lagoa), e foram medidos em torno de 2 a 5 meses após a avaliação de sobreano. As características FL1 e FL2 foram avaliadas em conjunto com peso ao sobreano (YW). Os componentes de variância e covariância e os valores genéticos foram estimados por inferência Bayesiana, por meio de modelo animal bi-característica (três análises: FL1-FL2, YW-FL1 e YW-FL2). O estudo de associação genômica ampla (GWAS) para FL1 e FL2 foi realizado utilizando o método weighted single-step GBLUP, a partir do qual foram estimados os efeitos dos SNPs. As janelas top 10 de 1 Mb que explicam a maior proporção da variância genética foram observadas para cada característica. As médias (erros padrão) a *posteriori* das estimativas de herdabilidade foram iguais a 0,18 (0,04) e 0,39 (0,07), para FL1 e FL2, respectivamente. A estimativa de correlação genética entre FL1 e FL2 (-0,47) foi de moderada magnitude e negativa, conforme esperado, considerando que o escore de classificação que favorece cada característica corresponde a valores numéricos atribuídos em sentidos opostos. A correlação genética estimada entre FL2 e YW (0,39) sugere que a média do peso ao sobreano da população em estudo não é alta a ponto de causar associação negativa com a qualidade de aprumo. Os valores de tendência genética estimados para FL1 e FL2 (-0,043 e 0,021 desvio-padrão genético/ano, respectivamente) foram favoráveis e indicam que a estratégia de descarte independente para problemas de aprumo está contribuindo para o progresso genético destas características na população em estudo. A proporção da variância explicada pelas janelas top 10 foi igual a 8,96% para FL1 e FL2. Importantes regiões genômicas foram identificadas nos cromossomos: BTA 1, BTA 2, BTA 6, BTA 7, BTA 8, BTA 10 e BTA 14 para FL1; e BTA 1, BTA 7, BTA 10, BTA 11, BTA 18, BTA 20, BTA 22, BTA 28 e BTA 29 para FL2. A janela 64 do BTA 8 e 40 do BTA 22 explicaram a maior proporção da variância para FL1 e FL2, respectivamente. Os cromossomos BTA 2, BTA 8 e BTA 11, apresentaram importantes genes candidatos para aprumo em bovinos Nelore: *cytochrome b reductase 1*, *cathepsin L*, *interleukin - 1 beta* e *interleukin - 1, alpha*.

Palavras chave: aprumo, correlação genética, cromossomo, inferência Bayesiana, single step GBLUP, tendência genética

GENETIC ANALYSIS OF FEET AND LEGS IN NELORE CATTLE

ABSTRACT - The aim of this study was to estimate variance components of two traits associated to feet and legs in Nelore cattle and identify putative genomic regions underlying the expression of these traits through genome-wide association (GWA) analyses. Feet and legs was evaluated by the assignment of visual scores at two different moments: FL1) binary trait measured at yearling (about 550 days of age), aimed to identify whether an animal had defects related to feet and legs (FL1=1) or not (FL1=0); FL2) feet and legs score ranging from 1 (less desirable) to 5 (more desirable) was assigned to the top 20% animals for the selection index adopted by the beef cattle breeding program PAINT (CRV Lagoa), which were measured around 2-5 months after the evaluation of yearling. The FL1 and FL2 traits were evaluated together with yearling weight (YW). The components of variance and covariance and breeding values were estimated by Bayesian inference, using two-trait animal models (three analyses: FL1-FL2, YW-FL1 and YW-FL2). The genome-wide association (GWA) analyses for FL1 and FL2 was performed using the weighted single-step GBLUP method, from which were estimated the SNP effects. Functional annotation was focused on the ten 1Mb windows explaining the largest fraction of the genetic variance for each trait. Posterior means (standard errors) of heritability estimates were equal to 0.18 (0.04) and 0.39 (0.07), for FL1 and FL2, respectively. The estimate of genetic correlation between FL1 and FL2 (-0.47) was of moderate magnitude and negative, as expected considering that the classification score that favors each trait represents numerical values assigned in opposite directions. The genetic correlation estimated between FL2 and YW (0.39) suggests that the average yearling weight of the studied population is currently not high enough to cause a negative association with feet and legs problems. The genetic trends estimated for FL1 and FL2 (-0.043 and 0.021 genetic SD/year, respectively) were favorable and indicated that the independent culling strategy for feet and legs problems is contributing to the genetic progress of these traits, in the population under study. The proportion of variance explained by the top 10 windows was equal to 8.96% for both FL1 and FL2. Highly significant chromosome regions include: BTA 1, BTA 2, BTA 6, BTA 7, BTA 8, BTA 10 and BTA 14 for FL1 and BTA 1, BTA 7, BTA 10, BTA 11, BTA 18, BTA 20, BTA 22, BTA 28 and BTA 29 for FL2. The window 64 of BTA 8 and 40 of BTA 22 explained the highest proportion of variance for FL1 and FL2, respectively. BTA 2, BTA 8 and BTA 11 presented important candidate genes for feet and legs related traits in Nelore cattle: *cytochrome b reductase 1*, *cathepsin L*, *interleukin - 1 beta* and *interleukin - 1, alpha*.

Palavras chave: Bayesian inference, chromosome, feet and legs, genetic correlation, genetic trend, single step GBLUP

CAPÍTULO 1 – CONSIDERAÇÕES GERAIS

Dotado de características climáticas e extensão territorial favoráveis à pecuária de corte, com cerca de 20% de sua área ocupada por pastagens, o Brasil firma-se como um dos maiores produtores de carne bovina e, nos últimos anos, como um dos maiores exportadores de carne (MAPA, 2015). Segundo dados da Companhia Nacional de Abastecimento (Conab), o Produto Interno Bruto do setor agropecuário deverá crescer cerca de 4,9%, em 2014 (CNA, 2014). Estima-se que o valor bruto da produção agropecuária, ao final de 2014, terá crescimento de 6,8% em relação a 2013. Espera-se, segundo a Conab, que a valorização do boi gordo permaneça em 2015, proporcionando remuneração adequada ao produtor.

De acordo com a Associação Brasileira dos Criadores de Zebu, os animais das raças zebuínas (*Bos taurus indicus*) representam entre 75 a 80% do rebanho bovino brasileiro, aproximadamente 167 milhões de animais (MAPA, 2015). Os primeiros animais zebuínos chegaram ao Brasil, originários da África, entre os séculos XVII e XVIII, juntamente com escravos, com o objetivo de fornecer alimento ou servir como objeto de troca (SANTIAGO, 1983), mas foi entre os anos de 1850 e 1890, que animais zebuínos originários da Índia, foram importados visando sua habilidade de adaptação às condições tropicais (MAGNABOSCO et al., 1997). Na década de 1960, foram introduzidos alguns touros importantes devido as suas características fenotípicas e que contribuíram para sua caracterização, conformação e melhoramento do rebanho nacional (ACNB, 2009). A boa adaptação às condições tropicais brasileiras se deve a sua grande capacidade de resistência a parasitas, eficiência na conversão de gramíneas tropicais, longevidade e fertilidade (KOURY FILHO, 2005).

No Brasil, onde a maioria dos rebanhos bovinos é criada em regime semi-extensivo (estima-se que apenas 3% do rebanho são terminados em sistema intensivo), em grandes áreas de pastagens, onde os animais percorrem grandes distâncias em busca de alimento e água, e estão sujeitos a grande variabilidade climática, bons aprumos são cruciais. Segundo Boggs, Merkel e Doumit (1998), animais com pernas muito retas podem apresentar dificuldade durante o processo de reprodução no momento da cobertura, e animais com pernas curvas tendem a

apresentar problemas de articulação. Animais que não se locomovem satisfatoriamente, não se alimentam bem, perdem em condição corporal, e conseqüentemente em desempenho produtivo e reprodutivo. Uma possível forma de minimizar problemas de aprumo seria conduzir avaliações genéticas desta característica e utilizá-la como critério de seleção. Nesse sentido, torna-se necessário conduzir estudos quantitativos para estimar a magnitude da variabilidade genética e da herdabilidade de problemas de aprumo.

Uma alternativa complementar à análise quantitativa seria utilizar informações de marcadores moleculares para aumentar a acurácia de predições dos valores genéticos, por meio de seleção genômica, onde se objetiva a utilização de toda informação de polimorfismos (usualmente do tipo SNP - *single nucleotide polymorphism*) que estejam em desequilíbrio de ligação com QTL's (*quantitative trait loci*), e/ou identificar possíveis regiões no genoma com efeito importante sobre a qualidade dos aprumos por meio de estudos de associação genômica ampla (GWAS) (MEUWISSEN & GODDARD, 2004; PEARSON & MANOLIO, 2008).

GWAS fundamentam-se no uso de marcadores genéticos moleculares, geralmente do tipo SNP, densamente distribuídos ao longo do genoma. A identificação de tais marcadores se baseia na detecção de polimorfismo resultante da alteração de um único par de base do genoma, conferindo vantagens importantes, tanto no poder para detectar variantes causais quanto na definição de regiões genômicas que abrigam estas variações (HIRSCHHORN & DALY, 2005).

1. REVISÃO DE LITERATURA

1.1. Característica Aprumo

O aprumo pode ser definido como a direção normal dos membros em toda a sua extensão ou, em particular, das suas diferentes regiões, de forma a sustentar solidamente o corpo do animal e permitir seu deslocamento fácil, podendo ser medido em diferentes fases (ex.: sobreano) (KOURY FILHO, 2005). Aprumo constitui importante item da avaliação fenotípica, pois depende de parâmetros como

proporções, direções, angulações e articulações dos membros anteriores e posteriores, além das estruturas de ossos, tendões e ligamentos (SIMIELLI FILHO, 2012). Animais que pisam tortuosamente, podem apresentar problemas ósseos e nos cascos, comprometendo seu desempenho produtivo e reduzindo a sua longevidade (KOURY FILHO, 2005).

Segundo Marega (2013), para a avaliação de aprumo, na prática, faz-se uso do bom senso e educação visual por parte do técnico, que podem ser aprimorados por meio de treinamento e do hábito de julgamento. Para uma correta avaliação, os animais são posicionados e observados de frente, de lado e por trás, estáticos e em movimento. De maneira geral, tanto pernas curvas quanto extremamente retas podem prejudicar a vida produtiva do animal no rebanho em virtude de problemas de locomoção. Quando avaliado de frente, o aprumo pode apresentar alguns desvios, caracterizando irregularidades posturais, como elevada ou reduzida distância entre os joelhos (Figura 1A e 1C) e elevada ou reduzida distância entre as pinças dos cascos (Figura 1B e 1C). Visto de lado, as principais irregularidades observadas são em relação ao joelho (Figura 1D e 1E), angulação dos cascos (Figura 1F), altura ou profundidade dos talões e força das quartelas. Visto por trás, os defeitos de aprumo mais comuns ocorrem em relação a distância entre os jarretes e medidas entre as pinças dos cascos.

A qualidade do aprumo está diretamente relacionada à saúde geral do animal e ao tempo de vida no rebanho, sendo que qualquer irregularidade postural pode determinar condições de desconforto de diferentes formas e graus. Sewalen et al. (2004), estudando animais da raça Holandesa, relataram que vacas que apresentam pernas muito retas ou muito curvas, têm sua longevidade afetada de maneira negativa, ocasionando menor vida produtiva, mesmo que o animal apresente capacidade genética favorável para outras características de relevância econômica.



A) Elevada distância entre os joelhos



B) Pinças voltadas para dentro



C) Reduzida distância entre os joelhos e pinças voltadas para fora



D) Pernas retas com reduzida curvatura



E) Pernas excessivamente curvas



F) Achinelamento das unhas

Figura 1. Exemplos de defeitos de aprumo que podem ser observados no animal quando avaliado de frente (Figura 1A, 1B e 1C) e de lado (Figura 1D, 1E e 1F).

Fonte: MAREGA, 2013.

Na bovinocultura de leite, afecções relacionadas a aprumo ocupam a terceira posição dentre os fatores que mais contribuem para o descarte involuntário de vacas em todo o mundo, sendo que em primeiro lugar estão as disfunções reprodutivas, como a sub-fertilidade e infertilidade, e em segundo lugar as afecções da glândula mamárias, como a mastite (MAREGA, 2013). Segundo este autor, dentre as características de seleção de um rebanho leiteiro, a qualidade do aprumo, é para muitos técnicos, criadores e selecionadores, a primeira característica a ser selecionada, pois possui relação direta ou indireta com todas as demais características, como por exemplo, capacidade corporal, força, e características de úbere. Souza et al. (2006) avaliando os prejuízos econômicos ocasionados por enfermidades podais em um rebanho de 100 fêmeas em lactação durante um ano, obteve custo com tratamento e redução na produção de leite de US\$52.69/vaca/ano, o custo adicional anual total no rebanho decorrente de sequelas de enfermidades foi de US\$12,536.70.

De maneira geral, ao nascerem, os animais possuem bons aprumos. À medida que se desenvolvem, o sistema locomotor sofre influência de fatores genéticos, que podem ou não se expressar durante esta fase da vida, transformando ou alterando a postura, além da influência do ambiente, como superalimentação, oferta e distribuição de alimento e água e a topografia do ambiente de pastagem, que podem afetar permanentemente os ossos e articulações. Animais criados a pasto tendem a apresentar menor incidência de problemas posturais, uma vez que seus pés e membros possuem reduzida capacidade de absorção de impactos causados por superfícies irregulares e abrasivas, considerando sua pequena área de apoio no solo, pouca capacidade de amortecimento, especialmente dos membros pélvicos, e seu peso excessivo (PLAUTZ, 2013).

Pesquisas relacionadas à característica aprumo são escassas em bovinos de corte, sendo a grande maioria da literatura sobre aprumos proveniente de estudos de conformação de bovinos de leite. A magnitude da herdabilidade estimada para aprumo varia de baixa a moderada (BERRY et al., 2004; HAGGMAN, JUGA, 2013; CHAPINAL et al., 2013), e pode estar relacionada a avaliação no momento da classificação e pontuação da característica, dependendo diretamente da qualidade da coleta dos registros mensurados.

1.2. Estudos de associação genômica ampla

Estudos de associação genômica ampla (GWAS) utilizam informação de polimorfismos existentes ao longo de todo o genoma, em conjunto com informações de fenótipo (e eventualmente informações de pedigree), para realizar uma análise de associação e, desse modo, identificar regiões no genoma que são importantes para características de interesse (ZHANG et al., 2012).

O método estatístico *Single Step GBLUP* (ssGBLUP), proposto por MISZTAL, LEGARRA e AGUILAR (2009), trata-se de uma modificação do método BLUP para combinar toda a informação genômica, fenotípica e de pedigree em um único passo para a predição dos valores genéticos. No ssGBLUP, a matriz de parentesco genômico (G) é estimada utilizando-se a informação dos genótipos dos animais, diferentemente do BLUP tradicional, em que a matriz de parentesco (A) é baseada na informação de pedigree. O ssGBLUP combina informações dos indivíduos genotipados e não genotipados, os quais são representados pelas matrizes A e G respectivamente, que são combinadas em uma matriz H (LEGARRA et al., 2009). A inversa de H tem forma simples e pode substituir a inversa de A em *softwares* pré-existentes usados para implementar o método BLUP (AGUILAR et al., 2010).

Uma questão importante na implementação do ssGBLUP é a definição da base/escala para a construção da matriz de parentesco genômico (G), que deve estar em escala compatível com a matriz de parentesco tradicional (A). Neste sentido, as frequências alélicas são usadas para construir a matriz G, podendo-se assumir valor constante para as frequências alélicas de todos os loci (usualmente 0,5), ou ainda utilizar-se as frequências específicas para cada loco, sejam estas calculadas com a amostra genotipada ou utilizando-se algoritmo para estimar a frequência alélica de cada loco na população base (VAN RADEN, 2008; AGUILAR et al., 2010; FORNI et al., 2010).

Além de ser utilizado para predição dos valores genéticos, o ssGBLUP também pode ser adaptado para GWAS (WANG et al., 2012). Estes autores propuseram a aplicação da metodologia “Weighted Single Step GBLUP”

(wssGBLUP), que permite combinar informações de pedigree, de fenótipo e de genótipo em um único passo, ponderando o efeito dos SNPs associados ao QTL de interesse o que, segundo os autores, aumentaria a acurácia, sobretudo na avaliação de características que apresentam alguns genes com efeito mais expressivo do que outros. Tal metodologia seria justificável, sobretudo, em situações em que apenas parte dos animais com informação fenotípica disponível é genotipada, eliminando a necessidade de múltiplos passos para converter a informação disponível em pseudo-fenótipos associados aos animais genotipados (e.g. valores genéticos preditos ou provas desregredidas) (VAN RADEN et al., 2009). Procedimentos em múltiplos passos, via de regra, resultam em problemas como uso de aproximações, perda de informação e heterogeneidade de variâncias que poderiam ser minimizados com a abordagem usada no ssGBLUP (VITEZICA et al., 2011).

Apesar da importância da característica aprumo em bovinos de corte, ainda são escassos estudos quantitativos para a estimação da magnitude da variabilidade e parâmetros genéticos da característica, evidenciando a necessidade de condução de pesquisa como forma mais eficiente de minimizar a incidência de problemas de aprumo nos rebanhos.

2. OBJETIVO

Estimar os componentes de variância, a herdabilidade e a tendência genética da característica aprumo em bovinos da raça Nelore, assim como realizar estudos de associação genômica ampla para essa característica, visando identificar possíveis regiões genômicas relacionadas com sua expressão e fornecer subsídios aos programas de melhoramento genético da raça, para a redução da ocorrência de animais com problemas de aprumo.

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CHAPTER 2 - GENETIC ANALYSIS OF FEET AND LEGS IN NELORE CATTLE

ABSTRACT - The aim of this study was to estimate variance components of feet and legs in Nelore cattle. Feet and legs was evaluated by the assignment of visual scores at two different moments: FL1) binary trait measured at yearling (about 550 days of age), aimed to identify whether an animal had defects related to feet and legs (FL1=1) or not (FL1=0); FL2) feet and legs score ranging from 1 (less desirable) to 5 (more desirable) was assigned to the top 20% animals for the selection index adopted by the beef cattle breeding program PAINT (CRV Lagoa), which were measured around 2-5 months after the yearling evaluation. The FL1 and FL2 traits were analyzed together with yearling weight (YW). The components of variance and covariance and breeding values were estimated by Bayesian inference, using two-trait animal models (three analyses: FL1-FL2, YW-FL1 and YW-FL2). Posterior means (standard errors) of heritability estimates were equal to 0.18 (0.04) and 0.39 (0.07), for FL1 and FL2, respectively. Results indicate that the incidence of feet and legs problems in this population could be reduced through selection. The estimate of genetic correlation between FL1 and FL2 (-0.47) was of moderate magnitude and negative, as expected, considering that the classification score that favors each trait represents numerical values assigned in opposite directions. The genetic correlation estimated between FL2 and YW (0.39) suggests that the average yearling weight of the studied population is currently not high enough to cause a negative association with feet and legs problems. The genetic trends estimated for FL1 and FL2 (-0.043 and 0.021 genetic SD/year, respectively) were favorable and indicated that the independent culling strategy for feet and legs problems is promoting favorable changes and contributing to the genetic progress of these traits in the population under study.

Keywords: Bayesian inference, beef cattle, genetic correlation, genetic trend, heritability

1. INTRODUCTION

Most beef cattle in Brazil are produced under semi extensive conditions. Having adequate locomotion is essential to allow good productive performance and welfare, because animals are required to walk in relatively large areas of grasslands searching for food and water. Proper feet and leg structure is also needed for natural service bulls to successfully breed cows as well as to ensure reproductive longevity. For instance, excessively straight-legged bulls are somewhat prone to suffer injuries during mating (BOGGS; MERKEL; DOUMIT, 1998), what would invariably result in economic losses.

For Nelore cattle, some Brazilian breeding programs have established a system to routinely evaluate feet and leg structure through visual scores. Currently, some breeding programs use feet and legs as an independent culling criterion, in which animals that phenotypically presents feet and legs problems are discarded or used less intensively in reproduction. However, this strategy can lead to remarkable economic losses because some of the culled animals have great genetic merit for other traits of economic relevance (e.g. growth and carcass traits).

A more efficient way to reduce the incidence of feet and legs problems, besides independent culling, would be to carry out genetic evaluations for these traits and use the expected breeding values as selection criterion, stressing the importance of studying the magnitude of genetic variability and the heritability of the trait, and possible genetic association with other traits undergoing selection.

Berry et al. (2004), Häggman & Juga (2013) and Chapinal et al. (2013), studying data of dairy cows, obtained heritability estimates for type traits related to feet and legs ranging from 0.03 to 0.22. In a recent study using data of Finnish Holstein cows, the estimated genetic correlations among six different feet and legs conformation traits ranged from -0.30 to 0.83 (HAGGMAN & JUGA, 2013). According to Perez-Cabal et al. (2006) the type traits that have more influence on the profits of dairy business, after considering production, are feet and legs.

Despite the importance that feet and legs have for the producers, there are few quantitative studies regarding this trait, being necessary more research in this

area to find possible solutions to reduce the incidence of feet and legs problems in the herds.

This study was carried out to estimate variance components, genetic parameters and genetic trends for two traits related to feet and legs in Nelore cattle, as well as to study their association with yearling weight, by using data from a commercial breeding program.

2. MATERIALS AND METHODS

Data. Phenotypic records for feet and legs as well as pedigree information were obtained from Nelore cattle participating in PAINT®, the beef cattle breeding program headed by the company CRVLagoa Ltda. The animals were raised in tropical pasture systems, in herds located in Brazil and Paraguay totaling approximately 300,000 animals with own records for yearling weight, collected between 2000 and 2013.

Feet and legs were evaluated by the assignment of visual scores to evaluate the overall structure for feet and legs at two different moments, leading to the definition of two different traits: FL1) a binary trait measured at yearling (about 550 days of age), aimed to identify whether an animal had defects related to feet and legs (FL1=1) or not (FL1=0); and FL2) feet and legs score ranging from 1 (less desirable) to 5 (more desirable) was assigned to the top 20% animals for the selection index adopted in this breeding program, which were candidates to receive CEIP (Special Certificate of Identification and Production) – an official certificate that testify the value of seedstock delivered by breeding programs, being a pre-requisite to commercialize seedstock from animals that are not registered in breeder associations in Brazil but are genetically evaluated as superior (top 20%) animals (HORIMOTO et al., 2007).

In the breeding program considered in this study, animals that receive score 1 for FL1 cannot be candidates to receive CEIP, and thus are not evaluated for FL2. The evaluation of FL2 occurs a few days after the animals are evaluated at yearling, when the results of routine genetic evaluations are released and the CEIP candidates

defined. Furthermore, animals having undesirable score for FL2 (FL2=1) are not allowed to receive the CEIP certificate, what quite often results in economic losses, due to the fact that genetically superior animals for growth and carcass traits have to be culled.

Statistical methods. In preliminary analyses, generalized linear mixed models were fitted in lme4 package in R software (R Development Core Team, 2011), in order to identify environmental effects influencing FL1 and FL2. Based on the results of such analyses, the final model for the genetic analysis of both traits included a fixed effect of contemporary group (CG) and the linear effect of yearling age as covariate. CG was defined based on the concatenation of the effects of herd, year, and season of birth, management group at weaning, date of measurement at yearling and management group at yearling. To be considered in the genetic analyses, CG was required to have a minimum of 10 animals as well as variability for the respective trait. Genetic connectedness among CG was verified using the AMC software (ROSO & SCHENKEL, 2006), being that only data from CG connected to the main set, i.e. presenting more than 10 genetic links to the main set, were kept in the analyses.

Variance component estimates and breeding values predictions were obtained using Bayesian inference. Three different bi-trait analyses were performed, adjusting a threshold-threshold (FL1-FL2) or a linear-threshold (YW-FL1; YW-FL2) animal model, using the THRGIBBS1F90 software (MISZTAL, 2014). Analyses considering YW were carried out aiming at evaluating possible correlated response of the selection for YW on feet and legs, and also to alleviate the effect of sequential selection in the analyses of FL2 (POLLAK & QUAAS, 1981). YW is routinely evaluated in the breeding program and its genetic proof comprises part of the selection index.

Two-trait model. The statistical model employed in the different analyses can be represented in matrix notation as:

$$\mathbf{y}_i = \mathbf{X}\boldsymbol{\beta}_i + \mathbf{Z}\mathbf{a}_i + \mathbf{e}_i, (1)$$

in which \mathbf{y}_i is a vector of observations (or liabilities, in the case of FL1 and FL2) for the i th trait ($i = 1,2$); $\boldsymbol{\beta}_i$ is a vector of systematic effects (CG and a linear effect of yearling age); \mathbf{a}_i is a vector of additive direct genetic effects; \mathbf{e}_i is a vector of random residual effects; and \mathbf{X} e \mathbf{Z} are incidence matrices that relate the phenotypic information of the i th trait to the effects $\boldsymbol{\beta}_i$ and \mathbf{a}_i , respectively. For feet and legs (FL1 and FL2), it is expected that the inclusion of the CG effect in the model accounts for part of the environmental effects and corrects for the effect of evaluator, given that a single technician evaluates all the animals of the same management group. The sex of the animal was not included in the definition of CG, given that the management groups presented animals of the same sex.

The assumptions of model (1) can be described as follows:

$$E \begin{bmatrix} y_i \\ a_i \\ e_i \end{bmatrix} = \begin{bmatrix} X\boldsymbol{\beta}_i \\ 0 \\ 0 \end{bmatrix}$$

The covariance structure between the random effects in (1) can be described as follows:

$$\mathbf{Var} \begin{bmatrix} \mathbf{a} \\ \mathbf{e} \end{bmatrix} = \begin{bmatrix} \mathbf{A} \otimes \boldsymbol{\Sigma}_a & \mathbf{0} \\ \mathbf{0} & \mathbf{I}_N \otimes \boldsymbol{\Sigma}_e \end{bmatrix},$$

in which $\boldsymbol{\Sigma}_a$ is the additive direct genetic covariance matrix, $\boldsymbol{\Sigma}_e$ is the residual covariance matrix, \mathbf{A} is the numerator relationship matrix, \otimes denotes the direct product between matrices, \mathbf{I} is an identity matrix, and \mathbf{N} is the number of animals. Since there was no variation in FL1 within levels of FL2 (i.e. FL1=0 for all animals evaluated for FL2), the residual covariance between FL1 and FL2 would not be estimable and thus was assumed to be zero.

In the analysis of FL1, considering that the variable in the underlying distribution is not observable, the parameterization $\sigma_e^2 = 1$ was adopted, to permit the identification of the variable in the likelihood function (GIANOLA & SORESENSEN,

2002). In the case of FL2, the thresholds t_1 and t_2 were kept fixed at 0 and 1, respectively, following Sorensen et al. (1995), so that σ_e^2 could be estimated.

Threshold Model. In the case of the analyses of FL1 and FL2, a threshold model was assumed.

Threshold models postulate that categorical traits are determined by nonobservable continuous variables in an underlying scale (hereafter, liabilities), so that a set of $(j-1)$ thresholds correspond to the discontinuities in the observable scale, for j mutually exclusive ordered categories. Thus initial threshold values are fixed as follows: $t_1 < t_2 < \dots < t_{j-1}$, under the assumption that $t_0 = -\infty$ and $t_j = +\infty$ (GIANOLA & FOULLEY, 1983). Thus, in the case of FL1 and FL2, it is assumed that the categories or scores for each animal i are defined by the liability U_i in the underlying scale, as follows:

$$\text{FL1: } \{ \text{FL1}_i = 0 \text{ if } U_i \leq t_1 \\ \text{FL1}_i = 1 \text{ if } U_i > t_1 \}$$

$$\text{FL2: } \{ \text{FL2}_i = 1 \text{ if } U_i \leq t_1; \\ \text{FL2}_i = 2 \text{ if } t_1 < U_i \leq t_2; \\ \text{FL2}_i = 3 \text{ if } t_2 < U_i \leq t_3; \\ \text{FL2}_i = 4 \text{ if } t_3 < U_i \leq t_4 \\ \text{FL2}_i = 5 \text{ if } t_4 < U_i \leq t_5 \}$$

Bayesian inference. A single chain with a length of 1,000,000 cycles was generated for each analysis, being that a conservative burn-in of 100,000 cycles and a thinning interval of 250 cycles were applied. The coda package of R software (R Development Core Team, 2013) was employed to assess the convergence of chains through visual inspection and also using Geweke's (GEWEKE, 1992) and Heidelberger & Welch (HEIDELBERGER & WELCH, 1983) tests.

Genetic trends were estimated by linear regression of the posterior means of predicted breeding values for each trait on the year of birth. The posterior means of predicted breeding values were standardized in terms of the posterior mean of the

genetic standard deviation. Attempts were made trying to associate individual inbreeding coefficient of the animals to their corresponding predicted breeding values for FL1 and FL2.

3. RESULTS AND DISCUSSION

For FL2, most of the animals were classified with scores ranging from 3 to 5 (71.2 %) (Table 1), suggesting that most animals presented satisfactory feet and legs structure. The incidence of animals presenting defective feet and legs was about 5% for FL1 and 7% for FL2 (Table 1), which means that considerable economic losses could have occurred as these animals are, in general, unable to perform their productive and reproductive function satisfactorily as a consequence of feet and legs problems.

Table 1. Summary statistics of feet and legs in Nelore cattle

Trait	Data structure				Score frequency distribution*					
	N	NCG	NS	ND	0	1	2	3	4	5
FL1	96836	2105	748	73272	92469 (95.5%)	4367 (4.5%)	-	-	-	-
FL2	14708	897	340	12920	-	1040 (7.1%)	3192 (21.7%)	6088 (41.4%)	3616 (24.6%)	772 (5.2%)

FL1 = feet and legs evaluated as a binary trait (scores assigned to all animals measured at yearling)

FL2 = feet and legs scores ranging from 1 (less desirable) to 5 (more desirable), (assigned to the top 20% animals for the selection index applied in this population)

N = number of observations

NCG = number of contemporary groups

NS = number of sires

ND = number of dams

*Absolute frequencies of each score are present followed by the relative proportion (in %).

The average estimates of direct heritability and genetic correlations for the traits FL1, FL2, and YW obtained from two-trait analyses are presented in Table 2.

Table 2. Heritability estimates (h^2) for FL1, FL2, and YW obtained in two-trait analyses (diagonal), genetic (above the diagonal) and environmental (below diagonal) correlations*, and their standard deviations (cited in brackets) for Nelore cattle.

Trait	FL1	FL2	YW
FL1	0.18 (0.04)	-0.47 (0.02)	0.04 (0.07)
FL2	-	0.39 (0.07)	0.39 (0.06)
YW	-0.19 (0.03)	0.11 (0.03)	0.47 (0.01)

* Estimates obtained as the average of the marginal posterior distribution of the parameters

FL1 = feet and legs evaluated as a binary trait (scores assigned to all animals measured at yearling)

FL2 = feet and legs scores ranging from 1 (less desirable) to 5 (more desirable) (assigned to the top 20% animals for the selection index applied in this population, composed by productive and reproductive traits measured at weaning and yearling)

YW = yearling weight (measured at around 550 days)

The heritability estimate of FL1 was lower than that of FL2 (posterior means about 0.18 and 0.39, respectively). Such result may be explained by a lack of systematic routine evaluation of feet and legs in more detail at yearling, so that only the most extreme cases end up being notified. Also the dichotomization of feet and legs evaluation in FL1 may cause some loss of information when compared to FL2, resulting in smaller variability. For polychotomous traits, similar type of dichotomization had been associated to loss of power in genetic studies (BRISBIN et al., 2010). Another aspect that could influence the differences between heritability estimates for FL1 and FL2 may be related to lower reproducibility of the evaluations made by the technicians responsible for performing the evaluation of feet and legs at yearling, when compared to more detailed evaluations carried out for FL2.

Heritability estimates and additive genetic variance obtained for FL2 (0.39 and 0.07) were similar to those obtained by Passafaro et al. (2013) (0.36 and 0.08), which analyzed data from a different Nelore population under a slightly different evaluation system (scores ranging from 1 to 6). Such findings suggest that feet and legs should respond to selection. Considering that FL2 has more evaluation categories than FL1, it is theoretically more appropriate to describe the variability between the animals for feet and legs. A disadvantage of FL2 is that it is evaluated just in part of the yearling

animals and possibly more prone to bias due to pre-selection. An alternative would be to treat both, FL1 and FL2, as complementary traits in routine genetic evaluation.

The estimate of genetic correlation between FL1 and FL2 (-0.47) was negative and of moderate magnitude. The sign of this estimate matched the expectation, considering that for FL1, the lower classification score (0) suggests that the animal does not present defective feet and legs, while for FL2, animals with better feet and legs are those that receive the highest score classification. The same factors described previously in speculating the reason for the difference between heritability estimates for FL1 and FL2 can also help to explain why the genetic correlation between FL1 and FL2 was not stronger. According to some authors (MISZTAL; SHORT; LAWLOR, 1992; DEGROOT et al., 2002; RENNÓ et al., 2003; BERRY et al., 2004; LAGROTTA et al., 2010), in dairy cattle, traits that compose the feet and legs section are negatively correlated, being the highest absolute value described by Berry et al. (2004), who obtained genetic correlation of -0.88 between the traits leg position and hoof angle.

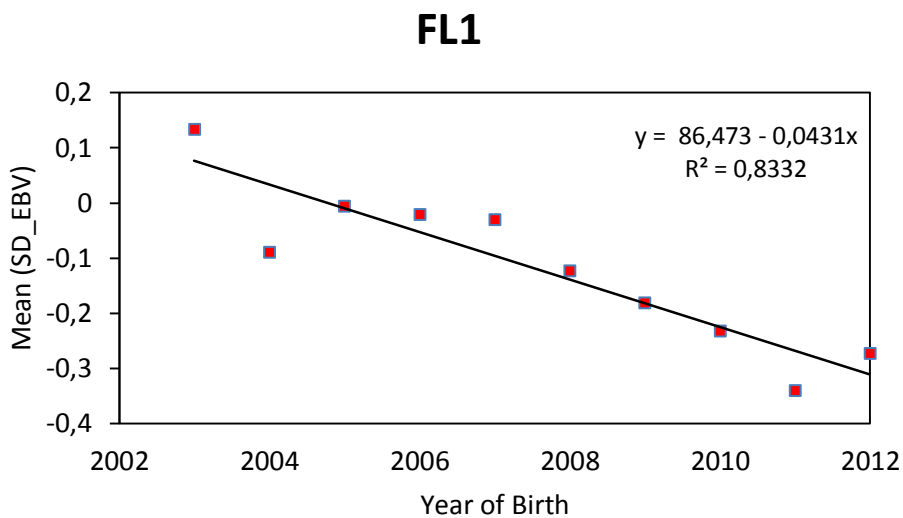
The estimate of genetic correlation between YW and feet and legs was of low (0.04) and moderate magnitude (0.39) for FL1 and FL2, respectively. The positive genetic correlation between YW and FL2 suggests that the selection for YW can lead to a desirable indirect genetic response for FL2, and is also an indicative that the average yearling weight of the studied population is currently not high enough to cause a negative association with feet and legs problems. Kuhlert and McDaniel (1996) and Campos et al. (2012) found estimates of genetic correlation close to zero between body weight and feet and legs associated traits in broiler chickens and Holstein cows, respectively. In the other hand, Wong-Valle et al. (1993), studying different lineages of broiler chickens, observed that the line with the highest incidence of feet and legs problems was the one presenting the larger body weight. Kapell (2013) obtained genetic correlations between leg traits and body weight in purebred broiler lines of low to moderate magnitude (0.09 to 0.25).

Estimates of environmental correlations of YW with FL1 (-0.19) and FL2 (0.11) were of low magnitude. The residual correlation between FL1 and FL2 was not estimated, because when the animals had some kind of defect of feet and legs, i.e.,

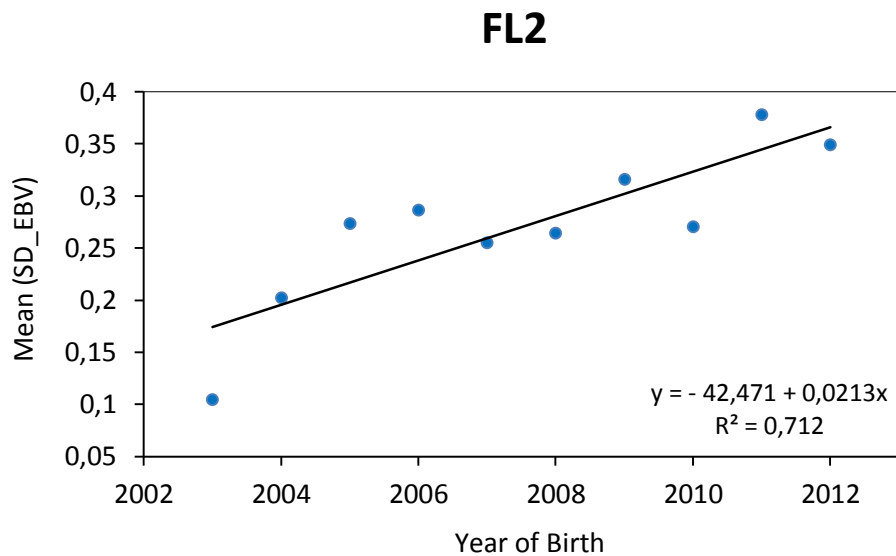
received score 1 for FL1, they were not candidates to receive the certificate of genetic superiority and, therefore, were not evaluated for FL2.

The genetic trends for FL1, FL2 and YW are presented in Figure 2. The coefficients of determination of the models used to regress the average breeding values for FL1, FL2 and YW over the year of birth of the animals were all higher than 0.70, indicating a reasonable fit of the linear regression. Genetic trends equal to -0.043 and 0.021 standard deviations per year were obtained for FL1 and FL2, respectively. Although relatively small, the genetic trends for FL1 and FL2 occurred in the desirable direction. This result suggests that the independent culling strategy for feet and legs problems adopted by the breeding program is causing favorable changes, contributing to the genetic progress of the population. The genetic trend for FL2 can also be a consequence of indirect response to selection, as YW was genetically positive correlated to FL2 and also presented a favorable genetic trend (Figure 2B). No genetic trends for feet and legs traits were found in the literature to serve as a basis for comparisons.

A)



B)



C)

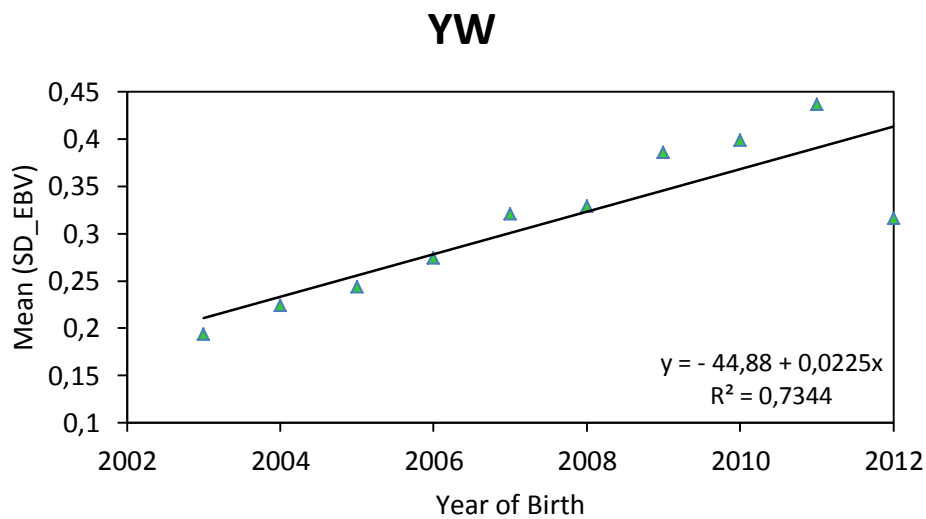


Figure 2. Genetic trends of feet and legs associated to traits FL1 (Figure 1A) and FL2 (Figure 1B) and of yearling weight (Figure 1C), for a population of Nelore cattle.

Aiming to complement the study, in an attempt to find association between individual inbreeding coefficient of the animals to their corresponding predicted breeding values, no significant associations were found (data not shown). However, it

is not clear at which extent this result is related to a lack of influence of inbreeding in feet and legs problems or due to the low quality of the available pedigree information, as roughly half of the calves controlled in this breeding program have unknown sire, due to the use of multiple sire mating.

4. CONCLUSION

The estimates of additive genetic variability and heritability obtained for the traits suggest that the incidence of feet and legs problems in this population could be reduced through selection. No antagonism was observed between feet and legs problems and yearling weight.

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CHAPTER 3 - GENOME-WIDE ASSOCIATION OF FEET AND LEGS IN NELORE CATTLE

ABSTRACT - The aim of this study was to identify putative genomic regions underlying the expression of two traits associated to feet and legs in Nelore cattle through genome-wide association (GWA) analyses. Feet and legs was evaluated by the assignment of visual scores at two different moments: FL1) binary trait measured at yearling (about 550 days of age), aimed to identify whether an animal had defects related to feet and legs (FL1=1) or not (FL1=0); FL2) feet and legs score ranging from 1 (less desirable) to 5 (more desirable) was assigned to the top 20% animals for the selection index adopted by the beef cattle breeding program PAINT (CRV Lagoa), which were measured around 2-5 months after the evaluation of FL1. Single-trait threshold animal models were used for GWA analyses of FL1 and FL2. The SNP effects were estimated using the weighted single-step GBLUP method. Functional annotation was focused on the ten 1Mb windows explaining the largest fraction of the genetic variance for each trait. The proportion of variance explained by the top 10 windows was equal to 8.96% for both FL1 and FL2. Highly significant chromosome regions include: BTA 1, BTA 2, BTA 6, BTA 7, BTA 8, BTA 10 and BTA 14 for FL1 and BTA 1, BTA 7, BTA 10, BTA 11, BTA 18, BTA 20, BTA 22, BTA 28 and BTA 29 for FL2. The window 64 of BTA 8 and 40 of BTA 22 explained the highest proportion of variance for FL1 and FL2, respectively. BTA 2, BTA 8 and BTA 11 presented important candidate genes for feet and legs related traits in Nelore cattle: the *cytochrome b reductase 1*, *cathepsin L*, *interleukin - 1 beta* and *interleukin - 1, alpha*.

Keywords: *Bos taurus indicus*, chromosome, feet and legs, single step GBLUP

1. INTRODUCTION

Feet and legs is one of the most important traits determining production in livestock. If an animal has bad legs, this problem has a major effect on longevity and productivity (PÉREZ-CABAL & ALENDA, 2002). Several dairy cattle studies consider conformation trait feet and legs as different subtraits like rear legs rear view, rear legs side view, foot angle, hocks, and bone quality. Reported heritability estimates for these traits range from 0.12 to 0.41 (VAN DORP et al., 1998; PÉREZ-CABAL & ALENDA, 2002; HIENDLEDER et al., 2003), so that feet and legs could be used as an indicator trait for other important traits, for example, claw disorders and lameness.

Previous studies from different countries have shown that one can detect QTL associated with feet and leg related traits in dairy cattle (ASHWELL et al., 1998; SCHROOTEN et al., 2000; BOICHARD et al., 2003; BUITENHUIS et al., 2007), aiming to use this knowledge as auxiliary information in the selection process.

Genome-wide association studies (GWAS) using single nucleotide polymorphism (SNP) markers allow identifying genomic regions affecting many economically important traits in livestock species. Recently, Wang et al. (2012) proposed an alternative approach of GWAS, the “Weighted Single Step GBLUP” method (wssGBLUP), which allows combining pedigree, phenotype and genotype information in a single step, weighting the SNP effects proportionally to their suggested relevance. In the wssGBLUP method, there is no need to restrict the use of phenotypes of animals that were not genotyped or include pseudo phenotypes. This is an advantageous method when working with complex models, such as threshold models, or using multiple traits, and in cases where there are many phenotyped animals, but few genotyped, a common situation in real databases, which is the case in this study.

The aim of this study was to identify putative genomic regions affecting two feet and legs related traits in Nelore cattle. For this, a GWAS using the wssGBLUP method was performed.

2. MATERIALS AND METHODS

Phenotypic and pedigree data. Phenotypic records and pedigree information were obtained from the commercial breeding program PAINT® (CRV Lagoa, Sertãozinho, SP, Brazil). Data on feet and legs related traits were collected between 2003 and 2013, from Nelore animals raised under tropical pasture systems in 169 different herds located in Brazil and Paraguay.

Feet and legs were evaluated by the assignment of visual scores to evaluate the overall structure for feet and legs at two different moments, leading to the definition of two different traits: FL1) a binary trait measured at yearling (about 550 days of age), aimed to identify whether an animal had defects related to feet and legs (FL1=1) or not (FL1=0); and FL2) feet and legs score ranging from 1 (less desirable) to 5 (more desirable) was assigned to the top 20% yearling animals (based on the selection index adopted by the breeding program), which were candidates to receive a certificate of genetic superiority (CEIP). The animals that receive score 1 for FL1 are not candidates to receive CEIP, and are not evaluated for FL2. The evaluation of FL2 occurs a few days after the animals are evaluated at yearling, when the results of routine genetic evaluations are released and the CEIP candidates defined.

Data from contemporary groups (CG) with less than 10 records and presenting no variability for the respective trait were discarded. CG were defined concatenating the effects of herd, year, and season of birth, management group at weaning, date of measurement at yearling and management group at yearling. Connectedness among CG was verified using the AMC software (ROSO & SCHENKEL, 2006). Data from disconnected CG, i.e. presenting less than 10 genetic links to the main dataset, were also discarded. After editing, the data contained 96836 records for FL1 and 14708 records for FL2, distributed over 2105 and 897 CG, respectively. The pedigree file contained a total of 188694 animals distributed over 5 generations.

Genotypes. A total of 1435 genotypes from 667 yearling animals, 402 sires and 366 dams were used in the genome-wide association (GWA) analyses. These animals constitute part of the genomic reference population of PAINT®, and they were chosen among those animals presenting progeny or own performance for FL1 or FL2. The sires were genotyped with the Illumina® BovineHD chip (HD), and the

yearling animals and dams were genotyped with the Illumina® BovineSNP50 v2 chip (Illumina Inc., San Diego, CA, USA) and had their HD missing genotypes imputed using the FImpute v2.2 software (SARGOLZAEI; CHESNAIS; SCHENKEL, 2014).

Subroutines in the software R (R Development Core Team, 2013) were used to perform the quality control (QC) of the genotypes. SNP markers from non-autosomal regions, mapped to the same position, presenting a p-value for the Hardy-Weinberg equilibrium test lower than 10^{-5} , with GC score lower than 0.15, call rate lower than 0.95 and minor allele frequency lower than 0.02 were discarded from the GWA analyses. The remaining number of SNPs after QC was 407,730. All samples presented a call rate higher than 0.9 and were kept for the analyses.

Genome-wide association analyses. Single-trait threshold animal models were used for GWA analyses of FL1 and FL2. The SNP effects were estimated using the weighted single-step GBLUP method proposed by Wang et al. (2012), which is advocated to be advantageous over other methods for allowing combining in a single-step genomic information with pedigree and phenotypes, from genotyped and non-genotyped animals, weighting the markers proportionally to their indicative importance for the respective trait (WANG et al., 2012).

The SNP effect estimates were obtained from the predicted breeding values, which were calculated according to the model:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}_a\mathbf{a} + \mathbf{e} ,$$

in which: \mathbf{y} is a vector of underlying liabilities of FL1 or FL2, $\boldsymbol{\beta}$ is a vector of systematic effects (CG and a linear effect of yearling age); \mathbf{a} is a vector of random additive direct genetic effects (breeding values); \mathbf{e} is a vector of random residual effects; and \mathbf{X} e \mathbf{Z}_a are incidence matrices that relate the liabilities in \mathbf{y} to the effects in $\boldsymbol{\beta}$ and \mathbf{a} , respectively. Given that the management groups presented animals of the same sex and that a single technician evaluated all the animals of the same management group, there was no necessity to include both effects (sex and technician) in the model.

The (co)variances of \mathbf{a} and \mathbf{e} were assumed as:

$$\text{var} \begin{bmatrix} \mathbf{a} \\ \mathbf{e} \end{bmatrix} = \begin{bmatrix} \mathbf{H}\sigma_a^2 & \mathbf{0} \\ \mathbf{0} & \mathbf{I}\sigma_e^2 \end{bmatrix},$$

in which: σ_a^2 and σ_e^2 are the additive direct and the residual variances, respectively, and \mathbf{H} is the matrix which combines pedigree and genomic information (AGUILAR et al., 2010), and \mathbf{I} is an identity matrix.

The underlying liabilities of FL1 and FL2 were defined as:

$$\text{FL1: } \{ \text{FL1} = 0 \text{ if } \mathbf{y} \leq t_1; \\ \text{FL1} = 1 \text{ if } \mathbf{y} > t_1 \};$$

$$\text{FL2: } \{ \text{FL2} = 1 \text{ if } \mathbf{y} \leq t_1; \\ \text{FL2} = 2 \text{ if } t_1 < \mathbf{y} \leq t_2; \\ \text{FL2} = 3 \text{ if } t_2 < \mathbf{y} \leq t_3; \\ \text{FL2} = 4 \text{ if } t_3 < \mathbf{y} \leq t_4; \\ \text{FL2} = 5 \text{ if } t_4 < \mathbf{y} \leq t_5 \},$$

in which: t_1 to t_5 correspond to thresholds that define, in the underlying scale, the mutually exclusive ordered categories of FL1 and FL2, under the assumptions that $t_1 < t_2 < \dots < t_{j-1}$, and $t_0 = -\infty$ and $t_j = +\infty$ (GIANOLA & FOULLEY, 1983). In the analysis of FL1, as σ_e^2 is not estimable, the parameterization $\sigma_e^2 = 1$ was adopted (GIANOLA & SORENSEN, 2002). In the case of FL2, the thresholds t_1 and t_2 were kept fixed at 0 and 1, respectively, so that σ_e^2 could be estimated (SORENSEN et al., 1995).

The solutions of the parameters of the above model were obtained by replacing the inverse of the numerator relationship matrix (\mathbf{A}^{-1}) in the regular mixed model equations (HENDERSON, 1984) by the inverse of the \mathbf{H} matrix (\mathbf{H}^{-1}) (MISZTAL et al., 2009, AGUILAR et al., 2010), using the Gibbs sampling program THRGIBBS1F90 (TSURUTA & MISZTAL, 2006). The default prior distributions were assumed for the parameters of the model, and a single chain with a length of 1,000,000 cycles was generated for each analysis (FL1 and FL2), being that a conservative burn-in of 100,000 cycles and a thinning interval of 250 cycles were applied. The coda package of R software (R Development Core Team, 2013) was

employed to assess the convergence of the chains using Geweke's (GEWEKE, 1992) and Heidelberger & Welch (HEIDELBERGER & WELCH, 1983) tests, besides visual inspection. Only the convergence of the variance components and heritability chains were assessed. Finally, the predicted breeding values and the other parameter estimates were obtained as the means of the marginal posterior distributions.

The SNP effect estimates ($\hat{\mathbf{u}}$) were obtained as a function of the predicted breeding values, using the formula (VANRADEN et al., 2009; STRANDEN & GARRICK, 2009):

$$\hat{\mathbf{u}} = \mathbf{DZ}'[\mathbf{ZDZ}']^{-1}\hat{\mathbf{a}}_g ,$$

where: \mathbf{D} is a diagonal matrix with weights for SNPs, \mathbf{Z} is an incidence matrix of genotypes for each locus, \mathbf{Z}' is the transpose of \mathbf{Z} , and $\hat{\mathbf{a}}_g$ is the vector of predicted breeding values of genotyped animals.

The $\hat{\mathbf{u}}$ vector and the \mathbf{D} matrix were iteratively recomputed using the following algorithm (WANG et al. 2012):

- 1) $t = 1$; $\mathbf{D}_{(t)} = \mathbf{I}$, where: t is the iteration number, $\mathbf{D}_{(t)}$ is the \mathbf{D} matrix at iteration t , and \mathbf{I} is an identity matrix;
- 2) Calculate the SNP effects at iteration t ($\hat{\mathbf{u}}_{(t)}$);
- 3) Recalculate the diagonal elements of \mathbf{D} as: $d_{i(t+1)}^* = \hat{u}_{i(t)}^2 2p_i(1-p_i)$ for all SNPs, in which p_i is the allele frequency of the second allele of the i th marker; and i , is the i th SNP.
- 4) Normalize $\mathbf{D}_{(t+1)} = (\text{tr}(\mathbf{D}_{(0)})/\text{tr}(\mathbf{D}_{(t+1)}^*))\mathbf{D}_{(t+1)}^*$;
- 5) $t = t + 1$;
- 6) Exit, or loop to step 2.

The algorithm was run over three iterations, resulting in an increasing shrinkage (from iteration 1 to 3) of the markers explaining lower variance. Wang et al. (2014) indicated that 3 iterations are sufficient to shrink toward zero the effect of supposedly unimportant markers. The application of the GWA analyses were performed using the BLUPF90 family programs (MISZTAL et al., 2012).

QTL mapping. The proportion of variance explained by SNPs within non-overlapping consecutive 1Mb windows was adopted as the criteria to identify potentially important genomic regions. A total of 2,522 windows, containing on average (standard deviation) 161.7 (± 47.6) SNPs, were considered. The top 10 windows, which explained the largest proportion of variance, were assumed as potentially important genomic regions. These windows were further inspected by consulting the genes and QTLs reported in the QTLdb database (HU et al., 2013) in the same genomic regions, using the UMD3.1 bovine genome assembly (ZIMIN et al., 2009) as the reference map. The presence of a QTL in the neighbouring windows (1Mb to the left and to the right of the top 10 windows) was also inspected as its effect can be captured by neighbouring SNPs due to linkage disequilibrium. The presence of a previous described QTL in the QTLdb database was double checked consulting the original references. Scientific papers published in journals indexed in PubMed and ScienceDirect were also consulted, searching for evidences to reinforce the importance of the genomic regions comprised by the top 10 1Mb windows. The genes within the top 10 windows were determined by the MapViewer tool of the National Center for Biotechnology Information (NCBI, 2015).

3. RESULTS AND DISCUSSION

Figures 1 and 2 show the Manhattan plots of the percentage of variance explained by 1Mb window for FL1 and FL2 obtained by wssGBLUP using w3. Manhattan plots presented here suggest that FL1 and FL2 are polygenic traits, influenced by many genes with relatively small effects. It was not possible to identify regions with great influence on the traits. This result could also be associated to the lack of sufficient information in the data to properly identify the important QTLs. However, some regions with indicative of importance were observed. A few peaks that explained 1% or more of the variance were found for both traits, being that FL1 presented more evident peaks when compared to FL2. According to Misztal et al. (2014), the existence of the peaks in Manhattan plots can be associated with many factors, including the structure of the data sets and changes in gene frequencies.

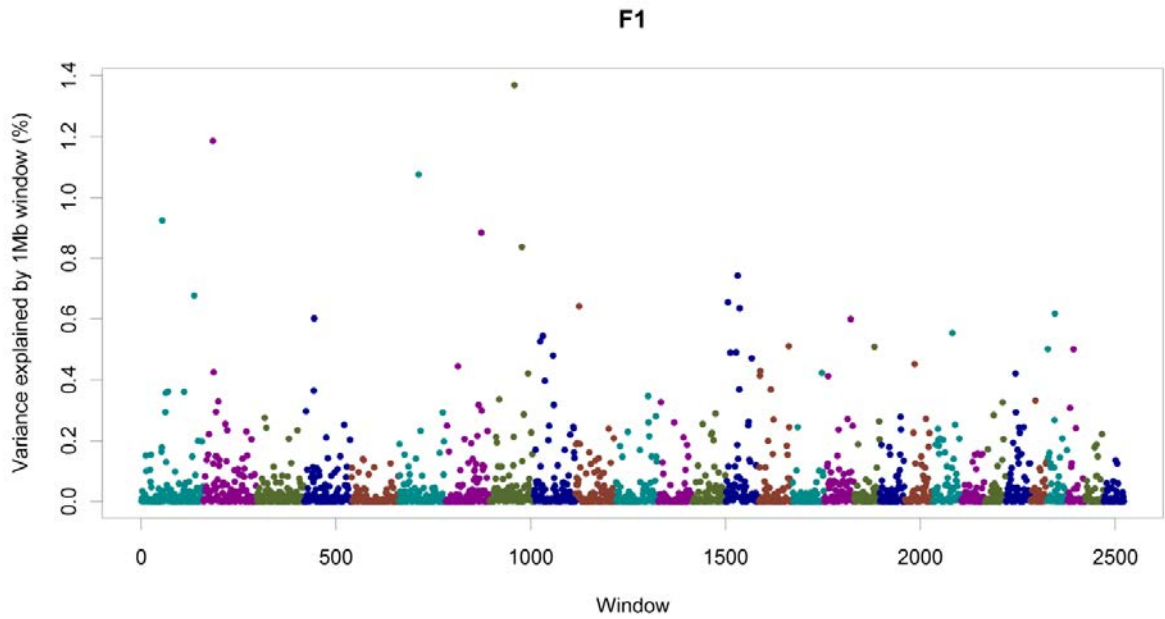


Figure 1. Manhattan plot for percentage of variance explained by 1Mb window for FL1 obtained by ssGBLUP using w3.

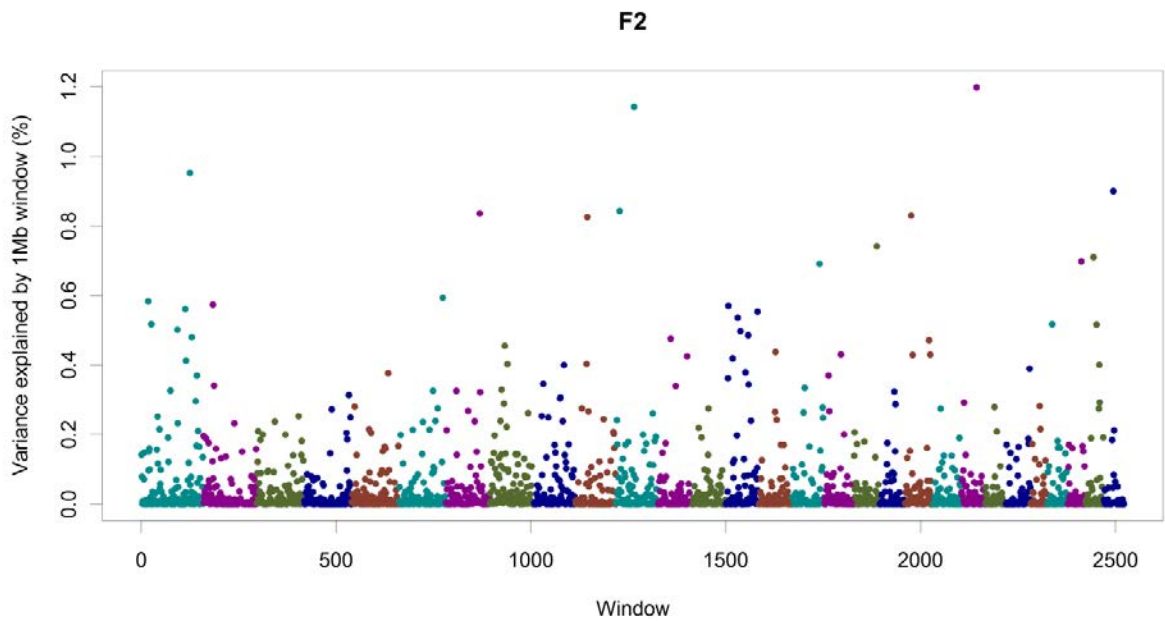


Figure 2. Manhattan plot for percentage of variance explained by 1Mb window for FL2 obtained by ssGBLUP using w3.

Tables 1 and 2 present the top 10 1Mb windows explaining the highest proportion of variance for the traits FL1 and FL2, respectively, obtained by ssGBLUP using w3. The proportion of variance explained by the top 10 windows was equal to 8.96% for both traits in w3. The window 64 of BTA 8 and 40 of BTA 22 presented the highest proportion of variance estimated for FL1 and FL2, respectively, whereas the most important SNPs of these windows explained 1.23 and 0.94% of the variance and are located in the base pair position 63,785,867 and 39,122,937, respectively. Some leading SNPs explained almost the total variance accounted by the respective window. In some cases, the leading SNP explained a percentage of the variance much smaller than the window, indicating that other SNPs of the window were important. FL1 presented important windows in 7 different chromosomes and FL2 presented top windows in 9 different chromosomes (Tables 1 and 2). There was no common top window between FL1 and FL2, which may be related to differences between these traits, considering that while FL1 is more associated to the presence of feet and leg problems, FL2 is more associated to the quality of the feet and legs in the animals.

Table 1. Top 10 windows explaining the highest proportion of variance of feet and legs binary score (FL1), obtained by ssGBLUP using w3.

Rank	Chr_Win	Var (%)	nSNPs	posSNP (bp)	varSNP (%)
1 st	8_64	1.37	185	63785867	1.23
2 nd	2_25	1.18	175	24367446	1.14
3 rd	6_51	1.07	184	50956746	1.05
4 th	1_54	0.92	167	53064069	0.92
5 th	7_92	0.88	201	91783596	0.83
6 th	8_83	0.84	197	82855034	0.52
7 th	14_31	0.74	165	30158402	0.31
8 th	1_136	0.67	243	135947945	0.62
9 th	14_6	0.65	259	5347162	0.33
10 th	10_10	0.64	216	9445786	0.36

Chr = chromosome; Win = 1Mb window within the chromosome; Var = variance explained by the SNPs within the window; nSNPs = number of SNPs within the window; posSNP = position (UMD3.1) of the leading SNP within the window; varSNP = variance explained by the leading SNP.

Table 2. Top 10 windows explaining the highest proportion of variance of feet and legs categorical score (FL2), obtained by ssGBLUP using w3.

Rank	Chr_Win	Var (%)	nSNPs	posSNP (bp)	varSNP (%)
1 st	22_40	1.20	180	39122937	0.94
2 nd	11_47	1.14	180	46647572	1.12
3 rd	1_125	0.95	121	124546798	0.81
4 th	29_25	0.90	188	24618881	0.89
5 th	11_10	0.84	214	9751032	0.18
6 th	7_88	0.84	118	87643539	0.81
7 th	20_16	0.83	105	15412961	0.33
8 th	10_32	0.83	85	31865789	0.63
9 th	18_59	0.74	130	58213152	0.22
10 th	28_21	0.71	165	20385511	0.42

Chr = chromosome; Win = 1Mb window within the chromosome; Var = variance explained by the SNPs within the window; nSNPs = number of SNPs within the window; posSNP = position (UMD3.1) of the leading SNP within the window; varSNP = variance explained by the leading SNP.

No previous studies reporting QTLs overlapping the top windows associated to feet and legs traits were found in Cattle QTL Database (CATTLE QTL DATABASE, 2015). Cole et al. (2011), in a GWAS using data of contemporary U.S. Holstein cows, found top 100 SNP effects for rear legs (side view) within some of the top windows identified in the present study, namely 1_125, 2_25, and 18_59. The authors also observed top 100 SNPs for feet and legs related traits within the following neighboring windows identified in the present study: 11_11 and 22_41 for feet and leg score, 11_48 rear legs (rear view), 20_15 for foot angle and rear legs (rear view). In a GWAS for claw disorders, Van Der Spek et al. (2015) detected significant and suggestive SNP in the window 10_32 and 14_7 associated with sole ulcer. The identification of coincident regions by different studies reinforces that some regions identified in the present study probably contain genes affecting feet and legs related traits in cattle.

Tables 3 and 4 present the annotated genes within the top 10 windows for the traits FL1 and FL2. Most of the genes are associated to metabolic and cellular

processes. FL1 presented a total of 85 annotated genes in the top10 windows, wherein the window with the largest number of genes is the 8_64. FL2 presented a total of 116 annotated genes in the top10 windows, wherein the window with the largest number of genes is the 18_59, with only one window without annotated genes (1_125). For FL2, important candidate genes in BTA11 were found, which may be associated with feet and legs traits. The *interleukin - 1 beta* (NCBI's IL1B) gene that is associated with a regulation of the glycoprotein secreted in the superficial layer of articular cartilage (Superficial zone protein - SZP) that contributes to effective boundary lubrication (DURAINÉ et al., 2008), and the *interleukin - 1, alpha* (NCBI's IL1A) gene that is proinflammatory cytokines responsible for causing matrix degradation and loss of mechanical properties of the articular cartilage (WILSON et al., 2007). The *cytochrome b reductase 1* (NCBI's CYBRD1) gene of BTA 2, are associated with mineral absorption. One of the mineral of larger important is the calcium, which plays innumerable roles in the organism, serving as a main component of bone as well as an intracellular messenger in muscle contraction/relaxation (NCBI, 2015). The *cathepsin L* (NCBI's CTSL) gene of BTA 8 is associated with the development of rheumatoid arthritis, a chronic autoimmune joint disease where persistent inflammation affects bone remodeling leading to progressive bone destruction, osteoporosis and infectious diseases (KAKEGAWA et al., 1993; ISHIBASHI et al., 1999).

Table 3. Annotated genes within the top 10 windows of feet and legs binary score (FL1).

Chr_Win	N	Genes
8_64	22	CCDC180, TDRD7, LOC785115, LOC101905141, TMOD1, TSTD2, LOC101905362, NCBP1, XPA, FOXE1, LOC100847313, C8H9orf156, HEMGN, LOC101905809, ANP32B, LOC101905751, LOC100847202, NANS, TRIM14, CORO2A, TBC1D2, GABBR2
2_25	16	TRNAG-UCC, PDK1, ITGA6, MIR2352, TRNAW-CCA, DLX2, DLX1, METAP1D, SLC25A12, HAT1, LOC101904968, DYNC1I2, LOC784052, LOC786733, CYBRD1, LOC101905180
6_51	2	TRNAS-GGA, LOC100298058
1_54	8	LOC101904329, CD47, IFT57, MYH15, LOC783477, KIAA1524, DZIP3, TRAT1
7_92	2	LOC100848524, LOC101904534
8_83	9	LOC101906474, DAPK1, LOC101906531, CTSL, LOC786322, FBP2, FBP1, LOC101907415, C8H9orf3
14_31	5	LOC101907975, LOC101907872, MIR124A-2, BHLHE22, CYP7B1
1_136	8	EPHB1, LOC101903747, LOC101903894, LOC101903829, KY, LOC101905906, CEP63, ANAPC13
14_6	5	LOC100296770, LOC101907283, COL22A1, LOC101907449, FAM135B
10_10	8	AP3B1, SCAMP1, LHFPL2, LOC101905812, LOC101905869, ARSB, ARSB, DMGDH

Chr = chromosome; Win = 1Mb window within the chromosome; N = number of genes within the window; Genes = NCBI symbol of annotated genes in *Bos taurus* genome (annotation release 103), using the *Bos taurus* UMD3.1 assembly.

Table 4. Annotated genes within the top 10 windows of feet and legs categorical score (FL2).

Chr_Win	N	Genes
22_40	5	CADPS, FEZF2, C22H3orf14, PTPRG, LOC100337379
11_47	26	LOC786288, TTL, POLR1B, CHCHD5, LOC101904088, LOC101904038, LOC101904134, SLC20A1, LOC101903687, NT5DC4, CKAP2L, IL1A, LOC101904177, IL1B, IL37, IL36G, IL36A, IL36B, IL36RN, IL1F10, IL1RN, PSD4, PAX8, LOC100294744, LOC101905095, LOC101905150
1_125	-	-
29_25	8	LOC100336918, LOC101904402, SLC6A5, PRMT3, HTATIP2, LOC518027, LOC101906541, LOC101906617
11_10	15	MRPS9, GPR45, TGFBRAP1, LOC100848208, C11H2orf49, FHL2, LOC100848188, LOC101903987, TACR1, POLE4, HK2, LOC101904209, LOC100297235, SEMA4F, M1AP
7_88	1	LOC100140939
20_16	7	LOC101906396, RNF180, LOC101906438, MIR320A-2, LOC101906491, LOC101906576, HTR1A
10_32	4	DPH6, LOC101903969, TRNAS-AGA, TRNAC-GCA
18_59	47	LOC617909, MIR99B, MIRLET7E, MIR125A, LOC100337268, LOC101907856, LOC101907942, HAS1, VN2R408P, LOC787554, ZNF613, LOC101902824, LOC101902702, ZNF432, ZNF432, ZNF614, LOC101903149, LOC100300607, ZNF350, LOC787309, BOSTAUV1R406, BOSTAUV1R407, LOC101908164, LOC100337475, BOSTAUV1R410, BOSTAUV1R411, LOC100847477, BOSTAUV1R413, PPP2R1A, LOC101903360, BOSTAUV1R414, BOSTAUV1R-PS409, BOSTAUV1R416, BOSTAUV1R-PS410, LOC101903564, LOC539675, VN1R2, LOC787057, LOC100848895, LOC101902345, LOC101904049, LOC101903979, LOC506495, LOC781189, LOC101904568, LOC101904503, LOC101904435
28_21	3	LOC101904967, LOC101905021, LOC781358

Chr = chromosome; Win = 1Mb window within the chromosome; N = number of genes within the window; Genes = NCBI symbol of annotated genes in *Bos taurus* genome (annotation release 103), using the *Bos taurus* UMD3.1 assembly.

4. CONCLUSION

The present genome-wide association study identified regions supposedly associated with feet and legs related traits in Nelore cattle. Some of these regions were located within or near regions previously associated to feet and legs related traits in other breeds, reinforcing the relevance of these regions. New supposedly important regions were also identified, which can serve as candidate regions in future studies.

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