

**UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL  
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PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOTECNIA**

**AVALIAÇÃO GENÉTICA DA LONGEVIDADE EM VACAS DA RAÇA  
HOLANDESA USANDO UM MODELO DE RISCOS PROPORCIONAIS  
WEIBULL**

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**TESE**


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para obtenção do Grau de


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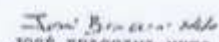
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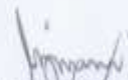
  
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“A mente que se abre a uma nova ideia, jamais voltará ao seu tamanho original”.

Albert Einstein

“A única maneira de fazer um bom trabalho é amando o que você faz. Se você ainda não o encontrou, continue procurando. Não se desespere. Assim como no amor, você saberá quando tiver encontrado”.

Steve Jobs

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## AVALIAÇÃO GENÉTICA DA LONGEVIDADE EM VACAS DA RAÇA HOLANDESA USANDO UM MODELO DE RISCOS PROPORCIONAIS WEIBULL<sup>1</sup>

Autor: Elisandra Lurdes Kern

Orientador: Prof. Jaime Araújo Cobuci; Co-orientador: Cláudio Nápolis Costa

**RESUMO** – A longevidade é uma característica relacionada à lucratividade da atividade leiteira. Contudo, sua seleção em rebanhos de vacas Holandesas no Brasil ainda é pouco considerada. Objetivou-se determinar os fatores não genéticos que influenciam a longevidade funcional em vacas Holandesas no Brasil, bem como estimar os parâmetros genéticos e conhecer a contribuição das características de tipo e da contagem de células somáticas (CCS) sobre o risco relativo de descarte das vacas. Utilizou-se um modelo de riscos proporcionais Weibull estratificado. Os efeitos fixos foram independentes do tempo, como a idade ao primeiro parto, e dependentes do tempo, como o efeito da região por ano de parto, classes de produção de leite por ano de parto dentro de rebanho, classes de percentagem de proteína e gordura dentro de rebanho, classes de produção de leite por número de lactações dentro de rebanho e variação nas classes de tamanho de rebanho. Os efeitos aleatórios foram: rebanho-ano, touro e de touro-avô materno. O risco de descarte aumentou com a idade ao primeiro parto, com o tamanho do rebanho, com o número de lactações e com o estágio de lactação. A produção de leite apresentou maior efeito sobre o risco de descarte. Vacas de baixa produção de leite, gordura e proteína apresentaram maior probabilidade de descarte em comparação à classe mediana. Vacas pertencentes às regiões do Paraná e São Paulo permaneceram mais tempo no rebanho do que as vacas de outras regiões. Os valores de  $h^2$  variam de 7,8% a 6,1% para a  $h^2$  equivalente e a efetiva, respectivamente. Observou-se tendência genética positiva para a longevidade. As características de tipo, escore final, angularidade, nivelamento da linha superior, textura do úbere e ligamento suspensório foram as características que se apresentaram mais relacionadas com a longevidade funcional. Foram observadas diferenças no risco de descarte dependendo do número de vacas classificadas para tipo dentro de rebanho. Até a 4ª lactação, o risco de descarte foi menor para vacas com baixa CCS em comparação a vacas da classe mediana. Já para vacas na 5ª lactação, a alta CCS conduziu ao menor risco de descarte. A rotina de avaliação genética é necessária para melhorar a duração da vida produtiva de vacas da raça Holandesa no Brasil. Características preditivas, tais como escore final, angularidade, nivelamento da linha superior, textura do úbere, ligamento suspensório e a CCS podem ser utilizadas para aumentar a confiabilidade dos valores genéticos dos touros para longevidade funcional.

Palavras-chave: análise de sobrevivência; características de tipo; escore de células somáticas; gado de leite; longevidade.

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<sup>1</sup>Tese de Doutorado em Zootecnia – Produção Animal, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil. (154 p.). Março 2017.

## GENETIC EVALUATION OF LONGEVITY IN HOLSTEIN COWS USING A WEIBULL PROPORTIONAL HAZARD MODEL <sup>2</sup>

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Advisor: Prof. Jaime Araújo Cobuci  
Joint Advisor: Cláudio Nápolis Costa

**ABSTRACT** - Longevity is a trait related to the profitability of dairy activity. However, its selection in Brazilian Holstein herds is still little considered. The aim of this study was to determine the non-genetic factors that influence functional longevity in Holstein cows in Brazil, as well as to estimate the genetic parameters and the contribution of somatic cell score (SCS) and type traits on the relative culling risk of cows. A piecewise Weibullproportional hazard model was used. The fixed effects were time-independent, as age at first calving, and time-dependent, as the interaction effects of region by year of calving, milk production class by year of calving within herd, within herd milk production class by lactation number, within herd fat and protein content, and variation in herd size class. The random effects were herd-year effect, additive genetic contribution from the sire and maternal grandsire of the cow. The relative risk increased with age at first calving, lactation number by stage of lactation, and herd size but lower risks were observed when herd size was increasing or decreasing, compared to stable herds. Milk production had a greater effect on the risk of culling. The relative risk increased as milk production, protein and fat decreased, but to a lesser extent for protein and fat compared to milk yield. Cows from Paraná and São Paulo regions remained longer in the herd than cows from the other regions. The  $h^2$  values varied from 7.8% to 6.1% for equivalent and effective  $h^2$ , respectively. A positive genetic trend of functional longevity was observed. The type traits, final score, angularity, top line, udder texture and suspensory ligament showed the strongest relationship with productive life. Differences in risk of culling were observed depending on the fraction of type-scored animals within a herd. The absence of type trait phenotypes was associated with a strong increase of culling risk for the cows. The impact of SCS on longevity was high in cows from 1<sup>st</sup> to 4<sup>th</sup> lactation with high SCS. Interestingly, for 5<sup>th</sup> lactation, cows with lower SCS have higher culling risk compared to cows with higher SCS. A routine of genetic evaluation is necessary to improve length of productive life of Brazilian Holsteins under local conditions. The use of early predictors correlated with longevity, as final score, angularity, top line, udder texture, suspensory ligament and SCS, may be recommended to increase the reliability of sires' estimated breeding values for functional longevity.

Key words: dairy cattle, longevity, somatic cell score, survival analysis, type traits

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<sup>2</sup> Doctoral thesis in Animal Science, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil (154 p.). March 2017.

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## RELAÇÃO DE ABREVIATURAS E SÍMBOLOS

ABCBRH	Associação Brasileira de Criadores de Bovinos da Raça Holandesa
AN	Angularity
BD	Body depth
BQ	Bone quality
CCS	Contagem de células somáticas
Cells/mL	Células por mililitro
CW	Chest width
DIM	Milk yield for days in milk
DVP	Duração da vida produtiva
EBV	Estimated breeding values
EVP	Escore de vida produtiva
FA	Foot angle
FTL	Fore teat length
FTP	Fore teat placement
FUA	Fore udder attachment
$h^2$	Effective heritability
$h_e^2$	Equivalent heritability
ICAR	International Committee for Animal Recording
Interbull	International Bull Evaluation Service
LF	Longevidade funcional e ou duração da vida produtiva funcional
LS	Loin strength
LV	Longevidade verdadeira ou Duração da vida produtiva
MA	Modelo animal
MS	Median suspensory
MT	Modelo touro
NL	Número de lactações iniciadas
NTDL	Número total de dias em lactação
PL	Productive life
PTA	Capacidade prevista de transmissão
PTL	Produção total de leite
RA	Rump angle
$r_g$	Correlação genética
RLS	Rear legs side view
RP	Riscos proporcionais
RR	Relative risk
RTP	Rear teat placement
RUH	Rear udder height
RUW	Rear udder width
RW	Rump width
SCS	Somatic cell score
SMG	Modelo touro avô materno
SOBRE	Sobrevivência até determinada lactação, ou idade e ou meses
STA	Stature
T	Variável positiva que indica o tempo de descarte
TL	Top line

UD	Udder depth
UT	Udder texture
VR	Vida no rebanho
VRF	Vida no rebanho funcional
$S(t)$	Função de sobrevivência
$\lambda(t)$	Função de risco



## **CAPÍTULO I**

## INTRODUÇÃO

O setor leiteiro tem um importante papel de ordem econômica e social no agronegócio brasileiro, com participação significativa no PIB da pecuária. A produção brasileira exhibe crescimento anual acima da média mundial, garantindo ao Brasil a quinta posição no ranking, atrás apenas da União Europeia, Índia, China e Rússia (USDA, 2016). Contudo, este crescimento não se reflete em eficiência produtiva, visto que a produtividade brasileira (~1,6 mil litros/vaca/ano) é, em muito, superada pelas obtidas na União Europeia (~6,0 mil litros/vaca/ano), nos Estados Unidos (~5,7 mil litros/vaca/ano), na China (~4,1 mil litros/vaca/ano) e, inclusive, na Índia (~2,0 mil litros/vaca/ano) (IBGE, 2015; USDA, 2016). Cabe ressaltar, no entanto, que a estatística da produção média de leite/vaca/ano no Brasil inclui informações de vacas de corte que também são ordenhadas, o que possivelmente justifica os baixos índices de produtividade acima relatados.

Para que a eficiência na produção animal seja alcançada, torna-se necessário o atendimento de questões que envolvem a escolha de animais de mérito genético superior e sua criação em um ambiente que permita a expressão de seu potencial genético. Animais eficientes produtivamente estão relacionados à geração de mais lucro na atividade leiteira. Segundo Peres-Cabal e Alenda (2002), o lucro é uma função da produtividade e do tempo que a vaca permanece produtiva no rebanho, sendo este tempo de permanência das vacas no rebanho conhecido como longevidade.

A longevidade é uma característica de grande importância na atividade leiteira, por estar envolvida indiretamente com várias outras características produtivas e reprodutivas e por sua relação com a lucratividade (Sewalem, et al., 2010). A relação da longevidade com a lucratividade é devida a fatores como: diminuição do custo de criação de novilhas de reposição pela maior permanência das vacas no rebanho; maior produção média e proporção de vacas adultas, as quais produzem mais leite do que aquelas no início da vida produtiva; seleção indireta para outras características, como a fertilidade, visto que, na ausência de estro, não há possibilidade de prenhez, e sem a prenhez, não haverá produção, sendo inviável economicamente (Galeazzi et al., 2010; Lagrotta et al., 2010; Sewalem, et al., 2010). Desta forma, quanto maior a produtividade e o tempo de permanência no rebanho, maior será a lucratividade da atividade leiteira.

O descarte involuntário, ou seja, aquele descarte causado por problemas de reprodução, saúde da glândula mamária, conformação, temperamento e facilidade de ordenha, é apontado como um dos principais causadores da redução da lucratividade das vacas. A diminuição das taxas de descarte involuntário permite que o descarte seja baseado no nível produtivo do animal, sendo este conhecido como descarte voluntário, resultando em rebanhos com maior mérito produtivo (Ducrocq et al., 1988).

A longevidade é uma característica afetada por diferentes fatores. Farabosco et al. (2009) e M'Hamdi et al. (2010) apontaram que as principais causas de descarte de vacas, são aquelas relacionadas à saúde da glândula mamária, baixa produção de leite e problemas de reprodução. Entretanto, também outros fatores, como as características lineares de tipo, em especial as

características relacionadas ao úbere e pés e pernas, apresentam impacto significativo sobre a longevidade em vacas leiteiras (Caraviello et al., 2003; Sewalem et al., 2005).

A relevância da longevidade pode ser evidenciada pela sua inclusão nos objetivos de melhoramento, nas avaliações genéticas e na formação de índices econômicos. De acordo com o Sistema Internacional de Avaliação de Touros (Interbull) de 2015, 21 países participam da avaliação genética para a longevidade na raça Holandesa. Nestes países, a avaliação genética nacional é realizada utilizando modelos de sobrevivência (10 países), ou modelo linear multicaracterística (7 países), ou mesmo modelo linear unicaracterística (2 países) (Interbull, 2015).

Vários autores têm apontado vantagens da metodologia de análise de sobrevivência sobre as convencionalmente utilizadas, como a de modelos lineares (Caraviello et al., 2003; Ducrocq, 2005; Sewalem, et al., 2010). Os modelos de sobrevivência possibilitam o uso de registros de vacas ainda vivas no momento das análises (vacas censuradas), a inclusão de variáveis dependentes do tempo que afetam a vida produtiva – tais como práticas de manejo que influenciam toda a vida produtiva da vaca, e não somente em pontos específicos –, permitem uma descrição precisa dos efeitos ambientais que influenciam a duração da vida produtiva da vaca e, por fim, modelam para a distribuição não linear dos dados de longevidade (Ducrocq, 2005).

Apesar dos modelos lineares serem relativamente simples, segundo Heringstad et al. (2000), a principal desvantagem de empregar tal modelo é que se assume a distribuição normal dos dados. Desta forma, em função da distribuição dos registros de longevidade, a escolha do tipo de análise a ser utilizada torna-se importante para que seja feita uma inferência mais próxima da variabilidade genética real de determinada característica.

Reconhecendo a importância econômica da longevidade no cenário da pecuária leiteira brasileira, alguns estudos envolvendo a raça Holandesa têm sido desenvolvidos para a estimação de parâmetros genéticos e de associação com outras características, como as características de tipo e produção de leite, utilizando diferentes medidas de longevidade analisadas com modelo animal linear, de limiar e também através da análise multivariada (componentes principais) (Kern et al. 2014a, b, c; Kern et al., 2015). Contudo, a avaliação genética para a longevidade dos animais da raça Holandesa ainda não é realizada no Brasil.

Considerando diversos fatores, como as vantagens da análise de sobrevivência, a inexistência de estudos com esta metodologia em vacas da raça Holandesa no Brasil, a possibilidade de utilização dos resultados na orientação de estratégias de avaliação genética da longevidade e a importância econômica da longevidade, entende-se que este estudo poderá beneficiar diretamente o produtor na busca de animais mais longevos e produtivos.

## REVISÃO BIBLIOGRÁFICA

### Longevidade

A permanência dos animais no rebanho, conhecida como longevidade, pode ser medida pela diferença entre dois períodos na vida do animal: i – ponto final, dado pela data de descarte ou morte do animal; e ii – ponto de origem, dado pela data de nascimento e/ou a data do primeiro parto. A longevidade que considera a data de nascimento como ponto inicial é dita como longevidade biológica, visto que compreende o período de crescimento e desenvolvimento da novilha. Quando o ponto inicial é a data do primeiro parto, a longevidade é chamada de longevidade produtiva, ou vida produtiva, pois considera o início da vida produtiva do animal. A longevidade produtiva, ou duração da vida produtiva, medida em dias, é comumente utilizada nos programas de melhoramento genético de bovinos leiteiros, pois remete ao período produtivo da vaca, sendo dividida, por Ducrocq et al. (1988), em dois tipos de longevidade: a verdadeira, definida como a habilidade de evitar o descarte devido a qualquer razão, sendo principalmente dependente da produtividade da vaca; e a funcional, sendo definida como a habilidade de adiar o descarte involuntário, ou seja, aquele independente da produção da vaca.

Segundo Kern et al. (2016), o uso da longevidade funcional, além de fornecer maior quantidade de informações da vida da vaca no rebanho, inclui informações adicionais, mais ou menos independentes das características de produção (leite, gordura e proteína), sendo, por consequência, mais útil e contribuindo economicamente para as metas dos programas de melhoramento leiteiro. De acordo com Queiroz et al. (2007), o estudo da longevidade produtiva, além de incluir informações sobre a morte e/ou descarte da vaca, engloba informações reprodutivas e produtivas, representado, assim, a eficiência do animal no sistema de produção. Contudo, a principal dificuldade do uso de medidas da vida produtiva é a presença de vacas vivas no momento do estudo. Esta particularidade é conhecida como censura, ou seja, vacas que não apresentam a data de morte e/ou descarte. A exclusão destes animais, ou considerá-los mortos ou descartados, conduz a resultados tendenciosos nas estimativas dos valores genéticos dos animais (Vollema et al., 2000). A problemática da censura pode ser modelada pela utilização da metodologia de análise de sobrevivência, a qual permite o estudo da longevidade de vacas ainda vivas e da não normalidade dos dados de longevidade.

Na literatura, outras formas de medir a longevidade produtiva são reportadas, tais como a produção total de leite na vida da vaca, número de lactações iniciadas, sobrevivência até determinado tempo e idade após o primeiro parto ou lactação (Forabosco et al., 2009; Imbayarwo-Chikosi et al., 2015). Mais da metade do total de países (24) participantes do Interbull (International Bull Evaluation Service) utilizam a medida de longevidade funcional (14) para a realização da avaliação genética da permanência das vacas no rebanho, seguida pela medida de sobrevivência até determinada lactação (4). Todas as medidas podem ser consideradas alternativas para medir a longevidade, apresentando, cada uma, suas características e peculiaridades. A escolha de qual medida de longevidade utilizar depende da facilidade de coleta das informações, do tempo necessário para a obtenção da

medida, da necessidade de pessoal treinado para a coleta e da metodologia de análise a ser utilizada.

A sobrevivência até determinada idade, ou *stayability*, é definida como a probabilidade de a vaca permanecer no rebanho até determinado tempo (por exemplo, em número de meses, a partir do primeiro parto), dada a oportunidade para este evento (Hudson & Van Vleck, 1981), apresentando comportamento binário de sucesso em que o valor 1 representa as vacas que permaneceram até determinada idade, e o valor 0 indica o fracasso, ou seja, que a vaca não atingiu a idade e/ou o tempo pré-estabelecido. Devido ao comportamento categórico dos dados, o estudo das medidas de sobrevivência requer o uso de um modelo animal de limiar. Esta medida pode ser uma alternativa ao tempo necessário de obtenção da medida de longevidade, não sendo necessária a espera da morte e/ou descarte da vaca do rebanho; contudo, apresenta informações restritas até determinada parte da vida produtiva da vaca, o que resulta em duvidosa descrição da real longevidade da vaca no rebanho.

### **Importância da Longevidade**

A lucratividade é o fator responsável pela manutenção de qualquer sistema de produção. Na atividade leiteira, o lucro é uma função da produtividade e do tempo que a vaca permanece produtiva no rebanho (Perez-Cabal & Alenda, 2002), sendo este tempo definido como longevidade.

A presença de vacas longevas no rebanho permite a redução dos custos de criação de novilhas de reposição devido ao aumento da proporção de vacas adultas, que produzem mais leite do que as vacas jovens; o aumento da produção média de leite do rebanho, devido à presença de vacas de maior mérito genético; e à menor incidência de vacas doentes e com problemas reprodutivos, o que diminui os custos com a aquisição de medicamentos e contratação de serviços de assistência médica veterinária, assim como reduz a necessidade de compra de maior número de doses de sêmen.

De acordo com Galeazzi et al. (2010), a seleção de animais longevos resulta na seleção indireta para melhoria da fertilidade das vacas, visto que no anestro não há ocorrência da prenhez, e, por consequência, não há produção de leite, o que torna estes animais inviáveis economicamente.

A relação da longevidade com a lucratividade é apresentada por vários autores. De acordo com Visscher et al. (1995), Vargas et al. (2002) e Sadeghi-Sefidmazgi et al. (2009), o aumento de 1% na taxa de sobrevivência de vacas Holandesas conduz a um lucro absoluto variando de US\$ 1,35 a 6,20 por vaca/ano. Rogers et al. (1988) observaram aumento na receita líquida por vaca/ano na ordem de US\$ 22,00 após uma redução de 2,9% nas taxas de abate involuntário, resultando em aumento na longevidade de vacas Holandesas. Estes valores econômicos positivos reforçam a incorporação da longevidade no objetivo de seleção para o gado leiteiro como uma estratégia para melhorar a seleção para o mérito econômico líquido (Vargas et al., 2002; Banga et al., 2014).

### **Fatores que afetam o descarte**

A longevidade é uma característica complexa influenciada por vários

fatores que afetam a tomada de decisão de descarte. O descarte de uma vaca do rebanho pode ser devido, por exemplo, à sua baixa produção de leite, gordura e proteína, sendo este definido como descarte voluntário. Quando o descarte ocorre em razão de problemas de saúde da glândula mamária, reprodução, locomoção, velocidade e temperamento à ordenha, e/ou dificuldade de parição, entre outros problemas que não envolvem as características de produção, o descarte é dito como involuntário (Ducrocq et al., 1988).

Para o aumento da eficiência econômica da atividade leiteira, a seleção dos animais deve preconizar a diminuição dos descartes involuntários, ou seja, da incidência de vacas com problemas de saúde e de reprodução, por exemplo. A redução dos descartes involuntários permitirá que os descartes voluntários, baseados na baixa produção de leite, sejam praticados (Ducrocq et al., 1988; Sewalem et al., 2005b). Desta forma, o produtor estará selecionando indiretamente as vacas com maior mérito genético produtivo, para as quais se esperam menores taxas de infertilidade, problemas de ligamento de úbere, de aprumo e de contagem de células somáticas. Adicionalmente, Ahlman et al. (2011) relatam que a prática do descarte involuntário favorece o bem-estar animal, visto que o descarte está relacionado a deficiências de saúde, locomoção e reprodução.

Um número considerável de covariáveis têm sido reportadas como importantes fatores que influenciam a permanência produtiva das vacas no rebanho, ou seja, que afetam o risco de descarte das vacas leiteiras. As principais covariáveis dependentes do tempo incluídas nos modelos de riscos proporcionais Weibull para o estudo da longevidade são: a produção de leite, gordura e proteína, estágio de lactação, número de lactações, variação no tamanho do rebanho e estação e ano de produção, sendo estes efeitos considerados dentro de rebanho. A variável idade ao primeiro parto é incluída como fator independente do tempo (Ducrocq, 2005; Terawaki & Ducrocq, 2009; Jenko et al., 2013).

Nas análises que utilizam o modelo de risco proporcional Weibull, os fatores que influenciam a ocorrência do evento de interesse, ou seja, o tempo até a morte e/ou descarte, são chamados de covariáveis. O efeito das covariáveis sobre a longevidade das vacas é expresso como o risco relativo de descarte, sendo este a razão entre o risco estimado de descarte, sob a influência de certos fatores ambientais, e o risco médio (ou risco de referência), que geralmente é definido com valor 1 (Sewalem et al., 2005b; M'hamdi et al., 2010).

Valores maiores que um indicam maior risco de descarte associado à covariável. Valores menores que um indicam menor risco de descarte. Por exemplo, quando o risco relativo de descarte para uma dada classe é de 3, significa que a vaca apresenta três vezes mais chance de ser descartada em comparação a uma vaca da classe de referência. Já quando o risco relativo de descarte para uma dada classe é de 0,5, a vaca pertencente àquela classe demonstra 50% menos chance de ser descartada do que a vaca da classe de referência (Sewalem et al., 2005b).

A produção de leite é um dos principais fatores que afetam a longevidade das vacas, tendo em vista que as principais decisões de descarte

são baseadas no nível de produção do animal. Segundo Páchová et al. (2005), vacas de baixa produção de leite apresentaram risco de descarte cinco vezes maior do que as vacas de produção mediana. Galeazzi et al. (2010) e M'hamdi et al. (2010) observaram que, à medida que houve o aumento da produção de leite, o risco de descarte das vacas diminuiu, possibilitando inferir que, para vacas de alta produção de leite, outros fatores podem influenciar o risco relativo de descarte das mesmas.

Em relação ao estágio de lactação e ao número de lactações, o risco de descarte aumentou continuamente ao longo das lactações, com maior valor de risco no final das mesmas e com o aumento do número de lactações (Ducrocq, 2005; Terawaki et al., 2006; Kern et al., 2016).

Contudo, Mészáros et al. (2008) e M'hamdi et al. (2010) observaram maior risco de descarte para vacas no início da lactação e a diminuição do risco com o aumento do número de lactações. Potočnik et al. (2011) observaram que vacas no início da primeira lactação e no final das demais lactações são mais suscetíveis ao descarte. De acordo com Terawaki et al. (2006), os descartes no início das lactações normalmente ocorrem devido a casos extremos, tais como produção de leite extremamente baixa ou graves problemas de saúde, locomoção ou mastite. Já os descartes no final da lactação são devidos à baixa rentabilidade da vaca no rebanho.

Em rebanhos em expansão e/ou estáveis, o risco de descarte foi menor se comparado àqueles que apresentam diminuição em seu número de animais (Ducrocq, 2005; Mészáros et al., 2008; M'hamdi et al., 2010). Comportamento semelhante foi observado por Kern et al. (2016) em rebanhos de vacas Holandesas no Brasil com mais de 50 vacas. Contudo, estes autores observaram que, para rebanhos com tamanho entre 5 e 20 vacas, o risco de descarte foi superior em rebanhos estáveis, quando comparados a rebanhos em crescimento ou em diminuição.

Dentre as principais causas de descarte apontadas pelos países participantes do Interbull (*International Bull Evaluation*), se destacam aquelas ligadas a problemas de reprodução, saúde do úbere e baixa produção de leite (Forabosco et al., 2009). Estas causas também foram apontadas por Ahlman et al. (2011) como as principais razões de descarte de vacas Holandesas criadas em sistemas orgânico e convencional. Ambos autores destacaram os problemas reprodutivos como sendo o principal fator que afeta a longevidade das vacas no rebanho.

Fatores como velocidade e temperamento à ordenha (Sewalem et al., 2010), facilidade ao parto (Sewalem et al., 2008) e idade no primeiro parto também podem influenciar a permanência das vacas nos rebanhos.

A velocidade de ordenha tem sido associada ao aumento dos índices de mastite e, conseqüentemente, da contagem de células somáticas, afetando, desta forma, a longevidade (Zwald et al., 2006). Vacas muito lentas e/ou nervosas à ordenha apresentaram maior risco de descarte do que as vacas classificadas como intermediárias para estas características (Sewalem et al., 2010). Vacas com partos que necessitaram de intervenção cirúrgica ou que apresentaram muita dificuldade foram 1,92 e 1,27 vezes, respectivamente, mais prováveis de descarte do que as vacas com partos sem nenhuma complicação (Sewalem et al., 2008).

A longevidade das vacas diminuiu à medida que aumentou a idade no primeiro parto (Nilforooshan & Edriss, 2004; Sewalem et al., 2005b; M'hamdi et al., 2010; Kern et al., 2016). M'hamdi et al. (2010) observaram risco relativo de descarte de 0,96, aos 21 meses de idade, e de 1,43, aos 39 meses de idade. O maior risco de descarte das novilhas com elevada idade no primeiro parto pode ser atribuído às práticas de manejo no rebanho e a possíveis problemas reprodutivos (Vukasinovic et al., 2001; Sewalem et al., 2005b). Além disso, em geral, novilhas tardias são menos rentáveis, uma vez que apresentam menor período de produção.

No estudo realizado por Vukasinovic et al. (2001) e Ducrocq (2005), os autores não encontraram efeito significativo da idade ao primeiro parto sobre a vida produtiva de vacas Holandesas. O efeito não significativo pode ser uma resposta à intensiva seleção que há muitos anos vem sendo realizada para a precocidade reprodutiva das vacas Holandesas.

### **Estimativas de herdabilidade para a Longevidade**

A herdabilidade para as diferentes medidas de longevidade, em geral, é consideravelmente baixa, com valores variando entre 0,002 a 0,19 (Tabela 1). Estas estimativas podem ser obtidas com diferentes modelos estatísticos, como modelo linear, de limiar, regressão aleatória e modelo de riscos proporcionais Weibull.

A herdabilidade com modelo linear tem demonstrado ser menor quando comparada a modelos de limiar (Boettcher et al., 1999; Kern et al., 2014a; Imbayarwo-Chikosi et al., 2015) e ao modelo de riscos proporcionais Weibull (Boettcher et al., 1999; Ducrocq, 2002; Roxstrom et al., 2003; Imbayarwo-Chikosi et al., 2015).

Uma maior estimativa de herdabilidade pode ser utilizada como indicadora de maior acurácia de seleção para a longevidade. Contudo, na Tabela 1, pode ser observado que, para todos os modelos, houve grande variação entre as estimativas de herdabilidade, sendo difícil apontar com precisão qual modelo gera maior herdabilidade. Esta dificuldade deve-se a diferenças entre as medidas de longevidade, entre as populações estudadas e às diferenças ambientais em que cada população foi criada.

De acordo com Van Pelt e Veerkamp (2015), nos modelos de regressão aleatória, as maiores herdabilidades são obtidas em idades mais elevadas. Kern et al. (2014a) também observaram comportamento semelhante utilizando medidas de sobrevivência com modelo de limiar. De maneira geral, o modelo de riscos proporcionais Weibull condiciona a maiores herdabilidades em comparação ao modelo de regressão aleatória (Imbayarwo-Chikosi et al., 2015).

Com o modelo de riscos proporcionais Weibull, a herdabilidade pode ser expressa sob diferentes formas: i – escala logarítmica; ii – escala original, que consiste na transformação da herdabilidade logarítmica usando uma extensão de série Taylor (Ducrocq, 1999); iii – herdabilidade efetiva, que considera a herdabilidade da característica na presença de censura; e iv – herdabilidade equivalente, que considera a proporção de registros não censurados no tempo t.

De acordo com Ducrocq (1997), Ducrocq e Skolner (1998) e Sasaki



(2013), a herdabilidade efetiva e a herdabilidade em escala original tendem a apresentar maiores valores do que a herdabilidade logarítmica. A herdabilidade equivalente é considerada mais realista, sendo utilizada para prever a confiabilidade esperada num dado ponto no tempo para um touro com N filhas, como uma função da proporção de filhas não censuradas (Yazdi et al., 2002; Ducrocq, 2005).

**Tabela 1.** Herdabilidade para diferentes medidas de longevidade obtidas com modelo linear, limiar, regressão aleatória e modelo de riscos proporcionais em animais da raça Holandesa.

Metodologia	Modelo	Medida de Longevidade	Herdabilidade	País	Referência
Linear	MT	LF	0,03 - 0,05	Alemanha	Wiebelitz et al., 2014
Linear	MA	LF, VRF	0,10	Espanha	Pérez-Cabal et al., 2006
Linear	MA	NL, NLF, LV, LF	0,03 - 0,05	República Tcheca	Zavdilova & Stipkova, 2012
Linear	MA	NL	0,06	África do Sul	Setati et al., 2004
Linear	MA	NL, PTL, NTDL, VR, LF	0,05 - 0,07	Brasil	Kern et al., 2015
Linear	SMG	SOBRE, VR, VRF	0,01- 0,10	EUA	Short & Lawlor, 1992
Linear	MA	EVP	0,05	Reino Unido	Pritchard et al., 2013
Regressão Aleatória	SMG	LV, SOBRE	0,002- 0,15	Holanda	Van Pelt et al., 2015
Regressão Aleatória	MA	LF	0,09 - 0,18	USA	Tsuruta et al., 2004
Regressão Aleatória	MA	LF	0,01 - 0,07	Reino Unido	Veerkamp et al., 2001
Limiar	MA	SOBRE	0,05 - 0,15	Brasil	Kern et al., 2014b
Limiar	MA	SOBRE	0,11	Espanha	Gonzalez-Recio & Alenda, 2007
Limiar	MT	LV	0,07	Canadá	Boettcher et al., 1999
RP Weibull de base única	MT	LV, LF	0,09 - 0,15	Canadá	Dürr et al., 1999
RP Weibull de base única	MT	LF	0,05 - 0,17	EUA	Caraviello et al., 2004b
RP Weibull de base única	MT	LF	0,12 - 0,19	Tunísia	M'hamdi et al., 2010
RP Weibull de base única	MT	LV	0,05 - 0,08	Japão	Sasaki et al., 2012
RP Weibull de base única	MT	LF	0,07	Irã	Najafabadi et al., 2016
RP Weibull de base única	SMG	LF	0,05 - 0,07	Espanha	Chirinos et al., 2007
RP Weibull estratificado	SMG	LF	0,11	França	Ducrocq, 2005
RP Weibull estratificado	SMG	LF	0,05 - 0,13	Japão	Terawaki & Ducrocq, 2009
RP Weibull estratificado	SMG	LF	0,07 - 0,078	Brasil	Kern et al., 2016

MT: modelo touro; MA: modelo animal; SMG: modelo touro avô materno; RP: Riscos proporcionais; NL: número de lactações iniciadas; PTL: produção total de leite; NTDL: número total de dias em lactação; LF: longevidade funcional e ou duração da vida produtiva funcional; LV: longevidade verdadeira ou duração da vida produtiva; VR: vida no rebanho; VRF: vida no rebanho funcional; SOBRE: sobrevivência até determinada lactação, ou idade e ou meses; EVP: escore de vida produtiva.

### **Preditores da Longevidade**

Muitas características são utilizadas como preditoras de outra determinada característica de interesse, o que permite que a seleção indireta para esta característica seja efetuada. Como preditoras da longevidade, podemos destacar as características lineares de tipo, a contagem de células somáticas e a fertilidade, por exemplo. A importância das características preditoras é a possibilidade de inclusão destas em “net merit indices”, ou a possibilidade de seu uso em combinação com a longevidade direta, como atualmente é realizado pela França, EUA e Bélgica (Forabosco et al., 2009).

A associação genética entre a longevidade e outras características pode variar entre as raças e entre populações da mesma raça criadas em ambientes distintos ao longo dos anos e, também, conforme os objetivos de seleção aplicados (Sasaki et al., 2013). Vacas registradas e vacas não registradas também apresentam diferenças nas estimativas de correlação genética da permanência das vacas no rebanho (Short & Lawlor 1992; Larroque & Ducrocq, 2001). Contudo, as correlações genéticas entre as medidas de longevidade verdadeira e funcional e as características produtivas e de tipo foram semelhantes (Cruickshank et al., 2002).

#### *Características Produtivas*

Os valores de correlação genética entre as características produtivas e a longevidade apresentam grande variação entre os estudos realizados, com valores de -0,19 a 0,84, para produção de leite e longevidade, de -0,21 a 0,35 para proteína e longevidade, e de -0,18 a 0,46, para gordura e longevidade (Vollema & Groen, 1997; Weigel et al., 1998; Cruickshank et al., 2002; Tsuruta et al., 2004; Samoré et al., 2010; Pritchard et al., 2013).

Os valores de correlação genética favorável e positiva (0,23 a 0,84) entre a produção de leite e a longevidade apresentados por Cruickshank et al. (2002), Vollema e Groen (1997) e por Weigel et al. (1998) podem ser resultado da realização de descarte voluntário baseado na produção das vacas, indicando que a seleção para vacas longevas conduz ao aumento da produção de leite. Contudo, Samoré et al. (2010) sugerem que o estresse causado pela alta produção de leite e seu efeito sobre o úbere pode reduzir a sobrevivência das vacas.

#### *Características do sistema mamário*

As características relacionadas ao sistema mamário apresentam grande influência sobre a permanência das vacas no rebanho, pois úberes caídos favorecem a contaminação por agentes patogênicos causadores de mastite e a ocorrência de lesões nos tetos, afetando a produtividade e resultando no aumento dos descartes involuntários, o que conduz a menores taxas de sobrevivência no rebanho. A relação entre úbere “pobre” e o descarte foi demonstrada por Poso e Mantysaari (1996) e por De Vlieghe et al. (2005). Nesses estudos, 34,8% e 10% das vacas, respectivamente, foram descartadas por problemas de úbere, sendo os 10% de descarte referentes apenas à primeira lactação.

Vários estudos têm apresentado correlações genéticas positivas entre as características do sistema mamário e as diferentes medidas de

longevidade, com destaque para profundidade de úbere, textura do úbere e ligamento do úbere anterior, variando os valores entre 0,15 e 0,43 (Cassandro et al., 1999; Cruickshank et al., 2002; Samoré et al., 2010; Kern et al., 2015).

A relação entre as características do sistema mamário e a lucratividade durante a vida das vacas foi examinada por Pérez-Cabal e Alenda (2002). Segundo os autores, positivas correlações genéticas foram encontradas para ligamento mediano (0,37), altura do úbere (0,29), ligamento do úbere (0,22) e sistema mamário (0,27), sendo esta última o agrupamento de mais de uma característica do sistema mamário.

Estes valores de correlação genética demonstram que vacas com úberes firmes, bem inseridos ao abdômen e com boa conformação resistem a altos níveis de produção, reduzindo, assim, os riscos de mastite e sendo, portanto, longevas e mais rentáveis (Cassell et al., 1990; Pérez-Cabal & Alenda, 2002).

Vacas com escore intermediário para profundidade de úbere apresentam maior probabilidade de permanência no rebanho em comparação a vacas de escore baixo, ou seja, vacas com úberes mais próximos do solo têm demonstrado maior risco de descarte involuntário (Larroque & Ducrocq, 2001; Caraviello et al., 2003; Zavadilová et al., 2011). Vacas com fraco ligamento suspensório (Larroque & Ducrocq, 2001; Schneider et al., 2003; Sewalem et al., 2004; Caraviello et al., 2004a; Zavadilová et al., 2011), úbere solto (Burke & Funk, 1993; Larroque & Ducrocq, 2001; Schneider et al., 2003; Sewalem et al., 2004, 2005a; Caraviello et al., 2004a), úbere baixo (Caraviello et al., 2004a; Sewalem et al., 2005a) e largura estreita do úbere (Caraviello et al., 2004a; Sewalem et al., 2005a) apresentaram alto risco de descarte.

O comprimento intermediário das tetas e a colocação das tetas são positivamente associadas com a longevidade funcional em vacas Holandesas (Schneider et al., 2003; Sewalem et al., 2004, 2005a; Zavadilová et al., 2011).

#### *Contagem de células somáticas*

A longevidade de vacas leiteiras pode ser influenciada pela contagem de células somáticas (CCS), seja através da remoção de animais clinicamente afetados, ou pelo o abate de animais com mastite subclínica com elevada CCS, com o objetivo de receber maior remuneração pela qualidade do leite (Caraviello et al., 2005). O estudo da CCS torna-se importante devido à sua relação com o aumento das taxas de mastite ( $r_g$  CCS e mastite 0,75 a 0,88 [Roxstrom & Strandberg, 2002]), aumento da produção de leite ( $r_g$  CCS e produção de leite de 0,13 a 0,45 [Carlén et al., 2004], correlação indesejável) e aumento dos descartes involuntários, resultando em vacas de menor longevidade no rebanho (Samoré et al., 2003).

Cranford e Pearson (2001) encontraram valores de correlação desfavorável entre PTA de touros para CCS e número de lactações totais e duração da vida produtiva de suas filhas. Samoré et al. (2003) reportaram correlação genética entre o PTA para CCS e o PTA para a taxa de descarte (longevidade) de 0,31, indicando que maiores valores de CCS foram associados a maior taxa de descarte, e conseqüentemente, menor longevidade. O antagonismo entre CCS e longevidade também foi observado em outros estudos, nos quais os valores de correlação genética variaram de

0,16 a 0,36 (Nielsen & Pedersen, 1995; Mrode et al., 2000; Roxstrom & Strandberg, 2002).

#### *Pés e Pernas*

As correlações genéticas entre diferentes medidas de longevidade e as características de pés e pernas variaram entre -0,18 e 0,23 (Cruickshank et al., 2002; Kern et al., 2015).

Vacas com pernas traseiras, vista lateral extremamente curvadas ou estreitas são menos longevas se comparadas a vacas de escore intermediário (Schneider et al., 2003; Caraviello et al., 2004a; Zavadilová et al., 2011).

A importância das características de pés e pernas para a longevidade das vacas está relacionada à taxa de descartes involuntários, causados por doenças que afetam a mobilidade das vacas. Onyiro et al. (2008) reportou moderada correlação genética da qualidade óssea (-0,21) e alta correlação para pernas traseiras, vista lateral (-0,63) com a dermatite digital, evidenciando que vacas sem a doença tinham boa qualidade óssea, ossos planos e adequado conjunto de pernas e pés, resultando em maior permanência das vacas no rebanho. Vacas de melhor qualidade óssea tendem a permanecer no rebanho, visto a correlação genética de 0,23 desta característica com a longevidade produtiva (Kern et al., 2015).

De acordo com Schneider et al. (2003), vacas classificadas com escores intermediários e altos, para qualidade óssea e ângulo do casco, apresentam menor risco de descarte do que vacas de baixo escore, ou seja, do que vacas com ossos grosseiros e com pouco ângulo de casco.

#### *Relação da longevidade com outras características de tipo*

As correlações genéticas entre as características de conformação e medidas de longevidade, em sua grande parte, foram negativas e moderadas, com valores entre -0,38 a 0,10 (Cruickshank et al., 2002; Zavadilová, M. Štípková, 2012; Kern et al., 2015).

Contudo, valor positivo e moderado (0,30) foi observado por Zavadilová, M. Štípková (2012) para escore de condição corporal. Vacas com escore baixo para estatura, largura do peito, tamanho e força lombar foram mais prováveis de descarte quando comparadas a vacas de escore intermediário e alto (Schneider, et al., 2003; Sewalem et al., 2004).

Zavadilová et al. (2011) reportaram que vacas grandes e profundas foram mais propícias ao descarte involuntário do que vacas com estatura pequena e profundidade corporal rasa. Buenger et al. (2001) e Caraviello et al. (2003) não encontraram forte relação entre tamanho e estatura com a longevidade funcional em vacas Holandesas. De acordo com Zavadilová et al. (2011), variações nos resultados dos estudos podem ser atribuídas a diferenças nas estratégias de descarte involuntário nas diferentes populações estudadas.

Vacas angulosas e de garupa larga apresentaram maior probabilidade de descarte involuntário quando comparadas àquelas da classe de referência (5) (Buenger et al. 2001). Resultado oposto foi observado por Dadpasand et al. (2008) para largura da garupa, assim, vacas com garupa estreita (escore 2) estão associadas ao aumento no risco de descarte, sendo

os escores 7 e 8 relacionados ao aumento da longevidade funcional. Contudo, Weigel et al. (1998), Caraviello et al. (2004) e Tsuruta et al. (2005) não encontraram relação significativa entre as características da garupa e a longevidade em vacas Holandesas nos USA. De acordo com Selawem et al. (2004), as características de garupa são associadas à facilidade de parto, sendo importante o produtor buscar vacas de escore intermediário, diminuindo assim os descartes involuntários.

As correlações genéticas entre escore final e medidas de longevidade foram próximas à zero, variando de -0,08 a 0,04 (Samoré et al., 2010; Zavadilová and Stípková, 2012; Kern et al., 2015). No entanto, estimativas superiores de correlação genética entre cinco medidas de longevidade e escore final foram observadas por Short e Lawlor (1992). Estes autores reportaram valores entre 0,30 (sobrevivência até 84 meses de idade) e 0,54 (vida no rebanho funcional) para vacas registradas, e 0,07 (sobrevivência até o 2º parto) a 0,19 (sobrevivência até 54 meses de idade) para vacas não registradas. Caraviello et al. (2003) e Sewalem et al. (2004) observaram forte relação linear entre escore final e longevidade em vacas Holandesas e vacas Jersey, respectivamente, em que vacas com alto escore final apresentaram-se mais longevas do que vacas de baixo escore.

### **Métodos de Análise da Longevidade**

Em razão de sua importância econômica, diferentes medidas (duração da vida produtiva, vida no rebanho, sobrevivência até determinado tempo, entre outras) e, conseqüentemente, métodos de analisar a longevidade são utilizados para a realização da avaliação genética desta característica, tais como a análise de sobrevivência, modelos lineares uni e multicaracterística, modelos de limiar e modelos de regressão aleatória (Forabosco et al., 2005; Interbull, 2015).

A escolha de qual método utilizar depende da medida de longevidade a ser estudada, da facilidade de obtenção das informações fenotípicas e genotípicas, da praticidade, do pessoal treinado e do tempo de execução das análises. De acordo com o serviço internacional de avaliação de touros de 2015, 21 países participam da avaliação internacional de touros para longevidade na raça Holandesa. Nestes países, as avaliações genéticas nacionais são baseadas tanto em modelos de análise de sobrevivência (10), modelos lineares multi (7), ou unicaracterística (2), ou modelo de regressão aleatória (1) (Interbull, 2015).

### **Análise de Sobrevivência no Melhoramento Animal**

A análise de sobrevivência é uma área da estatística que estuda variáveis aleatórias positivas (T), mensurando o comprimento do intervalo de tempo entre um ponto de origem e um ponto final. O ponto final é também conhecido como evento de interesse e/ou de falha. O intervalo de tempo até a ocorrência do evento de interesse é dito como tempo de sobrevivência (Kalbfleisch & Prentice, 2002). Os primeiros estudos utilizando a análise de sobrevivência ocorreram na medicina humana devido ao interesse de se conhecer o tempo de sobrevivência de um paciente em tratamento para determinada doença (Colosimo & Giolo, 2006). Na produção animal, a variável

T, ou variável resposta, é medida em dias, meses, ou anos entre o nascimento, ou o primeiro parto (ponto de origem) até a morte e/ou descarte (ponto final, evento de interesse, falha) da vaca (Ducrocq, 2001).

No estudo da longevidade mediante a utilização da análise de sobrevivência, algumas particularidades são importantes. A principal delas, e que justifica o uso desta análise, é a presença de censura (Ducrocq et al., 1988). Além dessa característica, também a influência das variáveis explicativas deve ser levada em consideração.

Estas variáveis são chamadas, na análise de sobrevivência, de covariáveis fixas. Na análise de sobrevivência, os efeitos aleatórios, quando incluídos no modelo, denominam o modelo como modelo de fragilidade. As covariáveis incluídas no modelo podem ou não variar com o tempo. Quando estas variam com o tempo, são consideradas como covariáveis tempo dependentes (Ducrocq et al., 1988; Caetano, 2011).

### *Censura*

Como já mencionado, a análise de sobrevivência mede a duração de um intervalo de tempo até a ocorrência de um evento de interesse. Contudo, nem sempre o evento de interesse pode ser observado. Este fenômeno é chamado de censura e consiste na observação parcial da resposta que foi interrompida por alguma razão, não permitindo a observação completa do tempo de falha (Larroque & Ducrocq, 2001; Colosimo & Giolo, 2006).

Em bovinos leiteiros, a censura no estudo da longevidade é bastante presente, sendo a duração da vida produtiva (DVP) a variável resposta mais comumente utilizada (Forabosco et al., 2009; Imbayarwo-Chikosi et al., 2015). A DVP mede o intervalo de tempo, em dias ou em meses, entre a data ao primeiro parto e a data de descarte ou morte da vaca. A data ao primeiro parto é conhecida, mas a data de descarte e/ou morte nem sempre está presente, visto que no momento do estudo a vaca ainda pode estar viva e/ou ter sido vendida a outro rebanho que não faz controle leiteiro, não conhecendo, assim, a data de ocorrência do descarte do animal (Ducrocq et al., 1988). Desta forma, a observação desta vaca é dita como censurada à direita.

Existem quatro diferentes tipos de informações imparciais – censura – que podem ser usadas nas análises de sobrevivência: i) a censura à direita, que ocorre quando o evento de interesse não é observado, ou seja, quando este ocorre após o término do estudo. Desta forma, apenas sabemos que o tempo de falha é maior que o tempo de censura; ii) censura à esquerda, que se dá quando o evento de interesse ocorre antes do início do estudo, mas não se sabe exatamente o momento; iii) censura intercalar, usada no estudo de populações naturais, em que o evento de interesse ocorre em um intervalo entre uma e outra observação, ou seja, em um ano o animal foi observado, mas no outro ano não, indicando que o evento de interesse ocorreu entre estes dois anos; iv) censura aleatória, ocorre se a observação for retirada no decorrer do estudo sem ter ocorrido o evento de interesse, ou se o evento de interesse ocorrer por uma razão diferente da estudada. A censura à direita é o tipo de censura mais frequente em bovinos leiteiros (Ducrocq, 2001; Colosimo & Giolo, 2006).

Apesar da ocorrência de dados censurados, as informações destes

animais precisam ser usadas na análise estatística, visto que, ainda que imparciais, fornecem dados da vida destes, e que a tentativa de exclusão dessas informações poderá resultar em conclusões viesadas sobre a permanência destes animais no rebanho (Ducrocq et al., 1988; Beswick et al., 2004; Colosimo & Giolo, 2006).

A inclusão conjunta de informações censuradas e não censuradas nas análises é uma das principais vantagens da análise de sobrevivência frente a outros métodos de estudo da longevidade.

#### *Covariáveis dependentes do tempo*

O ambiente, as práticas de manejo e o nível de produção variam ao longo da vida da vaca. Neste contexto, o objetivo da análise de sobrevivência está centrado na relação entre os tempos de sobrevivência e as covariáveis explicativas (Caetano, 2011).

O uso de modelos de sobrevivência possibilita a inclusão de variáveis dependentes do tempo que afetam a vida da vaca no rebanho, e permite considerar que as condições ambientais particulares influenciam a probabilidade de a vaca ser descartada a cada dia durante toda sua vida produtiva, e não apenas em momentos específicos da vida (Ducrocq, 2005). Por isso, torna-se necessária a inclusão das variáveis dependentes do tempo no estudo da longevidade das vacas (Zavadilová et al. 2011).

#### *Especificando o Tempo de Sobrevivência*

A variável aleatória não negativa ( $T$ ), usualmente contínua, representa o tempo de falha (variável de interesse) especificado em análise de sobrevivência pela sua função de sobrevivência, ou pela função de taxa de falha, também conhecida como função de risco (Larroque & Ducrocq, 2011; Colosimo & Giolo, 2006).

A função de sobrevivência  $S(t)$  é definida como a probabilidade de uma vaca sobreviver a um determinado tempo  $t$ . Em termos probabilísticos, a função de sobrevivência é descrita como:  $S(t) = P(T \geq t)$  (Colosimo & Giolo, 2006). Já a função de risco  $\lambda(t)$  representa a taxa de falha instantânea, ou descarte da vaca, no tempo  $t$ , dado que o descarte não ocorreu até o tempo  $t$ . Desta forma, considerando que a vaca permaneceu no rebanho até o tempo  $t$ , a função de risco fornece a taxa de risco de descarte para o próximo instante.

A função de risco  $\lambda(t)$  é definida como:  $\lambda(t) = \lim_{\Delta t \rightarrow 0} \frac{P(t \leq T < t + \Delta t | T \geq t)}{\Delta t}$ . Onde  $T$  é a variável positiva que indica o tempo de descarte (Ducrocq et al., 1988).

Diferentes funções de sobrevivência podem ter formas semelhantes, enquanto as respectivas funções de risco podem diferir drasticamente. Desta forma, a função de risco é mais informativa do que a função de sobrevivência, sendo um importante método para o estudo de dados de sobrevivência (Colosimo & Giolo, 2006).

#### *Modelo de Riscos Proporcionais*

Os modelos de sobrevivência são construídos a partir de dois componentes, um aleatório (função de risco base) e outro determinístico (variáveis explanatórias e vetor de regressão), sob o ponto de vista



paramétrico. Assim, o componente aleatório é representado por uma distribuição de probabilidade vinculada ao comportamento do tempo de sobrevivência, enquanto que o componente determinístico é representado pelo relacionamento entre os parâmetros desta distribuição e as covariáveis em estudo (Caetano, 2011). O modelo pode ser descrito de forma sucinta:  $\lambda(t; x) = \lambda_0(t) \exp\{x\beta\}$ , em que  $\lambda(t; x)$  é a função de risco de falha, ou descarte da vaca,  $\beta$  é um vetor de coeficientes de regressão,  $\lambda_0(t)$  é a taxa de risco base, ou seja, o risco dos indivíduos com covariáveis  $x=0$ . Por fim,  $\exp\{x\beta\}$  representa a parte paramétrica e depende do vetor de parâmetros de regressão  $\beta$ , considerando as covariáveis  $x$  (Cox, 1972).

Os modelos de sobrevivência podem ser paramétricos ou semi-paramétricos. O modelo semi-paramétrico é assim conhecido porque uma forma paramétrica é assumida somente para o termo que contém os efeitos das variáveis explanatórias. Logo, os parâmetros em questão são os coeficientes das variáveis explanatórias consideradas no modelo e que geralmente se quer estimar (Ramos, 2014).

A função de risco base arbitrária é tratada como não-paramétrica, sendo descrita da seguinte forma:  $\lambda(t) = \lambda_0(t)$ . Isso significa que não é necessário assumir que a variável resposta segue alguma distribuição. Como exemplo de modelo semi-paramétrico, temos o modelo de Cox.

No modelo paramétrico, tanto a taxa de risco quanto o termo que contém as variáveis explanatórias assumem uma forma paramétrica. Um modelo de sobrevivência paramétrico é aquele que assume que a variável resposta (tempo de sobrevivência) segue distribuição conhecida (Ramos, 2014). A distribuição mais utilizada para o estudo da longevidade em bovinos de leite é a distribuição Weibull. A Função de risco de base, seguindo a distribuição de Weibull, é descrita de seguinte forma:  $\lambda(t) = \lambda\rho(\lambda t)^{\rho-1}$ , em que  $\lambda$  e  $\rho$  representam os parâmetros de escala e forma da distribuição de Weibull, respectivamente (Ducrocq, 2005).

O modelo Weibull apresenta vantagens em comparação ao modelo de Cox, tais como inferências mais precisas sobre o tempo de sobrevivência, resultados relacionados diretamente com a duração da sobrevivência e modelando para funções de risco não constantes, ou seja, que variam ao longo da vida da vaca (Flynn, 2012; Imbayarwo-Chikosi et al., 2015). Contudo, o modelo de Cox é mais flexível, visto que a função de risco de base não é restrita a uma forma específica ou distribuição.

Embora o modelo Cox permita estudar simultaneamente o efeito de diversas covariáveis na longevidade e permita o isolamento dos efeitos destas covariáveis, o modelo de Weibull assume que a função de risco base,  $(\lambda_0(t))$ , pode ser parametrizada de acordo com um modelo específico para a distribuição dos tempos da longevidade (Walters, 2009; Imbayarwo-Chikosi et al., 2015).

O nome “modelo de riscos proporcionais” é devido às seguintes funções, em que  $\lambda_A(t)$  e  $\lambda_B(t)$ , de risco dos animais A e B, associados com o vetor covariável  $x_A$  e  $x_B$ , respectivamente. A relação entre estes dois riscos dada na equação abaixo, uma constante que não depende do tempo e na qual as funções de risco dos animais A e B são proporcionais (Caetano, 2011).

$$\frac{\lambda_A(t)}{\lambda_B(t)} = \frac{\lambda(t; x_A)}{\lambda(t; x_B)} = \frac{\lambda_0(t) \exp\{x_A \beta\}}{\lambda_0(t) \exp\{x_B \beta\}} = \exp\{(x_A - x_B) \beta\}$$

A equação acima é chamada de risco relativo (razão de risco) de um indivíduo A e apresenta o evento de falha comparado a um indivíduo B.

Supondo que a constante é igual a 3, em qualquer ponto do tempo, o animal A será três vezes mais provável de descarte do que o animal B (Caetano, 2011).

O modelo de riscos proporcionais costuma ser empregado para se estimar o risco de um indivíduo levando-se em consideração uma ou mais variáveis explanatórias.

Um modelo de sobrevivência paramétrico (modelo Weibull) é aquele que assume que tanto a variável resposta (tempo de sobrevivência) quanto o termo que contém os efeitos das variáveis explanatórias seguem distribuição conhecida.

A função de risco base ( $\lambda_0$ ) é tratada como paramétrica quando utiliza a distribuição de Weibull (Colosimo & Giolo, 2006). A função de risco de base pode ser única ( $\lambda_0$ ), quando uma única função de Weibull é assumida em toda a vida da vaca, ou pode ser estratificada ( $\lambda_{0,ls}$ ), quando mais de uma função de Weibull são obtidas ao longo da vida da vaca (Ducrocq, 2005).

Considerando um exemplo em vacas Holandesas, se a produção de leite é diferente entre as lactações nos diferentes rebanhos, a pressuposição de uma base comum a todas as lactações nos diferentes rebanhos não é mais válida.

No melhoramento animal, o objetivo principal da utilização de modelos é a identificação das diferenças genéticas entre os animais. Para que isso seja possível, é necessária a inclusão do termo do componente aleatório referente a cada indivíduo.

Os modelos de riscos proporcionais podem ser estendidos incluindo os efeitos genéticos (aleatório), como em modelos lineares mistos. Modelos mistos de sobrevivência são conhecidos como modelos de fragilidade devido à inclusão de um componente aleatório criado para modelar a variabilidade de fatores individuais não observados que não são computados pelos outros preditores do modelo, além dos efeitos fixos e da função de risco de base (Colosimo & Giolo, 2006; Caetano, 2011). Assim, fragilidade é o termo utilizado para estimar o risco ou a probabilidade de sobrevivência de cada indivíduo, ou seja, os efeitos aleatórios (por exemplo, genéticos) dos animais (Kleinbaum & Klein, 2005).

Dentre os países membros do Interbull que realizam a avaliação genética para a longevidade, mais da metade utilizam o modelo de riscos proporcionais Weibull, sob um modelo touro ou touro-avó materno (Interbull, 2015).

Nestes países, a função de risco  $\lambda(t)$  no tempo  $t$ , vem sendo definida como uma função de risco base Weibull estratificado na forma de  $\lambda_{0,ls}(t) = \lambda \rho (\lambda t)^{\rho-1}$ , com os parâmetros de escala ( $\lambda$ ) e forma ( $\rho$ ) diferindo para combinações de lactação ( $l$ ) e estágio de lactação ( $s$ ), o que resulta em diferentes funções de Weibull – uma para cada intervalo de combinação. O modelo de Weibull pelo qual são obtidos os componentes de variância genética

e os valores genéticos dos touros para a longevidade funcional pode ser obtido via modelo touro-avô materno que segue:

$$\lambda(t) = \lambda_{0,ls}(t) \exp\{\sum_m f_m(t) + hys_k(t) + s_i + 0.5 mgs_j\}$$

Na equação,  $\sum_m f_m(t)$  é a soma dos efeitos ambientais fixos, dependentes (por exemplo, o nível de produção de leite ao longo da vida) e independentes (como a data ao primeiro parto) do tempo.  $hys_k(t)$  e  $s_i + 0.5 mgs_j$  representam a parte aleatória do modelo, em que  $hys_k(t)$  é o efeito de rebanho-ano com distribuição log-gamma, e  $s_i + 0.5 mgs_j$  é a contribuição aditiva genética do touro  $i$  e do avô materno do touro  $j$  da vaca.

Nos estudos de melhoramento genético animal, o modelo de riscos proporcionais Weibull tem sido analisado utilizando o software Survival Kit (Ducrocq et al., 2010; Mészáros et al., 2013). Este software foi desenvolvido devido à carência de programas que utilizem a análise de sobrevivência para a estimação dos valores genéticos dos animais de produção, visto que a maioria dos programas apresentam banco de dados extremamente grandes, em que os animais estão sob seleção.

O Survival Kit utiliza a abordagem Bayesiana para obtenção das estimativas dos valores genéticos e das herdabilidades, gerando números confiáveis mesmo com banco de dados extremamente grande, como ocorre na área de melhoramento animal. Os dados podem ser analisados sob um modelo de Cox ou modelo de Weibull, permitindo a inclusão de variáveis explanatórias contínuas ou descontínuas (tempo dependentes) e risco base estratificado. Os efeitos aleatórios podem assumir distribuição normal, normal multivariada (com uma matriz de parentesco) ou log-gama (Ducrocq & Casella, 1996; Ducrocq et al., 2010; Mészáros et al., 2013).

O Survival Kit permitiu grandes avanços no estudo da análise de sobrevivência na área do melhoramento animal pela possibilidade que oferece de modelar para a censura dos dados, ajustar a distribuição não normal dos dados de sobrevivência e incorporar efeitos dependentes do tempo nos modelos de riscos proporcionais Weibull. Contudo, o software é incapaz de ajustar para a estimação de correlação genética entre características (Veerkamp et al., 2001; Holtsmark et al., 2009), um requisito para aumentar a acurácia das avaliações dos animais. Ainda assim, ele permite a realização de análises de associações fenotípicas entre as características, possibilitando conhecer quais fatores e/ou características mais contribuem para o risco de descarte do animal (Larroque & Ducrocq, 2001; Zavadilová et al., 2011), sendo estas informações utilizadas para compor índices de seleção para a longevidade das vacas. Outra restrição do software é a não capacidade de processar modelos animais completos, o que restringe as análises a modelos de touro e de touro-avô materno (Holtsmark et al., 2009).

## HIPÓTESES E OBJETIVOS

### Hipóteses

A duração da vida produtiva – longevidade funcional – de vacas Holandesas no Brasil é influenciada pelos fatores não genéticos dependentes e independentes do tempo.

A duração da vida produtiva das vacas Holandesas no Brasil apresenta baixa herdabilidade.

As características lineares de tipo influenciam o risco relativo de descarte das vacas Holandesas manejadas no Brasil.

O escore de células somáticas contribui para o aumento da taxa de descarte das vacas Holandesas criadas no Brasil.

### Objetivos

Determinar os fatores não genéticos dependentes e independentes do tempo que influenciam a duração da vida produtiva de vacas Holandesas criadas nas condições brasileiras.

Estimar a herdabilidade equivalente e efetiva para a longevidade funcional em vacas Holandesas utilizando um modelo de riscos proporcionais Weibull estratificado.

Determinar a contribuição das características relacionadas ao sistema mamário, pernas e pés, garupa, corporais e escore final sobre o risco relativo de descarte das vacas.

Determinar a influência do escore de células somáticas sobre o risco de descarte das vacas Holandesas no Brasil.

## **CAPÍTULO II<sup>1</sup>**

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## Survival analysis of productive life in Brazilian Holstein using a piecewise Weibull proportional hazard model

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### ABSTRACT

The objectives of this study were to assess the most important factors that influence productive life (PL) of Brazilian Holstein cows and to estimate genetic parameters for PL using a piecewise Weibull proportional hazard model. Records of PL from first calving to last recording (culling) of 132,922 cows coming from 945 herds were used. They had to have their first calving occurring between 1989 and 2013 and they were daughters of 6,804 sires. The model included the time-dependent effects of region within year, class of milk production within herd-year, class of milk production within lactation number, class of fat and protein contents within herd and (variation in) herd size as well as the time-independent fixed effect of age at first calving, the random effects of herd-year, sire and maternal grand sire. All fixed effects had a significant effect on PL ( $P < 0.001$ ). The relative risk (RR) for within herd class of milk yield varied from 3.16 for the worst 20% class to 0.41 for the best 20% class. RR also increased as protein and fat decreased, but to a lesser extent compared to milk yield. Significant effects on PL were found for region-year,

with large yearly changes in some cases. RR increased with age at first calving and with herd size but lower risks were observed when herd size was increasing or decreasing, compared to stable herds. The Weibull shape parameters (and therefore RR) increased with lactation number and with stage of lactation. The sire genetic variance estimate was  $0.030 \pm 0.002$  which corresponds to an equivalent heritability estimate of 6.1% accounting for censoring. A positive genetic trend of PL was observed. These results may contribute to the development of a routine genetic evaluation necessary to improve PL of Brazilian Holsteins.

**Keywords:** dairy cattle, longevity, genetic analysis, survival, environment effect.

## 1. Introduction

Longevity is an important economic trait which considerably influences overall profitability of animal production. Profitability in dairy cattle is associated with a decrease of involuntary culling, caused by problems in reproduction, udder health and workability, and with an increase in voluntary culling mainly due to a low production level. Two distinct definitions for longevity were proposed by Ducrocq et al. (1988): True longevity defined as ability to avoid culling for any reason, which mainly depends on productivity, and functional longevity defined as the ability to delay involuntary culling. The use of functional longevity provides new information, more or less independent from production traits, and consequently more useful in breeding programs. In order to improve the economic efficiency of dairy production, inclusion of functional longevity in breeding goals has received increasing attention in the last two decades.

According to the International Bull Evaluation of December 2014, 21 countries are participating to the International Bull Evaluation for longevity in the Holstein breed. In these countries, the national genetic evaluations are based either on a survival analysis model (10 countries), or on a multitrait linear model (7 countries) or a single trait linear model (2 countries) (Interbull, 2015). The use of a survival model for longevity is supported by 1) the possibility to use records of cows that are still alive at the time of study (censored), 2) the possibility to include time-dependent variables that affect herd life such as herd management practices (Terawaki et al., 2006), which allow to consider the particular environmental conditions of a cow

influencing her probability of being culled each day during her entire productive life and not only at some specific moments, 3) the ability to cumulate information during periods of different lengths from one animal to the other, leading to higher heritability compared with linear models applied to survival at a specific time point (Sewalem et al., 2005; Forabosco et al., 2006). The estimates of theoretical heritability for longevity (i.e., in absence of any censoring) using survival models varied from 0.05 to 0.18 (Forabosco et al., 2009).

In survival analysis, a model is usually chosen for the hazard function, i.e., the probability that a cow may be culled on a particular day given she was alive the previous day. This allows to combine records from all cows, whether they are still alive (censored) or already culled. A very flexible model for the overall (baseline) hazard function is the piecewise Weibull model, in which a different Weibull function (a generalization of the exponential distribution) is defined within each stage of lactation of each lactation (Ducrocq, 2005) and not over the entire productive life of the cow. Terawaki et al. (2006) indicate that the piecewise Weibull model can better cope with the cyclic change of hazard over each lactation, leading to smoother hazard functions.

Survival models also permit a precise description of the environmental factors influencing risk of culling and therefore length of productive life and how they vary over time. For example, Ducrocq (2005) and Jenko et al. (2013a) reported differences in hazard due to the effects of year of production in different regions, of change in herd size and class of milk production in France and Slovenia, respectively. Ducrocq (1994) reported how the probability of being culled changed according to the different levels of milk production, size and variation in size herd in French Normande cows.

The objectives of this study were to evaluate the environmental factors that affect the productive life of Brazilian Holstein cows and estimate the genetic parameters for this trait using a piecewise Weibull proportional hazard model.

## **2. Material and methods**

### *2.1 Data*



Productive life records defined as the number of days between first calving and last test day record were obtained from the Brazilian Association of Holstein Cattle Breeders (ABCBRH) and its affiliated state agencies. Records from milk production of Holstein cows with at least a first calving occurring between 1989 and 2013 were included in the analysis. Cows younger than 20 or older than 40 months at first calving and cows without information on date of first calving were excluded from the data. In total, the analyzed data file included 132,922 productive life records of daughters of 6,804 sires from 945 herds, covering 5 regions (region 1- Minas Gerais, 2- Other states of Brazil, 3- Paraná, 4- São Paulo, and 5- Rio Grande do Sul).

The analysis was focused on culling early in life which are the most damaging for the herd profitability. For this purpose, when a cow had more than five lactations, her productive life record was assumed to be censored on the day of her sixth calving. Cows that were still alive on December 31, 2013 and with less than six calvings were also considered as censored at this date.

According to Jenko et al. (2013b), very long lactations are often related to poor recording of culling date or to abortions. In order to avoid favoring animals with long lactations, 5,176 cows with their last lactation longer than 800 days were censored after 800 days of lactation. For the other long lactations, it was assumed that cows were dried off at 800 days. To account for a possible preferential treatment in their new herd, cows sold to another herd were considered as censored on the day of their last milk record in their first herd. When the exact culling date was known, it was used. However, it was often missing and then, the last known recording date was used as culling date.

The pedigree was traced back only for the sires of the cows. The pedigree file included 6,804 animals. Seven unknown sire or maternal grand sire groups were created based on year of birth, sex and country of origin. Four groups of country of origin were created : the first group included Canada and the United States; the second group included Brazil, Argentina and Uruguay; the third group represented Europe (France, Spain, Italy, Belgium, New Zealand, Germany and the Netherlands); and the fourth group corresponded to sires with unknown origin.

## 2.2 Model

The hazard function  $\lambda(t)$  at time  $t$  was defined as a piecewise Weibull baseline hazard function of the form  $\lambda_{0,ls}(t) = \lambda\rho(\lambda t)^{\rho-1}$  with scale parameter  $\lambda$  and shape parameter  $\rho$  differing for each combination of lactation  $l$  (1 to 5) and stage of lactation  $s$  (1 to 4) resulting in 20 different Weibull functions, one per interval combination. Stages of lactation from 0 to 270 days, 271 to 380 days, 381 to dried date, and a separate dry period were defined according to  $\tau$ , the number of days since the most recent calving. These limits, also used in other studies (Ducrocq, 2005; Ojango et al., 2005; Terawaki et al., 2006; Jenko et al., 2013a), were determined after plotting the empirical within lactation estimate of the hazard function (Kalbfleisch and Prentice, 1980) derived from the within lactation Kaplan-Meier (Kaplan and Meier, 1958) estimates of the survival curves. To be considered as dry in lactation  $l$ , a cow had to have a known  $l + 1$  calving date. With this definition, no cow could be considered as uncensored (culled) during their dry period. Hence, the apparent risk of being culled during a dry period was 0. With this, cows with a longer length of life due to long dry periods were not given any extra advantage.

The analysis of productive life was performed using the Survival Kit version 6 software (Mészáros et al., 2013). The genetic value for functional longevity was approximated by accounting for production level in the model, i.e., for the main source of voluntary culling. The sire genetic variance and the breeding values of sires for functional longevity were estimated. The final model used can be written as:

$$\lambda(t) = \lambda_{0,ls}(t) \exp\{\sum_m f_m(t) + hys_k(t) + s_i + 0.5 mgs_j\}$$

where  $\sum_m f_m(t)$  is the sum of fixed environmental effects. One of these effects, age at first calving, was assumed to influence the whole productive life: it was time independent and was estimated for each one-month class of age at first calving between 20 and 42 months. All the other fixed effects were time-dependent. These included the interaction effects of region by year of calving (from 1989 to 2013); milk production class by year of calving within herd milk production class (from 1- worst (1- 20%), 2- (21 - 40%), 3- (41 – 60%), 4- (61 – 80%), 5- best (81 – 100%) for milk production, and 6- unknown); within herd milk production class by lactation number (1- first lactation, 2- later lactations); within herd fat content (from 1- worst (1- 20%) 2-

(21 - 40%), 3- (41 – 60%), 4- (61 – 80%), 5- best (81 – 100%) and 6- unknown); within herd protein content (from 1- worst (1- 20%) 2- (21 - 40%), 3- (41 – 60%), 4- (61 – 80%), 5- best (81 – 100%) and 6- unknown); and variation in herd size class (with only one class for herds with less than 5 cows, three classes of variation in herd size (decrease by more than 10%, stable size, increase by more than 10% for herds with 5 to 19 cows) and five classes of variation in herd size (decrease by more than 15%, decrease by 5 to 15%, stable size, increase by 5 to 15%, increase by more than 15% separately for herds with 20 to 49 cows and 50 cows or more)).

$hys_k(t)$  was the random herd-year effect with a log-gamma distribution with shape and scale parameters, both equal to  $\gamma$  in order to force its mean to be 1, and  $s_i + 0.5 mgs_j$  is the additive genetic contribution from the sire  $i$  and of the maternal grand sire  $j$  of the cow.

The statistical significance of the fixed effects in the proportional hazard model was tested using likelihood ratio tests. The estimates of these fixed effects were expressed as relative culling risks, determined as the ratio between the risk of culling under a particular class of environmental factor and a specific reference class.

The effective heritability ( $h^2$ ) of the model is the theoretical heritability in complete absence of censored information. The effective heritability was obtained from the sire variance ( $\sigma_s^2$ ), and from the variance of the log-gamma distribution of the herd-year effect, which is equal to  $\psi^{(1)}(\gamma)$ , the value of the trigamma function of  $\gamma$ . For a sire-maternal grand-sire model, it was calculated as Jenko et al. (2013a) as:

$$h^2 = \frac{4\sigma_s^2}{\frac{5}{4}\sigma_s^2 + \Psi^{(1)}(\gamma) + 1}$$

A more realistic and practical “equivalent heritability” (Yazdi et al., 2002, Meszaros et al., 2010) accounting for  $p(t)$ , the proportion of uncensored records at time  $t$ , was also calculated using the overall Kaplan-Meier estimate of  $p(t)$  for the first five years of productive life:

$$h_e^2(t) = \frac{4\sigma_s^2}{\frac{5}{4}\sigma_s^2 + \Psi^{(1)}(\gamma) + \frac{1}{p(t)}}$$

The equivalent heritability  $h_e^2$  is important because it allows the prediction of the expected reliability at a given time point for a sire with  $N$  daughters as a function of his proportion of uncensored daughters (Yazdi et al., 2002). The actual approximate reliability ( $R_i^2$ ) for a particular sire  $i$  is a function of the actual number  $N_{u(i)}$  of uncensored daughters:

$$R_i^2 = \frac{N_{u(i)} h^2}{(N_{u(i)} - 1) h^2 + 4} = \frac{N h_e^2(t)}{(N - 1) h_e^2(t) + 4}$$

The estimated breeding values (EBV) were standardized as  $EBV = -\hat{a}/\sigma_s$ , i.e., dividing the predicted sire effects  $\hat{a}$  by the sire genetic standard deviation ( $\sigma_s$ ) and multiplying them by -1 so positive EBV values correspond to sires transmitting better longevity, i.e., lower risk of being culled (Ducrocq, 2005).

The genetic trend was estimated by computing average EBV by year of birth of sires.

### 3. Results

In total, 38,902 cows had a censored record, which represents 29 % of the data set. From these, 12.8% were censored because they reached their 6<sup>th</sup> calving, 6.3% because they were still alive at the end of the study and 80.9% because reached their herd was no longer under milk recording.

Figure 1 represents the survivor curves of the whole population in the different regions. In Paraná (region 3) and to a lesser extent, in São Paulo (region 4), cows lived longer than in the other regions. This appears to be mainly related to a much higher culling rate at the end of the first lactation in the other regions. The small steep drop at 800 days in region 5 (Rio Grande do Sul) is related to a higher proportion of first lactation cows with very large lactation lengths, which were bounded at 800 days during data preparation. This may be related to a

locally less exhaustive recording of calving dates. Region 1 and 2 showed a similar curve shape.

In general, the values of the hazard function derived from the Kaplan-Meier estimate of the within lactation survivor curve (Figure 2) were lower in the first two lactations. After a slight rise at the beginning of the lactation, the hazard was relatively stable until about 270 days in first lactation, whereas it was slowly increasing for the other lactations. After 270 days, a sharp increase in hazard rate was observed for all lactations until about 380 days for lactation. Then the increase was not as strong. The large fluctuations observed after 380 days of lactations are due to the small number of cows which are still lactating. These observations are consistent with the choice of 270 days and 380 days as within-lactation cut-off points for the parametric piecewise Weibull distribution.

The evolution of the hazard function over time is illustrated in Figure 3 which reports the estimates of the Weibull parameter  $\rho$  for the different stages of lactation for all lactations. The Weibull parameter  $\rho$  describes the shape of the baseline hazard function: when it is larger than 1, the hazard function is increasing with time and the larger the parameter is, the steeper the increase. It can be seen that  $\rho$  was much lower during the first (1.53 - lactation 1 to 1.86 - lactation 5) and third stages (1.83 - lactation 5 to 2.56 -lactation 1) of lactation compared with the middle one (4.38 - lactation 1 to 5.00 - lactation 2). The stable values of the  $\rho$  estimates for a given period over lactations illustrate the cyclic culling pattern. The much higher estimate of  $\rho$  between 270 and 380 days of each lactation reflects the fact that cows are at a much higher risk to being culled during this period. From the last test date of the lactation to her next calving, the cow was considered dried (fourth "stage of lactation"). Then, her risk of culling was implicitly assumed to be zero, since this status was conditional on the existence of a new calving. This was chosen to avoid giving extra credit to cows with a long dry period.

The different fixed effects were tested using likelihood ratio tests comparing the full model with a model excluding the effect (Table 1). All the effects were found to be highly significant.

The relative risk of culling for the region by year of production effect shown on Figure 4 demonstrates the clear differences between regions observed in Figure 1. Region 1 (Minas

Gerais state) showed the highest relative risk values compared to the other regions especially after 1999, with high peaks between 2005 and 2009. Region 3 (Paraná state) showed a relatively constant and low risk of culling over the years, consistent with the higher survivor curve in Figure 1. In region 4 (São Paulo state), the relative risk went down until 2004. In region 2 including animals from other states of Brazil, important changes in relative risk over the years were observed.

From 2005 to 2012, high and low peaks in culling risk were observed in particular for regions 1, 3 and 4. From 2007 to 2011 milk price showed a considerable increase in Brazil compared to previous years, particularly in 2007 (Cepea, 2015). This higher milk price is the factor probably explaining the decrease in relative risk of culling in regions 3 and 4. In contrast, region 1 showed a sharp peak with a high relative risk in 2007. In 2005, the Minas Gerais Holstein association started to penalize farmers who stopped milk recording of animals without providing an exit record or a culling code of the animals as reported by C. F Mendonça Júnior (pers. comm.)<sup>1</sup>. This probably forced farmers to register many previous cullings. This can explain the drop in records and the increase in relative risk of culling. Moreover, 2007 was marked by a severe drought which contributed to decrease food supply forcing farmers to dispose of part of their herd. Finally, a important milk adulteration scandal broke in this region in 2007, leading to a decrease in milk collection for some cooperatives and consequently a drop of milk sales (Seagri, 2015). All these scenarios combined can explained the higher risk of culling observed in this period in region 1.

The effect of milk production on the relative risk of culling of cows showed that cows without production information had a higher risk of being culled than cows with average milk production (RR=1.18 in 2010 to 8.46 in 1995 – but these values were influenced by a relatively low number of animals culled before being actually milked). With a few exceptions (e.g., in 2007, 2008 and 2011) the relative risk for animals were substantially higher in class 1 (20% worst of the herd) and class 2 (20% to 40% worst) (RR values ranging from 1.49 (2007) to 3.16 (2012) and 1.02 (2008) and 1.63 (2013), respectively), than the average class (Figure 5). In

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<sup>1</sup> Personal communication from Cleocy Fam de Mendonça Junior, technical superintendent of Associação de Criadores de Gado Holandês de Minas Gerais, Juiz de Fora, Minas Gerais, Brazil, 2015.

contrast, the relative risk for animals of classes 4 and 5 (best 20% to 40% and best 20%) had relatively lower culling risk, with RR values ranging from 0.65 (2011) to 0.98 (2012) and from 0.41 (2011) to 0.87 (1991).

Risk of culling increased almost linearly with age at first calving (Figure 6). However, cows with a very early first calving (20 and 21 months of age) showed an increased risk of culling compared to cows calving for the first time around 22 to 23 months.

The estimated relative risks for protein and fat percent classes are presented in Figure 7, using class 3 (average) as reference. Animals without fat and protein percent information had respectively a 1.48 and 1.17 higher risk of being culled, probably partly because the absence of measurements was most often related to a very short lactation and not necessarily to a culling reason linked to milk components. Cows with low fat or protein content levels (class 1) showed a higher relative risk of culling (1.09 and 1.20, respectively). On the other hand, cows with a high fat or protein had a slightly lower risk of culling (RR= 0.96 and 0.92 respectively). Interestingly, protein percent appeared to be more influential than fat percent.

The relative culling risks for different classes of herd size and variation in herd size are displayed in Figure 8. Herds with fewer than five animals showed a very low relative risk (RR=0.34) in comparison with the reference class (Class 4.3). Cows in stable herds with between 5 and 20 animals (class 2.2) had a higher relative risk (RR=0.92) of being culled than cows in expanding or shrinking herds of the same size (RR=0.70 and 0.66, for class 2.3 and 2.1, respectively). When herd size was between 21 and 50 animals (classes 3.1 to 3.5) or more than 50 animals (classes 4.1 to 4.5), it was observed that expanding herds showed significantly lower relative risks compared with shrinking herds (e.g., RR=0.62 and 0.70 for classes 3.5 and 4.5 (increasing by more than 15%) vs RR= 1.18 and 1.22 for classes 3.2 and 4.2 (decreasing by 5 to 5%)). As for class 2.1, class 3.1 (herds with 21 to 50 cows with a decrease by more than >15%) had a distinct behavior with a lower relative risk (RR=0.93 than the reference).

The estimates of the effect of the interaction between lactation and milk yield class revealed that adult cows (with more than one lactation) and low milk production had a relatively higher risk to be culled (RR=1.19 (class 1) and 1.04 (class 2)) than first lactation cows with an average milk production (class 3). The opposite was found for cows in higher producing classes

(RR=0.91 and 0.76 for adult cows in class 4 and 5). Adult cows without known milk production for a particular lactation (likely to have been culled early in lactation) showed a lower risk of being culled (RR=0.74) than first lactation cows without known production.

The estimates of the  $\gamma$  parameter of the log-gamma distribution, the resulting variance of the herd-year random effect, the sire genetic variance and the effective heritability for length of productive life (i.e., assuming no censoring) are shown in Table 2. Figure 9A. A represents the Gram-Charlier approximation of the posterior density of the sire variance. It can be seen that the small standard deviation of the Bayesian estimation of the sire variance leads to a peaked, nearly symmetric distribution, reflecting an accurate estimation of the genetic component of the model.

Figure 9B displays the equivalent heritability during the first five years of productive life, which takes censoring into account. It shows a continuous increase with time (days since the first calving) with a sharp increase during the years and a progressive stabilization around 7% after 5 years. The theoretical heritability estimate in absence of censoring is 7.8%.

Figure 10 presents the expected increase in reliability of EBV with the increase over time of the number of uncensored daughters by sire. For example, without any other information coming from related sires, a bull needs to have 118 uncensored daughters to get a reliability of 70% only based on progeny information.

The genetic trend measured as the average EBV per year of birth of the bulls is shown in Figure 11. The EBV continuously increased over the years. This improvement (a positive increase in EBV corresponds to a decrease in risk of culling) was quite modest: about +0.25 genetic standard deviation in 30 years, i.e., less than 0.01 genetic standard deviation per year.

#### **4. Discussion**

The empirical hazard function observed for Brazilian Holsteins was similar to equivalent studies e.g., in French Holsteins (Ducrocq, 2005), Japanese Holsteins (Terawaki et al., 2006) or in Brown cattle (Jenko et al., 2013a), where cows showed a risk of culling slowly increasing during most of the lactation with a sharp increase at the end of lactation. According to Terawaki et al. (2006), culling during the first part of the lactations normally occurs due to



extreme cases, such as very low milk production or severe functional problems. At the end of lactation, heavier culling occurs because milking the cow is no longer profitable, even though culling decisions to low production, infertility or recurrent health problems may have been taken earlier.

The choice of a piecewise Weibull model within lactation number and stage of lactation accounts for these strong changes in risk of culling during productive life and ensures a better fit compared to the use of a single baseline hazard function (Ducrocq, 2005; Terawaki et al., 2006). As a result, several  $\rho$ , the Weibull shape parameters, were estimated. These values were similar in size and variation over the lactation to the ones reported by Ducrocq (2005) for Holstein cows in France.

#### 4.1. *Fixed effects*

The estimated effects of region within year of production revealed a different evolution of risk of culling over the years, with periods of regular increase or decrease of culling rate compared to the region arbitrarily chosen as reference (region 5). The large environmental variations in food availability, climate, and in management practices between the different studied regions as well as other factors such as milk price and particularities of milk recording of the associations in the region are the most likely reasons responsible for the very large variation in risk of culling of Brazilian cows over the years. This was observed in other studies. For example Caraviello et al. (2004) reported differences in relative risks in various geographic regions in the USA, which also had an impact on the parameters of the Weibull distribution as well as on heritability estimates. These authors mentioned factors such as herd size, housing facilities, feeding system, and heat stress, which can affect the voluntary and involuntary culling policies in individual herds. A possible improvement of the model here would have been a definition of different shape parameters ( $\rho$ ) for each region and year. The more parsimonious model considered here implicitly assumes that all regional differences are accounted for through the region x year effect.

Milk production has been identified as one of the main factors that affect the productive life of cows, because of its relationship to profitability in the dairy industry. The effect

of milk production by year on risk of culling showed a similar trend for all milk production classes over the years, but with a large variability in the magnitude of relative risks. A larger relative risk of culling related to cows in low milk production classes indicate that they are more likely to be voluntarily culled. Correcting for this effect in the model is tantamount to comparing culling risks of cows with comparable milk production. As such, the sire (genetic) effect on culling risk can be interpreted as an approximation of the effect of this sire on functional productive life ending in an involuntary culling. Factors that significantly affect functional longevity of cows are related to reproductive and health mammary gland problems (Forabosco et al., 2009). In this study, cows with high milk production had a lower relative risk than average cows, reflecting a lower voluntary culling.

Voluntary selection for milk components can be observed through the relative risk of culling associated with fat and protein content. In this study, cows with worst protein and fat percentage had a significantly higher risk of culling compared to average cows. For other classes of protein and fat percentage, differences in risk of culling were limited. Similar results were observed by Sewalem et al. (2005) and Ducrocq (2005) with Holstein cows in Canada and France, respectively, for protein content. However our estimate of the effect of fat content was contrary to the one reported by Sewalem et al. (2005). This reflects that over the years, Holstein cows in Brazil were also selected for increased fat content.

Age at first calving significantly influenced length of productive life. The occurrence of late first calving is usually a consequence of reproductive, nutritional and health problems and these factors may continue to affect the risk of culling later. In addition, it impacts lifetime production due to fewer calvings and lactations (Dürr et al., 1999; Sewalem et al., 2005). Our estimates of age at first calving effects were similar to those reported by M'hamdi et al. (2010) and Sewalem et al. (2005). The larger risk observed for young cows (20 months) compared with cows calving around 22 to 24 months of age may be related to major occurrence of dystocia in younger cows (Martinez et al., 1983). It was not observed in the case of French Holstein (Ducrocq, 2005).

The effect of herd size variation was included to take into account the fact that in expanding herds, cows are intuitively less likely to be culled in order to increase herd size from

on-farm stock (Mészáros et al., 2008; Potočník et al., 2010). Conversely, a decrease of the average number of cows in the herd often reflects a voluntary increase in culling rate. The estimates obtained here showed that overall culling rate is higher in larger herds in Brazil but also that culling policies may be different in small herds (less than 20) compared to larger herds, as observed by Ducrocq (2005). Surprisingly, in such herds, culling risk after correcting for all other factors is lower for shrinking herds as well. A possible explanation is that in these herds, voluntary culling for low production is minimal and cannot be decreased further to compensate an involuntary culling, so farmers try to keep their cows longer.

#### *4.2. Estimation of variance of random effects and genetic parameters*

The variance of the herd-year random effect was larger than most published values, for example the one reported by Ducrocq (2005) and Terawaki et al. (2006) in Holstein cows in France and Japan, respectively. It indicates that the data analyzed correspond to herds with a more diverse management system. According to Sewalem et al. (2005), a smaller population size and/or sires with their daughters in a few herds can result in an inflation of the environmental (herd-year) variance. The sire genetic variance and effective heritability ( $h^2$ ) were generally smaller than the ones reported in other countries in Holstein. The  $h^2$  values estimated using The Survival Kit for national evaluations varied from 0.05 (Hungary) to 0.22 (Czech Republic) (Interbull, 2015). The distinct values of  $h^2$  resulted from differences in management practices and populations but also on the model used and data edits. According to Terawaki and Ducrocq (2009), the sire variance and the effective heritability can be also influenced by total number of records and of herds in the data set and by the similarity in distribution of herd size.

The estimates of equivalent heritability, i.e., when the variable proportion of censored daughters per sire is accounted for, showed a constant increase during the first 5 years of productive life of the cows. These values were rather low (around 4%) after 2 years of productive life. This is in fact the consequence of an initially low number of uncensored daughters. These low values have a substantial impact on the reliability of bulls with young

daughters: as seen in Figure 10, larger progeny groups are needed to reach a given level of reliability when the equivalent reliability is lower.

The genetic trend was extremely small, but it showed a favourable trend over the years. However, genetic trends slightly unfavourable have been reported for functional longevity (Jenko et al., 2013a). According these authors, this genetic trend behavior is not surprising, given the negative genetic correlation between functional longevity and milk production traits for which selection has been intensively practiced in the past years. Functional longevity is determined by decisions related to involuntary culling, independent from level of milk production. A decrease in involuntary culling is favourable for selection to production, resulting in larger gains in production due to more opportunities of voluntary cullings, based in milk yield.

## **5. Conclusions**

This study shows that Brazil can use with profit survival analysis models to implement a genetic evaluation on length of productive life in Holstein cows. It was shown that the environmental factors considered in this study have a large influence on risk of culling of Holstein cow in Brazil, especially within herd level of milk production and regional effects. The piecewise Weibull baseline model adequately mimicked the cyclic change of hazard over successive lactations, in particular an initially slow increase in hazard and a much faster increase at the end of each lactation. Heritability values obtained were relatively low, in the lower range of values reported in the literature. A routine genetic evaluation is necessary to improve length of productive life of Brazilian Holsteins under local conditions. The use of early predictors correlated with functionality or udder health may be recommended to increase the reliability of sires' estimated breeding values for functional longevity.

## **Acknowledgments**

The authors are grateful to the Brazilian Association of Holstein Cattle Breeders (ABCBRH) for supplying the data, which were essential to the development of this study. The first author expresses her gratitude to the Unité Mixte de Recherche de Génétique Animale et Biologie Intégrative (GABI) of the Institut National de la Recherche Agronomique (INRA) in

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**Table 1.** Likelihood ratio test statistics ( $-2\Delta LL$ ) for fixed environmental effects comparing the complete model with one where the effect is excluded (degrees of freedom (DF) and statistically significance ( $p$ )).

Effects	DF	$-2\Delta LL$	$P(0.001)$
Region by year of calving	86	4203.3	<0.001
Milk production class by year of calving	125	4174.4	<0.001
Within herd milk production class by lactation number	5	380.3	<0.001
Age at first calving	22	919.3	<0.001
Within herd protein percent class	5	534.4	<0.001
Within herd fat content class	5	133.7	<0.001
Variation in herd size class	13	2695.7	<0.001

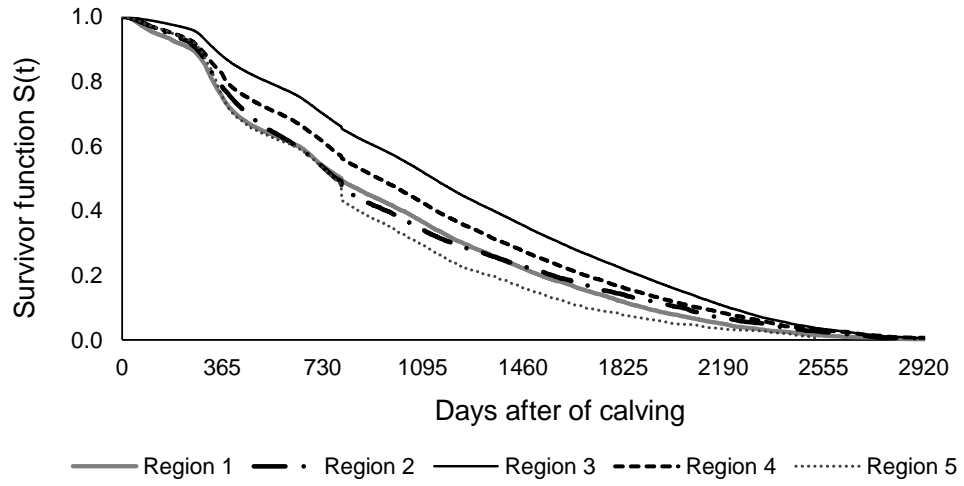


**Table 2.** Estimates of parameters related to the herd-year and sire random effects genetic and non-genetic random effect parameters.

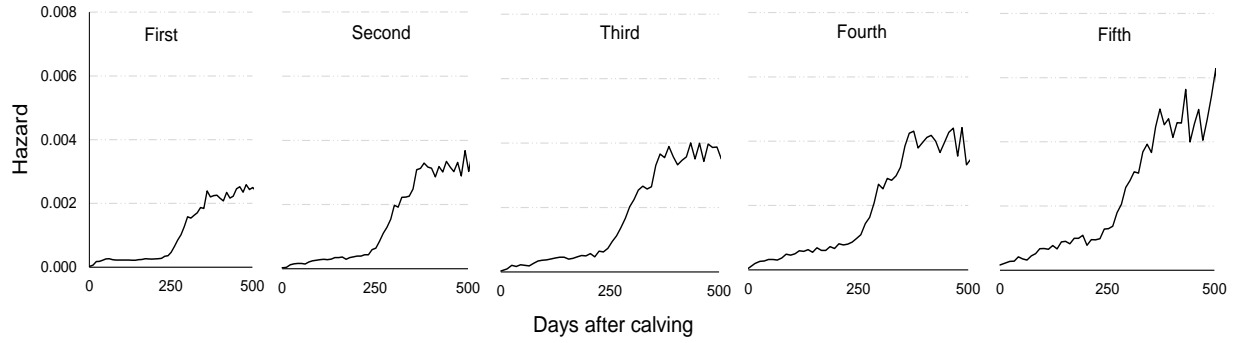
<b>Variables</b>	<b>Values</b>
$\gamma$	2.414 $\pm$ 0.248
$\psi^{(1)}(\gamma)$	0.512
$\sigma_s^2$	0.030 $\pm$ 0.002
$h^2$	0.078

$\gamma$  = shape and scale parameters (assumed to be equal) of the log-gamma distribution (of the herd-year-effect),  $\psi^{(1)}(\gamma)$ = variance of the herd-year random effect;  $\sigma_s^2$ = sire genetic variance;  $h^2$ = effective heritability.

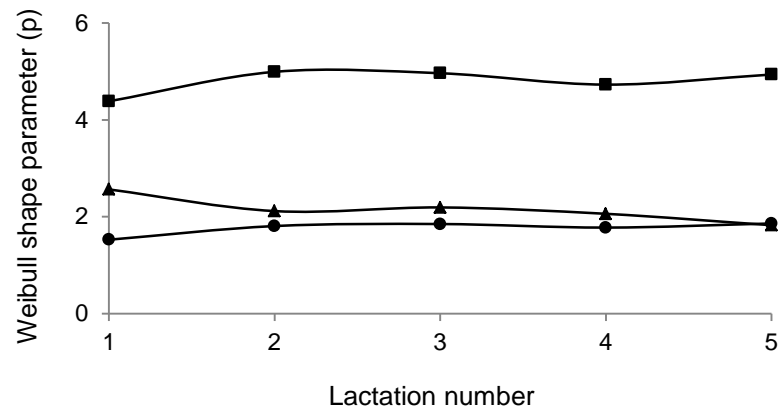
**Figure 1.** Survivor curves after first calving in the 5 regions (region 1- Minas Gerais, 2- Other states of Brazil, 3- Paraná, 4- São Paulo, and 5- Rio Grande do Sul).



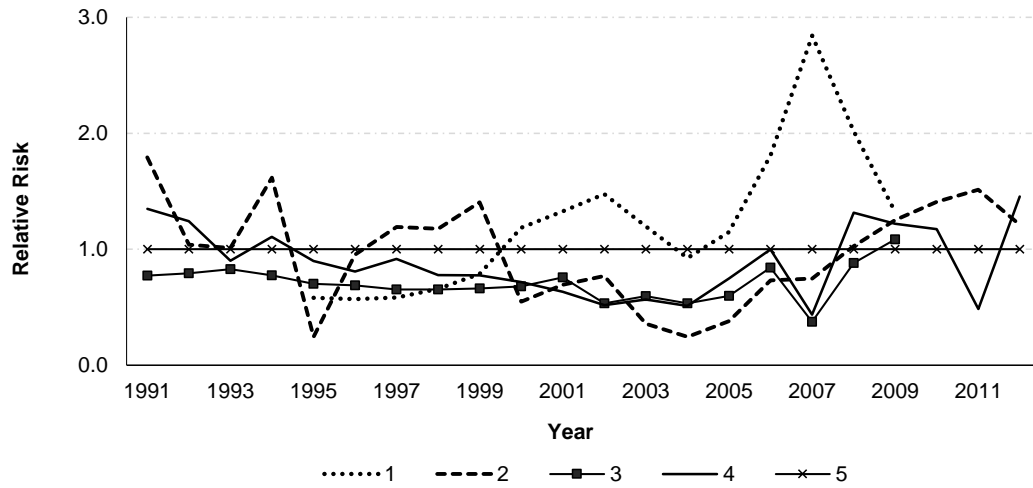
**Figure 2.** Empirical hazard function derived from the Kaplan-Meier estimator of survival function for the first five lactations.



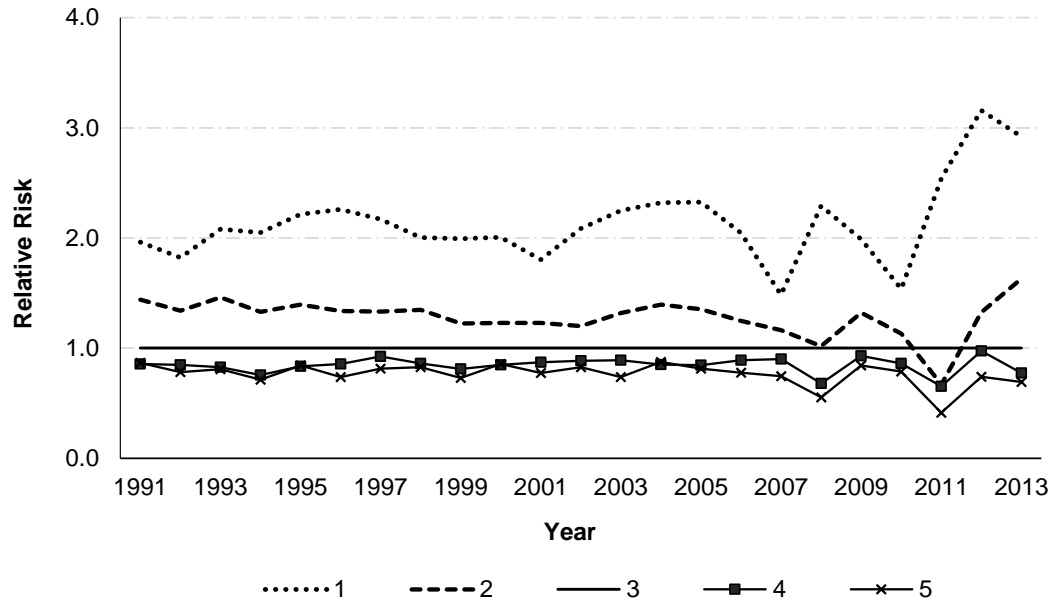
**Figure 3.** Estimates of the Weibull shape parameter  $\rho$  as a function of lactation number (1 to 5) combined with stage of lactation ((●)= 0 to 270 days after calving, (■)= 271 to 380 days, (▲)= more than 380 days).



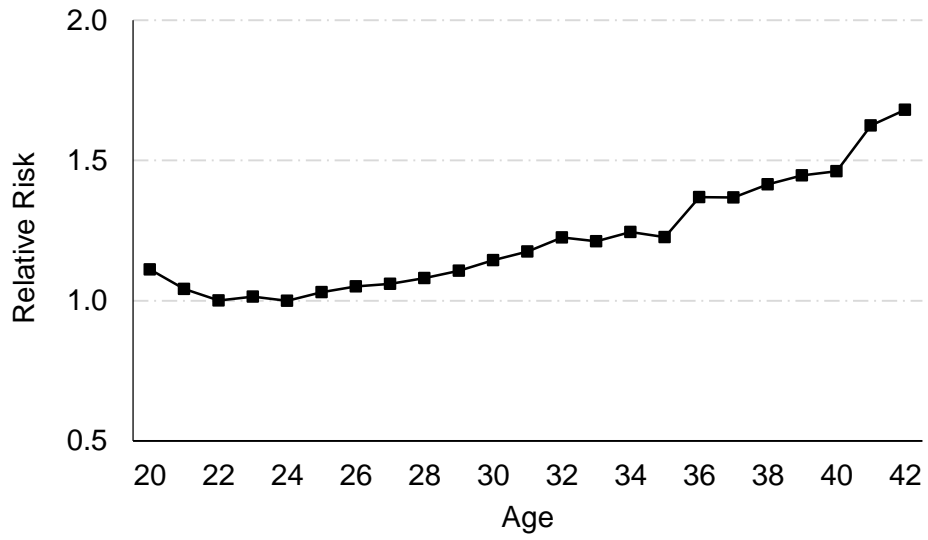
**Figure 4.** Estimates of the relative risk of culling for regions 1 to 4 by year compared with region 5 chosen as a reference (relative risk=1).



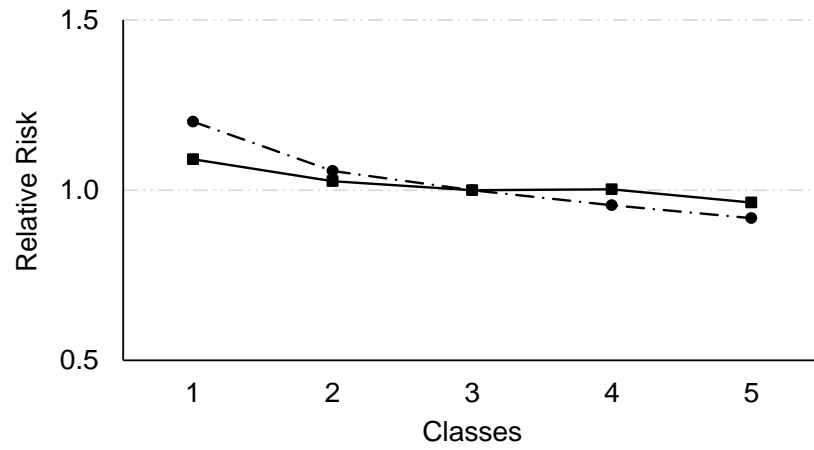
**Figure 5.** Estimates of the relative risk of culling for the five milk yield classes (1- worst 20%; 5 – best 20%;) by year (1989 to 2013) compared with the average milk production class (class=3) used as reference.



**Figure 6.** Estimate of the relative risk of culling for age at first calving affect (reference: 24 months of age).

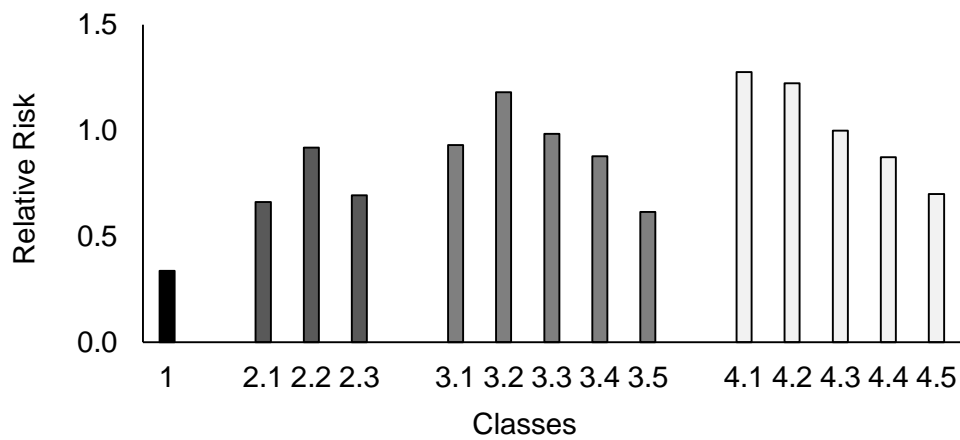


**Figure 7.** Estimates of relative risk of culling for five classes of protein (dashed line) and fat (continuous line) percent production effect (1- worst, 5 – best) compared with the average production class (class 3) used as reference.

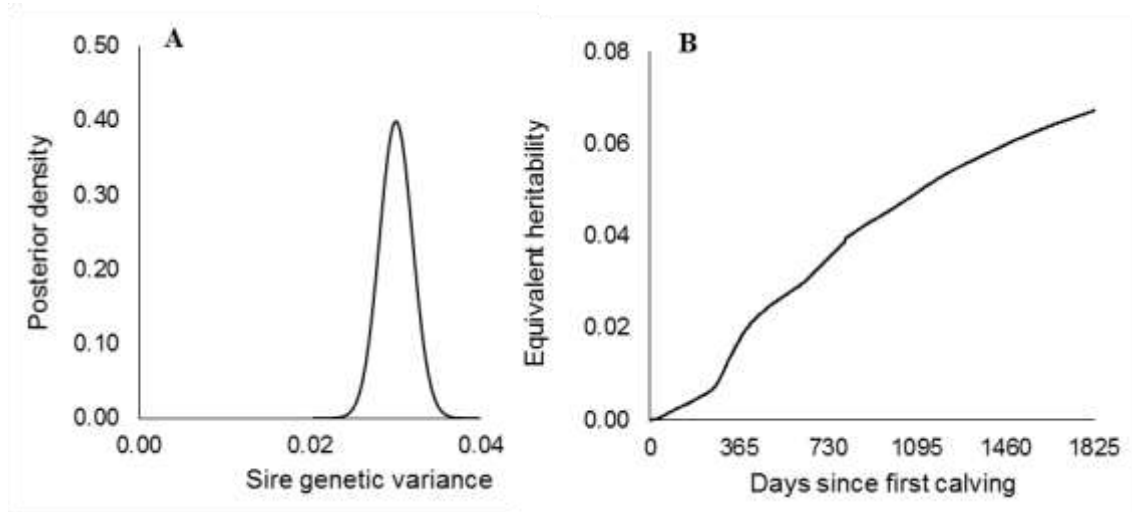




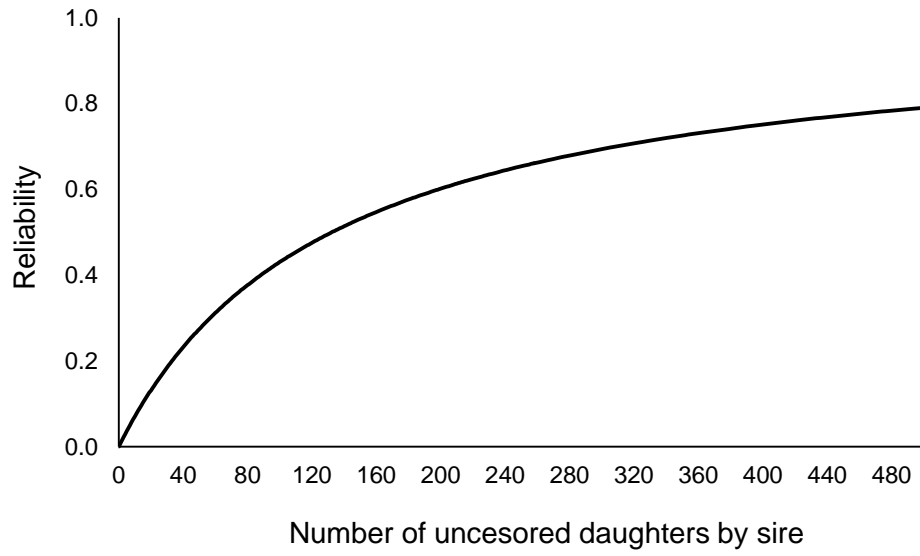
**Figure 8.** Estimates of the relative risk of culling for the interaction effect of herd size by variation of herd size (reference: stable herd size with more than 50 cows). Herd size: **1**- less than 5 cows, **2.1 to 2.3**- from 5 to 20, **3.1 to 3.5**- from 21 to 50, **4.1 to 4.5**- more than 50 cows. Variation in herd size: **1, 2.2, 3.3** and **4.3** -stable herd, **2.1**- decrease by more than 10%, **2.3**- increase by more than 10%, **3.1** and **4.1**- decrease by more than 15%, **3.2** and **4.2**- decrease between 5 to 15%, **3.4** and **4.3**- increase between 5 to 15%, **3.5** and **4.5**- increase by more than 15%.



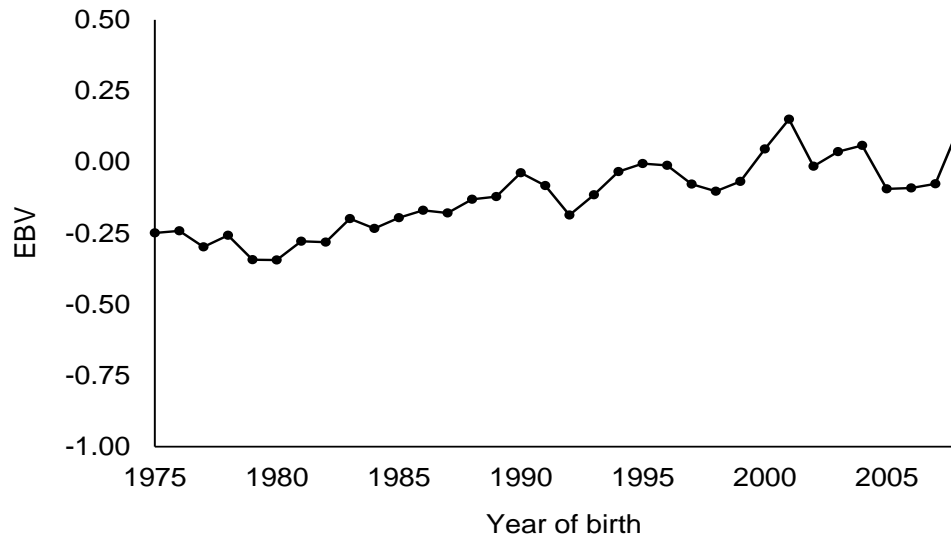
**Figure 9.** Gram-Charlier approximation of the posterior densities of the sire genetic variance (A) for length of productive life and evolution of the equivalent heritability (B) as a function of number of days since first calving, derived from the Kaplan-Meier estimate of the global survivor function.



**Figure 10.** Approximate reliability of sire breeding values based on number of uncensored daughters.



**Figure 11.** Estimate of the genetic trend for length of productive life: average estimated breeding values (EBV) of bulls per year of birth, in genetic standard deviations.



## **CAPÍTULO III<sup>1</sup>**

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## **Phenotypic relationships between type traits and length of productive life in Holstein cows using a piecewise Weibull proportional hazard model**

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### **ABSTRACT**

Longevity is an important trait given its relationship with dairy cow profitability. Type traits have been used as indirect predictors for productive life. The objective of this study was to evaluate the relationship of 20 type traits and final score on length of productive life in Brazilian Holsteins, using a piecewise Weibull proportional hazard model. Records on length of productive life of 54,633 cows and type traits of 78,289 cows from 915 herds were included in the analysis. Three analyses were performed to study the relationship between length of productive life and each type trait using proportional hazard models: i) productive life was corrected for within herd level of production as a proxy for functional longevity, where were included the time-dependent effects of region within year, class of milk production within herd-year, class of milk production within lactation number, class of fat and protein contents within herd and (variation in) herd size as well as the time-independent fixed effect of age at first calving and the type trait score; ii) the effects related to production level as class of milk production within herd-year, class of milk production within lactation number, class of fat and

protein contents were omitted from the first model (true longevity) and iii) with the first model, the effect of type was also studied considering five classes of percentage of type-scored cows within the herd. The herd-year effect, sire and maternal grand sire were included as random effects in all analyses. All analyses were performed using the computer program Survival Kit v. 6. Type traits were found to have a low association with functional longevity. However, final score, angularity, top line, udder texture and suspensory ligament showed the strongest relationship with productive life. Differences in risk of culling were observed depending on the fraction of type-scored animals within a herd. When type traits were available only for a small fraction of the herd, these cows had a better chance of remaining longer in the herd. The absence of type trait phenotypes was associated with a strong increase of culling risk for the cows. In conclusion, type traits were not found to be good indirect predictors of productive life in Brazil, probably because, in most herds, type-scoring was not exhaustive and was performed on cows with better type traits.

**Keywords:** conformation traits, dairy cattle, longevity, culling risk, survival analysis.

## 1. Introduction

Productive efficiency of dairy cattle has a strong relationship with increased longevity. Longer productive life lead to lower replacement and treatment costs associated with fertility and health disorders or problems related to cow morphology, such as lameness related with poor feet and leg conformation as well as clinical or subclinical mastitis with poor udder conformation (Cassell and Weigel, 1994; Ducrocq et al.,1988; Essl, 1988).

Two different definitions of cow longevity were introduced by Ducrocq et al. (1987): true longevity characterizes the aptitude for a cow to remain in her herd, which depends on productivity. Functional longevity represents the ability to delay involuntary culling, for example because of sterility, lameness, mastitis or other diseases. When cows are selected for better functional longevity, involuntary culling is decreased and a higher voluntary culling rate is possible, hopefully resulting in higher herd profitability.

Direct selection for longevity has always been a challenge, ranging from the choice of a proper measure of productive life compatible with short generation intervals up to the choice of an appropriate method of analysis accounting for the fact that environmental factors influencing culling change with time. Survival analysis offers the possibility of objectively combining information from live (censored) and culled (uncensored) animals, to model the nonlinear and time-dependent factors influencing length of productive life. Survival analysis models usually rely on the description of the evolution of a hazard function throughout the cow's productive life, i.e., the evolution of her daily probability of being culled, given that she is alive (Ducrocq, 2005).

Proportional hazards model have been proposed, which separate the description of, on one hand, the baseline hazard function of the whole population and, on the other hand, the time-independent or time-dependent effects of genetic or non-genetic effects affecting the baseline hazard function. Because of its computational simplicity, a Weibull distribution is often chosen for the baseline hazard function. This is implemented in the Survival Kit package (Meszaros et al., 2010) for example. The use of a piecewise Weibull distribution allows a finer description of the cyclic nature of the culling rate of cows from one lactation to the next (Ducrocq, 2005).

This survival analysis model is currently used in a number of countries for the genetic evaluation of bulls due to the productive life of their daughters (Interbull, 2015). However, according to Buenger et al. (2001), the reliability of young sire evaluations for productive life remains limited, because a majority of their daughters are still alive at the end of their first lactation. The problem increases since nowadays we have genotyped young bulls. Indeed, a large proportion of censored records lead to a low accuracy of productive life evaluations (Vukasinovic et al. 1999). To increase this accuracy, it is desirable to combine information on traits correlated with longevity which can be recorded early in life (Larroque and Ducrocq, 2001; Buenger et al., 2001; Forabosco et al., 2009).

Type traits play an essential role in breeding and selection decisions in dairy cattle. According to Short and Lawlor (1992) and Dekkers et al. (1994), the main objective of type classification is to identify and select desirable type traits associated with improved herd life. Numerous studies investigated the relationship between type traits and longevity to justify direct



selection on some to improve longevity (Jong et al., 1999; Larroque and Ducrocq, 2001; VanRaden, 2001; Morek-Kopec and Zarnecki, 2012). Type trait scores are most often collected during first lactation, i.e. early in life. They are relatively easy to measure and have higher heritability than length of productive life.

Several studies using different approaches showed the beneficial impact of type traits on longevity in dairy cattle, especially udder, feet and leg traits (Larroque and Ducrocq, 2001; Buenger et al, 2001, Caraviello et al., 2003; Sewalem et al., 2005; Dadpasand et al., 2008). However, Terawaki et al. (2006, 2009) reported that the herd policy relative to type traits recording has a strong influence on the length of productive life of cows in Japan. These authors showed that the fraction of type-scored cows in the herd influences the risk of culling and the heritability of length of productive life in Japanese Holstein cows.

A routine genetic evaluation of Holstein cattle for linear type traits has been carried out by Embrapa Dairy Cattle (Costa et al., 2012) since 2003 and involves the evaluation of final score and 20 elementary type traits divided into 4 groups: body traits, rump traits, feet and legs and udder system. A genetic evaluation of Holstein bulls for productive life of their daughters in Brazil has been investigated (Kern et al., 2016) but is not routinely carried out. To promote future evaluations, an important step is to understand the relationship between productive life and other traits, in particular with potential early predictors such as type traits. The hypothesis of this study was that some type traits can be used as early predictors of longevity in Holstein cows in Brazil. This study was performed with the objective to evaluate the effect of type traits on productive life in Brazilian Holstein cows, using a piecewise Weibull proportional hazard model.

## **2. Material and methods**

### *2.1 Data*

Length of productive life and type traits records were obtained from the Brazilian Association of Holstein Cattle Breeders (ABCBRH) and its affiliated state agencies. Two longevity traits were defined as in Ducrocq et al. (1988): true longevity which is measured as the

number of days from first calving to culling, and functional longevity which is approximated by correcting true longevity for within-herd–year level of production.

The type traits database was merged with production data. As a result, 54,633 cows had both type and production traits (milk yield adjusted to 305 days, fat and protein contents) while 78,289 cows had only production data. Production and type traits of Holstein cows with first calving occurring between 1989 and 2013 were included in the analysis. Cows younger than 20 or older than 40 months at first calving and cows without date at first calving were excluded from the analysis, as well as daughters of sires with fewer than five daughters.

Focusing on the effect of type traits on culling early in life (which most strongly impacts profitability), a cow that had more than five lactations was assumed to be censored on the day of her sixth calving. When the exact culling date was missing, the last known milk recording date was used as culling date. Cows that were still alive on December 31, 2013 were also considered as censored. To avoid biases due to potential poor recording of calving dates, lactation lengths were bounded at 800 days. Cows sold to other herds were censored on the day of their last milk record in the first herd, to account for a possible preferential treatment in their new herd. Overall, 33% of the cows had a censored record.

The linear classification system used by the ABCBRH included 20 type traits expressed on a scale from one to nine points (Table 1) as well as final score expressed on a scale from 1 (poor) to 6 (excellent), where body traits received a weight of 22%, feet and legs 26%, mammary system 42%, and rump traits 10%. The impact of each type trait on functional longevity was studied separately. In total 132,922 cows, daughters of 6,084 sires in 915 herds were included in the analysis.

## *2.2 Model*

The type traits are often presented as good predictors of longevity. In this way it is important to know the relationship between type traits and true and functional longevity in Holstein cows in Brazil. Three analyses were performed to evaluate the relationship between length of productive life and each type trait using proportional hazard models where the hazard  $\lambda(t)$  of a cow at time  $t$  (i.e., the risk of culling given she is alive just prior to  $t$ ) was modeled using a

piecewise Weibull hazard function. In the first analysis, an approximation of functional longevity was studied, in which all environmental effects found significant in the analysis of Kern et al. (2016) and described below were analyzed with each type trait presented in Table 1. In the second analysis, the effects related to level of production were omitted to study the impact of each type trait on true longevity. In the third analysis, the functional longevity and all effect included in the first analysis were considered, with except of change of the effect of each type trait by the effect of 5 classes of herd percentage of type-scored cows (1= 0 to 10%, 2= from 11 to 30%, 3= from 31 to 50%, 4= from 51 to 70%, 5= from 71 to 100% of herd percentage of type-scored cows). The analysis of the effect of percentage of type-scored cows was performed because not all cows in a herd were scored for type.

The full model used for the three analysis above described the relationship between type traits and longevity in Holstein cows in Brazil can be write as:

$$\lambda(t) = \lambda_{0,ls}(t) \exp\{\sum_m f_m(t) + hys_k(t) + s_i + 0.5 mgs_j\} \quad [1]$$

where  $\lambda(t)$  is the baseline hazard function of the cow at time  $t$ , defined as a piecewise Weibull hazard function of the form  $\lambda_{0,ls}(t) = \lambda\rho(\lambda t)^{\rho-1}$  with scale parameter  $\lambda$  and shape parameter  $\rho$  differing for each combination of the  $l$ th lactation (1 to 5) and the  $s$ th stage of lactation  $s$  (1 to 4) resulting in 20 different Weibull functions, one per interval. Stages of lactation were defined according to the number of days from the most recent calving, with changes within each lactation at time 0, 270, 380, and day when dried-off.

In the three analysis described above, the model included the following fixed environmental effects ( $\sum_m f_m(t)$  is the sum of fixed environmental effects): Age at first calving, was assumed to influence the whole productive life: it was time independent and was estimated for each one-month class of age at first calving between 20 and 42 months. All the other fixed effects were time-dependent. These included the interaction effects of region by year of calving (from 1989 to 2013); variation in herd size class (i - with only one class for herds size with less than 5 cows, three classes of variation in herd size (ii - decrease by more than 10% in herd size, iii - stable size, iv - increase by more than 10% for herds size with 5 to 19 cows) and five classes of variation in herd size (v - decrease by more than 15% in herd size, vi - decrease by 5

to 15%, vii - stable size, viii - increase by 5 to 15%, iv - increase by more than 15%, separately for herds size with 20 to 49 cows and also to herds size with 50 cows or more)); and type trait score of  $p$  ( $p$  generally from 1 to 9) (Table 1). The 20 type traits and the final score were considered separately, one at a time. A particular “missing” class was used for cows without type score. In the third analysis, this fixed effect were changed by herd percentage of type-scored cows effect as describe above.

For the second analysis, the following fixed effects time-dependent were omitted when studying the impact of type traits on true longevity: milk production class by year of calving within herd milk production class (from 1- worst (1- 20%), 2- (21 - 40%), 3- (41 – 60%), 4- (61 – 80%), 5- best (81 – 100%) for milk production, and 6- unknown); within herd milk production class by lactation number (1- first lactation, 2- later lactations); within herd fat content (from 1- worst (1- 20%) 2- (21 - 40%), 3- (41 – 60%), 4- (61 – 80%), 5- best (81 – 100%) and 6- unknown); within herd protein content (from 1- worst (1- 20%) 2- (21 - 40%), 3- (41 – 60%), 4- (61 – 80%), 5- best (81 – 100%) and 6- unknown).

The following random effects were also considered in the three analysis:

$h_{yq}(t)$  is the  $q$ th random herd-year effect assumed to follow a log-gamma distribution (with shape and scale parameters both equal to  $\gamma$  in order to force its mean to be 1),

$s_r + 0.5 mgs_s$  represents the additive genetic contribution from the sire  $r$  and of the maternal grand sire  $s$  of the cow.

Estimates of all fixed effects were expressed as relative culling risks, defined as the ratio between the risk of culling under a particular environmental factor and a specific reference class. For example, the risk ratio associated with a score of  $p=8$  for one of the 20 type traits was defined as  $\exp\{t_p - t_5\}$ , score 5 being chosen as the reference class.

The overall influence of each type trait on functional or true longevity was assessed using likelihood ratio tests, comparing a full model including each particular type trait with a reduced model without any type trait. For true longevity, in the second analysis, corrections for production traits were omitted from the model. All analyses were performed using the Survival Kit version 6 software (Meszaros et al., 2010).

### 3. Results

All type traits included in the model had a highly significant effect ( $P < 0.001$ ) on risk of the cow being culled. However, this was due to the existence of a “missing” class independent of type trait: this missing class represents contemporary cows which were not type-scored (Tables 2 and 3) and had a much higher relative risk of being culled (1.48 to 1.62) than a cow in the reference class. This motivated the extension of our analysis to consider the percentage of typed cows in the herds as in Terawaki and Ducrocq (2009).

The relative contribution of each type trait to minus twice the log-likelihood ( $-2\log L$ ) of the model for functional longevity (corrected for production) or true longevity (not corrected for production) is illustrated in Figure 1, after removing the contribution of the missing type class. The relative contributions were ranked in decreasing order and expressed as a percentage of the contribution of final score, which is the trait that has caused the largest change in  $-2\log L$ . Whether a correction for production traits was applied or not, the contribution of type traits on productive life (PL) was in general small and with limited variability, with a few exceptions considered below. Final score, angularity and top line were the most important traits related to PL, followed by some udder traits such as udder texture and median suspensory ligament.

#### 3.1 *Relative risk of culling*

Among body traits, angularity and top line had a relatively strong relationship with productive life corrected or not for production (Figure 1), but angularity had a larger contribution when production was not corrected for, suggesting that it was also associated with better production. The opposite was observed for top line. Except for stature, high scores for body traits tended to be associated with a decrease in risk of culling (Table 2). There was no clear relationship between stature and productive life (Figure 1). When the fraction of type-scored cows was accounted for (figure 2), there were few or no scores below 3 or 4 when less than 30% of the herd was scored, probably because of a pre-selection of the animals to be scored based on these criteria.

Relative risk estimates for feet and leg traits are shown in Table 2. Bone quality and rear leg set displayed a linear relationship with functional productive life, with slightly higher

relative risk estimates for lower scores. The trend for foot angle was so small that none of the scores showed a significant difference from the reference score. Cows with straight rear legs and coarse bone had an increased relative risk of culling. In herds with over 70% of cows recorded for type traits, cows with highly sickled legs (score 9) showed a higher risk of culling (Figure 3) which is surprising because for other classes of percentage of type-scored cows in the herd, higher scores were associated with a lower relative risk. Low variation in the risk of culling for bone quality was observed when more than 70% of cows were type-scored.

Except for rump angle, rump traits showed a low relationship with functional longevity (Table 2). For loin strength and rump width, the higher scores are associated with a lower relative risk. For rump angle, there is an intermediate optimum score corresponding to the reference class (score 5). An approximately linear trend in estimated relative risk of culling for rump width and loin strength was observed in herds with more than 70 % of type-scored cows. However, large fluctuations of the effect of the same traits were found for cows in herds with less than 50% percent of type-scored animals (Figure 3) illustrating the impossibility of drawing clear conclusions with partial performance recording.

Traits related to mammary system presented a low to moderate relationship with productive life (Figure 1). Among udder traits, udder texture, median suspensory ligament and rear udder width had the largest effect on longevity. Despite this relatively low impact of udder traits on productive life, fore udder attachment, rear udder width, udder depth and median suspensory ligament showed a nearly linear relationship with functional longevity, with a higher relative risk for cows with a low score, while the other udder traits exhibited either no trend or an intermediate optimum (Table 3).

Cows with close rear teats, extremely narrow rear udder, deep and fleshy udder were more likely to be culled compared to cows with opposite characteristics. High scores for median suspensory ligament were associated with lower culling rates. These observations are supported by some obvious problems caused by extreme scores. For example, when a cow has close rear teats, it is more difficult to put teat cups into place and they fall on the ground more easily. Another example is the incidence of mastitis which may be increased for cows with fleshy udder, needing more time to be milked.

Due to the large number of udder traits, only traits with the largest effects here or in previous published works are displayed for the analysis showing the influence of percentage of type-scored cows in the herd (Figure 4). In general, a higher variability in relative risk was observed for extreme scores (2 or 9) and for class 1 which has a low percentage of type-scored cows. This can be explained by a lower number of observations of these cases. Low scores (scores 2 and 3) for fore udder attachment, rear udder width, udder texture and udder depth tended to be associated with a higher relative risk for herds with better data i.e., with a higher percentage of type-scored cows (classes 4 and 5). Relative risks for fore udder attachment, rear udder width and udder depth decreased when the corresponding score increased.

Figure 5 shows a clear linear relationship between class 3 and 6 for final score and length of productive life. Cows with final score equal to 6 had a relative risk about 20% lower than the reference class (class 5), whereas cows with a low score (1-3) were 1.5 times more likely to be culled. Final score was, by far, the trait with the largest impact on productive life, with or without correction for production (Figure 1). When percentage of type-scored cows in the herd was accounted for (Figure 6), a curious result was observed: when less than 10% of the herd was type-scored, the relative risk of culling appeared to be slightly better for cows with a low final score than cows with a better final score. In contrast, when higher proportions of cows were scored, the impact of final score on productive life was large.

The effect of each type traits on the estimates of the  $\gamma$  parameter of the log-gamma distribution, the resulting variance of the herd-year random effect, the sire genetic variance and the effective heritability for functional length of productive life (i.e., assuming no censoring) were generally similar between all type traits studied (Table 4).

#### **4. Discussion**

To avoid biases in genetic evaluations of type traits, all first lactation cows in a herd must be recorded together for type traits. This is an ICAR (International Committee for Animal Recording) recommendation (ICAR guidelines, 2014, Article 7, p6 and section 5.1.5.1, p 218). However, in Brazil, breeders can choose which cows are scored – for example the ones that they consider as “best”.

Frequently not all animals in the herd may have been recorded for type traits and the worst animals may not have been included in our analysis. As a consequence, the absence of a type score had a very large influence on the cows' survival for all type traits. This illustrates that selection of cows among breeders of the Holstein Association in Brazil is heavily based on type, probably because of its impact on sales of live animals rather than on production. Clearly, when type information was available only for a small fraction of the herd, these cows were not chosen at random and had a better chance of remaining longer in the herd. A similar situation was reported by Terawaki et al. (2006; 2009) in Japanese Holsteins. These authors draw attention to the percentage of cows with a type score in a herd is considered as a criterion that reflects the herd's breeding goals and management. Consequently, the percentage of scored cows in each herd appears to be important to consider in the model for the genetic analysis of functional longevity.

In the recent years, several studies compared the relative contribution of type traits on productive life variability. In contrast with the present study, other studies systematically found that udder traits had the largest impact on productive life, in particular udder depth and fore udder attachment (Larroque and Ducrocq, 2001; Buenger et al., 2001; Caraviello et al. 2003; Schneider et al., 2003; Sewalem et al., 2005; Dadpasand et al., 2008; Morek-Kopec' and Zarnecki, 2012). Some, however, also reported a significant contribution of final score and dairy character (or angularity). The high impact of angularity and top line on longevity found here can be explained by the breeder preferences and by the manner in which data collection is carried out, as described above.

With such a system of data collection, the available observations cannot properly describe the actual effect of type on productive life for the whole herd. For body traits, a more or less continuous decline in relative risk is observed except for body depth for classes 4 and 5 (more than 50% scored cows).

A clear linear relationship between angularity and productive life was also reported by Sewalem et al. (2004) and Dadpasand et al. (2008) in Canadian and Iranian Holstein, respectively. However, Caraviello et al. (2004) and Buenger et al. (2001) found an intermediate optimum for angularity and functional longevity.



A linear relationship for foot angle and bone quality was reported by Schneider et al. (2003) in Canadian Holstein and by Caraviello et al. (2004) for foot angle in US Holstein. However, Morek-Kopec et al. (2012) in Poland and Berry et al. (2005), in New Zealand found an intermediate optimum for feet and legs traits in Holstein cows.

In relation to rump traits, other studies found a small to moderate impact of rump traits on longevity, in most cases with the lowest relative risks for intermediate scores and the highest for extreme scores (Buenger et al., 2001; Larroque and Ducrocq, 2001; Berry et al., 2005; Morek-Kopec and Zarnecki, 2012).

Among type traits, udder traits are generally considered the most influential for profitability on a farm. However, in the present study, this logical relationship was not reflected by a longer herd life, probably because few animals with bad udders were type-scored. As such, they were included in the “missing” class which is characterized by a much higher relative culling risk. Several studies showed a strong relationship between udder depth, fore udder attachment and median suspensory ligament and longevity (Larroque and Ducrocq, 2001, Buenger et al., 2001; Sewalem et al., 2004 and 2005; Dadpasand et al., 2008). The first two authors reported a difference in length of productive life of about a year between extreme categories for udder depth.

Final score was the trait with the largest impact on productive life. Similar results using the same methodology were reported by Schneider et al. (2003) in Quebec Holstein cows, Caraviello et al. (2003, 2004) in Jersey and Holstein in US and by Sewalem et al. (2004, 2005) in Canadian Holstein and Jersey cows. Sewalem et al. (2004) interpreted these results considering that final score represents a composite trait, combination of all individual type traits, with udder traits - which were the most closely related to survival in their case - receiving the largest weight.

The variance of the herd-year effect, sire genetic variance and effective heritability ( $h^2$ ) were larger than published values with the same data set, statistical model in Holstein cows in Brazil (Kern et al., 2016), when the effect of each type traits was not included. It indicates that the type traits are important factors related to herd's breeding goals and management policy in Holstein population in Brazil. Terawaki and Ducrocq (2009) and Terawaki et al. (2006) also

reported that type traits might result in diverse management policy in herds in Japan, leading to increase in the estimate of heritability when the percentage of cows type scored in herd also increase.

## **5. Conclusions**

The objective of this study was to measure the impact of type traits on length of productive life of Brazilian Holsteins. Both the hierarchy of traits and the magnitude of their effects were different from that previously reported in the literature: Udder traits were found to be relatively unimportant while final score, top line and angularity had a large effect. The most likely interpretation is that the current type recording system in Brazil allows the farmer to choose the animals should be scored, leading to a biased assessment of the relative importance of type traits. Cows with no type scores are at a much higher risk of being culled than type-scored cows, illustrating a strong selection of the animals to be recorded. Within herd exhaustive type recording is required for a more precise evaluation of the relationship between type traits and longevity. The correction or not for production traits led to low differences between both traits on contribution of type traits. However, the selection to better functional longevity is related a decreased in involuntary culling and a higher voluntary culling rate, hopefully resulting in higher herd profitability.

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**Table 1.** Description of linear type traits.

Trait	Description of evaluation	Score			
		1	5	9	Ideal
<b>Mammary system traits</b>					
Fore udder attachment	Attachment to abdominal wall	Extremely weak	Intermediate	Extremely strong	9
Fore teat placement	Teat placement from the center of the quarter	Extremely outside	center	Extremely inside	5
Rear teat placement	Teat placement from the center of the quarter	Extremely outside	center	Extremely inside	5-6
Fore teat length	Length of the front teat	Extremely short	Intermediate	Extremely long	5
Rear attachment height	Distance between milk secreting tissue and the base of vulva	Extremely low	Intermediate	Extremely high	9
Rear attachment width	Width at milk secreting tissue	Extremely narrow	Intermediate	Extremely wide	9
Udder depth	Distance from the udder floor to the hock	Extremely deep	Intermediate	Extremely shallow	5-6
Udder texture	Softness and expandability	Extremely fleshy	Intermediate	Extremely soft	9
Median suspensory ligament	Depth of cleft	Extremely weak	Intermediate	Extremely strong	9
<b>Feet and legs traits</b>					
Foot angle	Toe angle	Extremely low	Intermediate	Extremely steep	7
Bone quality	Flatness of bone	Extremely coarse	Intermediate	Extremely flat	9
Rear legs side view	Degree of curvature (side view)	Extremely straight	Intermediate	Extremely curved	5
<b>Rump traits</b>					
Rump angle	Height of pin bones relative to height of hook bones	Extremely high	Intermediate	Extremely low	5-6
Loin strength	Strength of vertebrae between back and rump	Extremely weak	Intermediate	Extremely strong	9
Rump width	Distance between the most posterior point of pin bones	Extremely narrow	Intermediate	Extremely wide	9
<b>Body traits</b>					
Stature	Measured from top of the spine in between hips to ground	Extremely short	Intermediate	Extremely tall	7
Chest width	Measured from the inside surface between the top of the front legs	Extremely narrow	Intermediate	Extremely wide	7
Body depth	Depth of body at the rear rib	Extremely shallow	Intermediate	Extremely deep	7
Angularity	Appearance of angularity	Extremely rounded	Intermediate	Extremely angular	9
Top line	Relation between the posterior and anterior stature of the animal in dorsal line	Extremely low	leveled	Extremely high	5-6-7
Final Score	Balance between the type traits according to its importance within each section	Extremely poor	Intermediate	Excellent	6

**Table 2.** Relative risk of culling for body, feet and legs and rump traits, according the (reference: score 5).

Score	STA	CW	BD	AN	TL	FA	BQ	RLS	RA	LS	RW
1	-	-	-	-	-	-	-	-	-	-	-
2	-	0.97	-	-	1.41*	1.03	1.25	1.08	0.75	0.89	0.92
3	0.89	1.01	1.03	0.93	1.14*	1.02	1.14	1.05	1.08*	1.10*	1.07
4	0.97	0.99	0.95	0.98	1.08*	1.02	1.02	1.05	1.01	1.00	0.96
5	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
6	1.00	0.94*	0.97	0.94*	0.94*	1.02	1.00	0.98	1.02	1.01	0.98
7	0.99	0.92*	0.91*	0.92*	0.84*	1.00	1.00	0.95*	1.06*	0.98	0.95*
8	1.00	0.85*	0.87*	0.80*	0.74	0.78	0.94*	0.88*	1.06	0.95	0.90*
9	1.01	0.60*	0.70*	0.77*	-	-	0.93	0.86	1.28	0.86*	0.91*
missing	1.60*	1.52*	1.51*	1.48*	1.57*	1.61*	1.58*	1.58	1.62*	1.58*	1.54*

*Body traits* - stature (STA), chest width (CW), body depth (BD), angularity (AN) and top line (TL); *Feet and legs* - foot angle (FA), bone quality (BQ) and rear legs side view (RLS); *Rump traits*- rump angle (RA), loin strength (LS) and rump width (RW). Values with an asterisk are significantly different from the reference class at a level of 0.05. Only classes with a minimum of 50 uncensored failures are reported.

**Table 3.** Relative risk of culling for mammary system traits (reference score 5).

Score	FUA	FTP	RTP	FTL	RUH	RUW	UD	UT	MS
1	-	0.97	-	-	-	-	1.10	-	0.88
2	1.14*	1.14	-	1.19	1.09	1.16	1.13	0.99	0.95
3	0.99	0.98	1.00	1.06*	1.04	1.03	1.07	0.99	1.08
4	1.03	1.04*	1.07	1.03	1.08	1.05*	1.04	0.97	1.02
5	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
6	0.98	0.97	1.01	1.02	1.00	1.02	0.96*	0.99	0.96
7	0.96	0.94	0.98	0.97	0.99	0.99	0.94*	0.97	0.95*
8	0.92*	1.00	0.98	1.00	0.94*	0.96	0.90	0.88*	0.89*
9	0.78*	-	0.95	1.09	0.99	0.87*	-	0.87*	0.86*
missing	1.56*	1.61*	1.59*	1.61*	1.59*	1.61*	1.61*	1.54*	1.53*

Fore udder attachment (FUA), fore teat placement (FTP), rear teat placement (RTP), fore teat length (FTL), rear udder height (RUH), rear udder width (RUW), udder depth (UD), udder texture (UT), median suspensory (MS). Values with an asterisk are significantly different from the reference class at a level of 0.05. Only classes with a minimum of 50 uncensored failures are reported.

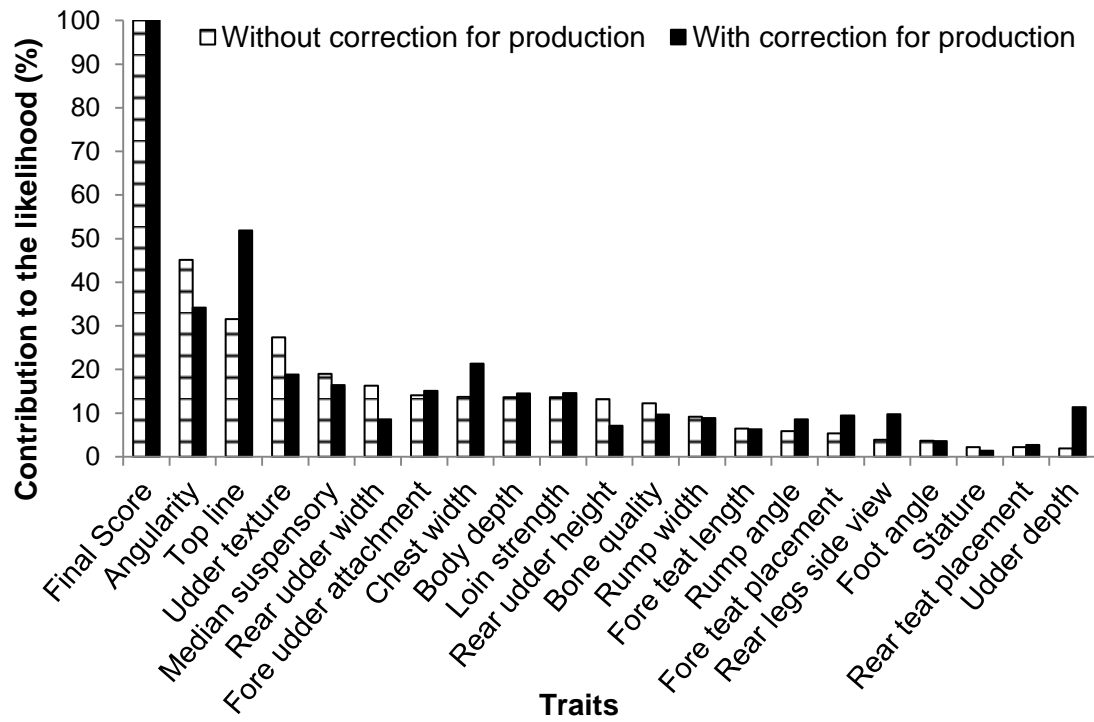


**Table 4.** Estimates of parameters related to the herd-year and sire random effects genetic and non-genetic random effect parameters for functional longevity analyzed including the type traits effect.

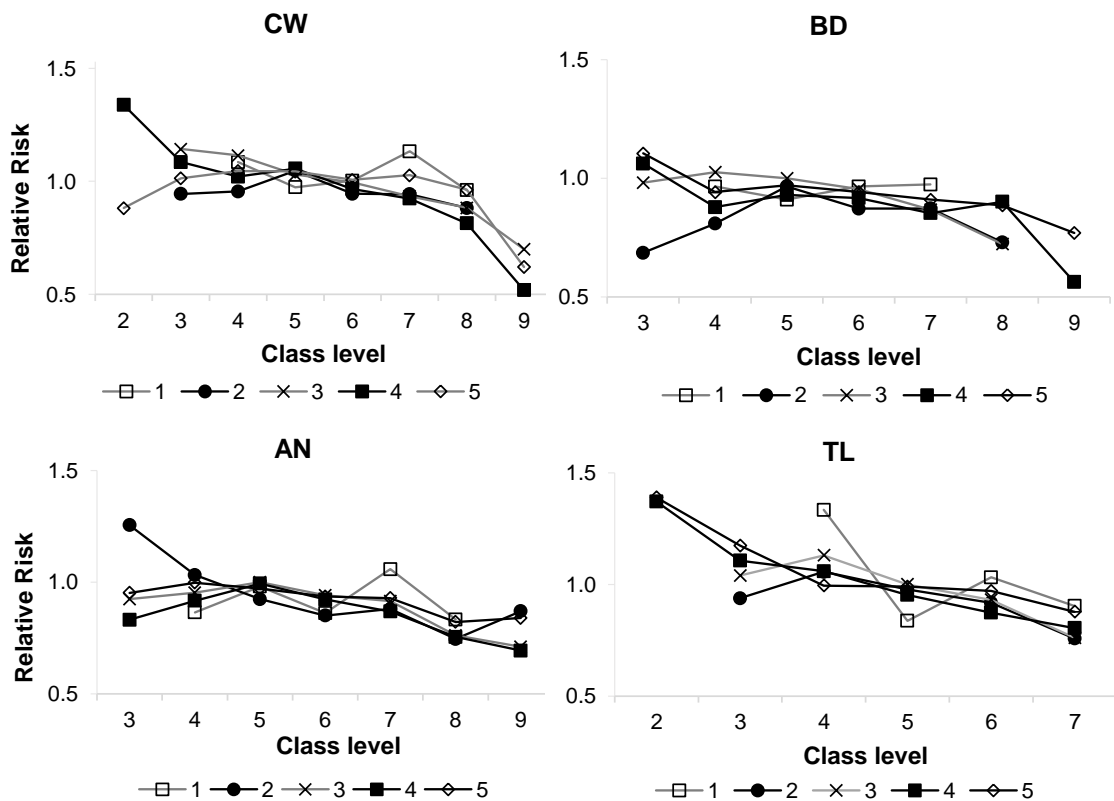
Type Traits	STA	CW	BD	AN	TL	FA	BQ	RLS	RA	LS	RW	FUA	FTP	RTP	FTL	RUH	RUW	UD	UT	MS	FS
$\gamma$	1.901	1.900	1.901	1.908	1.905	1.901	1.902	1.902	1.901	1.906	1.902	1.901	1.900	1.902	1.901	1.902	1.900	1.900	1.903	1.901	1.905
$\psi^1(\gamma)$	0.687	0.688	0.688	0.684	0.686	0.688	0.687	0.687	0.688	0.685	0.687	0.688	0.688	0.687	0.688	0.687	0.688	0.688	0.687	0.687	0.686
$\sigma_s^2$	0.034	0.034	0.033	0.035	0.035	0.034	0.034	0.034	0.034	0.035	0.035	0.034	0.034	0.034	0.034	0.035	0.034	0.034	0.034	0.034	0.035
$h^2$	0.080	0.080	0.077	0.081	0.081	0.079	0.080	0.080	0.080	0.080	0.080	0.079	0.079	0.079	0.079	0.080	0.080	0.079	0.080	0.080	0.082

$\gamma$  = shape and scale parameters (assumed to be equal) of the log-gamma distribution (of the herd-year-effect),  $\psi^1(\gamma)$  = variance of the herd-year random effect;  $\sigma_s^2$  = sire genetic variance;  $h^2$  = effective heritability. *Body traits* - stature (STA), chest width (CW), body depth (BD), angularity (AN) and top line (TL); *Feet and legs* - foot angle (FA), bone quality (BQ) and rear legs side view (RLS); *Rump traits*- rump angle (RA), loin strength (LS) and rump width (RW). *Mammary system* - Fore udder attachment (FUA), fore teat placement (FTP), rear teat placement (RTP), fore teat length (FTL), rear udder height (RUH), rear udder width (RUW), udder depth (UD), udder texture (UT), median suspensory (MS) and Final score (CF).

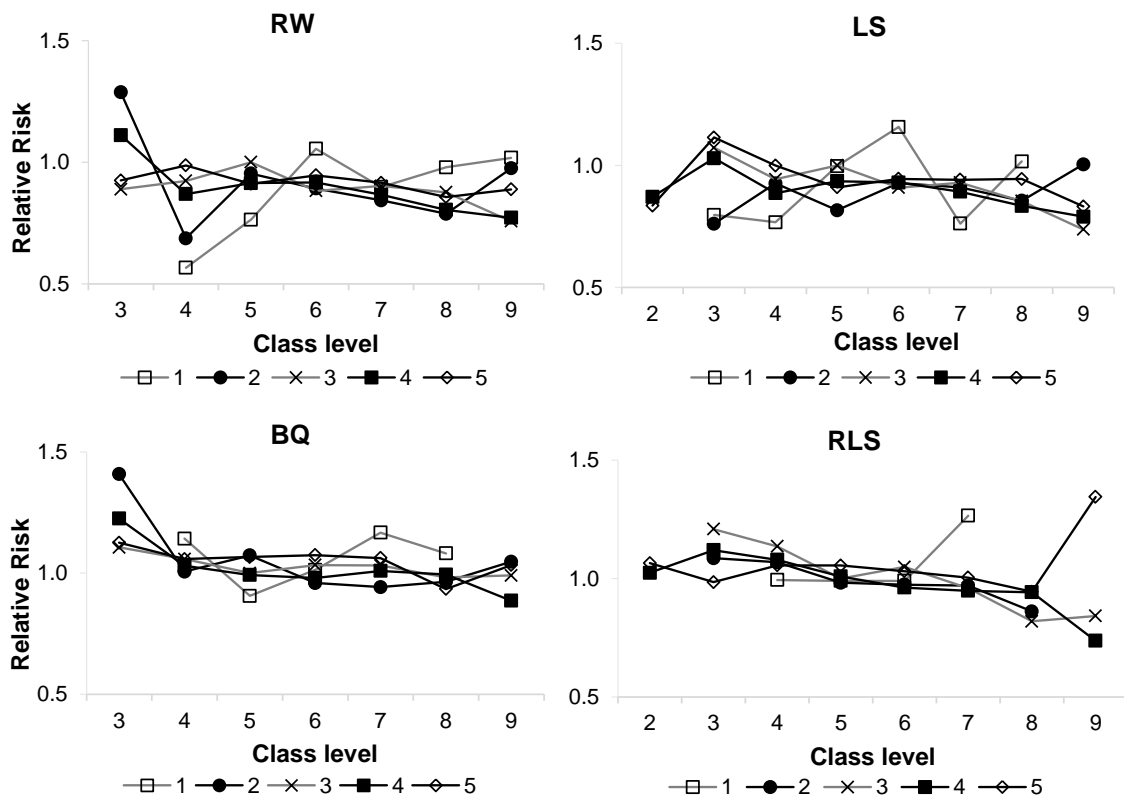
**Figure 1.** Contribution to the likelihood of each linear type trait and final score of the productive life (as a percentage of the contribution of the most important trait and excluding the contribution of “missing” score class).



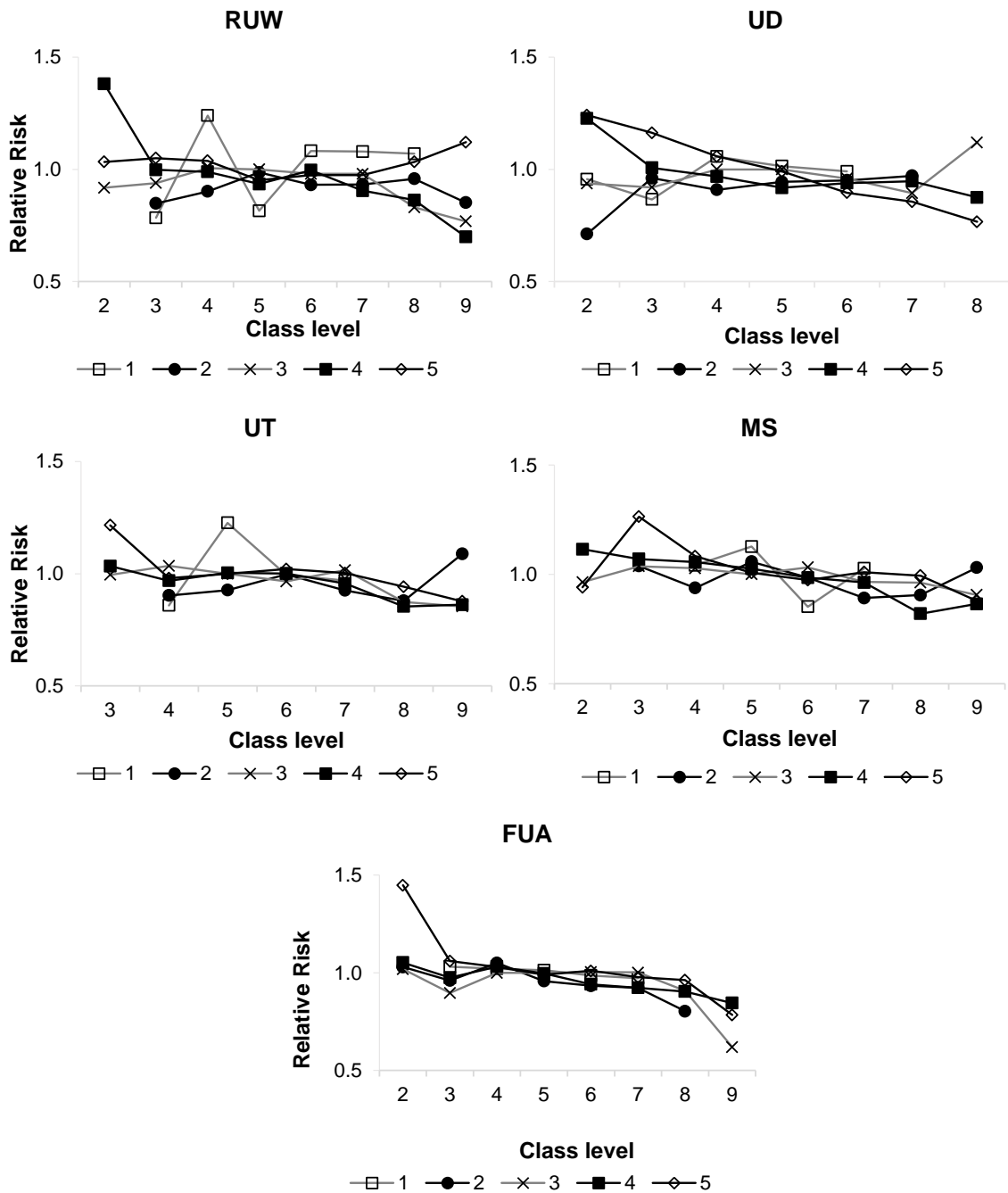
**Figure 2** Relative risk of culling for the body (chest width (CW), body depth (BD), angularity (AN) and top line (TL)) traits according to 5 classes of herd percentage of type-scored cows (1= 0 to 10%, 2= from 11 to 30%, 3= from 31 to 50%, 4= from 51 to 70%, 5= from 71 to 100% of herd percentage of type-scored cows) for Brazilian Holsteins (reference: score 5 in class 3 of herd percentage of type-scored cows). Only classes with a minimum of 40 uncensored failures are reported.



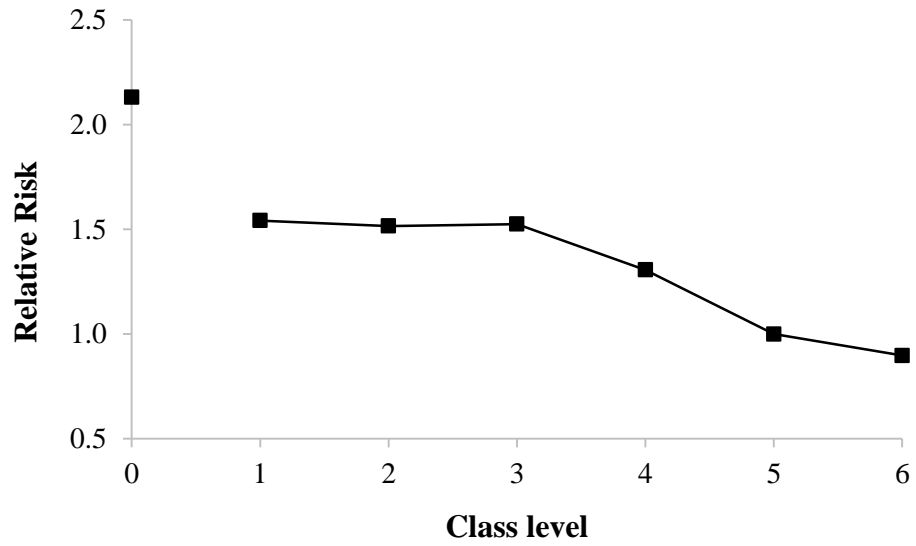
**Figure 3.** Relative risk of culling for some rump (loin strength (LS) and rump width (RW)) and feet and legs (bone quality (BQ) and rear legs side view (RLS)) traits according to 5 classes of herd percentage of type-scored cows (1= 0 to 10%, 2= from 11 to 30%, 3= from 31 to 50%, 4= from 51 to 70%, 5= from 71 to 100% of herd percentage of type-scored) for Brazilian Holsteins (reference score 5 in class 3 of herd percentage of type-scored cows). Only classes with a minimum of 40 uncensored failures are reported.



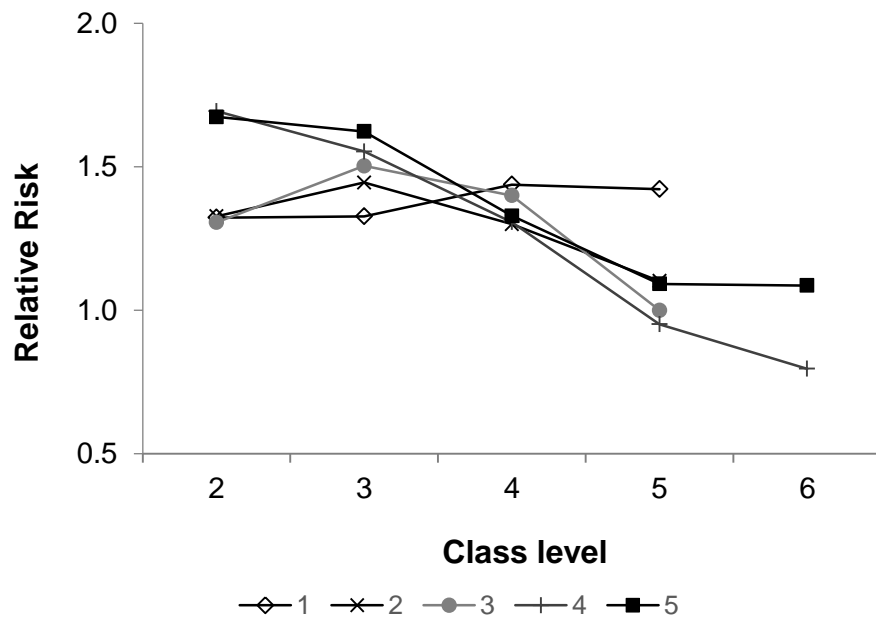
**Figure 4.** Relative risk of culling for the mammary system (fore udder attachment (FUA), rear udder width (RUW), udder depth (UD), udder texture (UT), and median suspensory (MS)) traits according to 5 classes of herd percentage of type-scored cows (1= 0 to 10%, 2= from 11 to 30%, 3= from 31 to 50%, 4= from 51 to 70%, 5= from 71 to 100% of herd percentage of type-scored) (reference final score 5 with class of herd percentage of type-scored cows 3). Only classes with a minimum of 40 uncensored failures are reported.



**Figure 5.** Relative risk of culling for final score for Brazilian Holsteins (reference: score 5). Only classes with a minimum of 40 uncensored failures are reported.



**Figure 6.** Relative risk of culling for final score according 5 classes of herd percentage of type-scored cows (1= 0 to 10%, 2 = from 11 to 30%, 3= from 31 to 50%, 4= from 51 to 70%, 5= from 71 to 100% of herd percentage of type-scored cows) for Brazilian Holsteins (reference: final score 5 in class 3 of herd percentage of type-scored cows). Only classes with a minimum of 50 uncensored failures are reported.



## **CAPÍTULO IV<sup>1</sup>**

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**Analysis of the relationship between somatic cell score and longevity in Brazilian  
Holstein cattle**

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**Running title:** Association between somatic cell and longevity

**Title of the manuscript:** Analysis of the relationship between somatic cell score and longevity in Brazilian Holstein cattle

## **ABSTRACT**

**Objective:** The aim of this study was to explore the impact of somatic cell count (SCC) on the longevity of Brazilian Holstein cows by using a piecewise Weibull proportional hazards model.

**Methods:** Records on length of productive life of 131,330 cows from 915 herds were included in the analysis. The statistical model included the effects of age at first calving, it was time independent. All the other fixed effects were time-dependent. These included the interaction of region by year of calving; variation in herd size class; milk production class by year of calving within herd; within herd milk production class by lactation number; within herd fat content; within herd protein content and somatic cell score (SCS).

**Results:** The overall mean number of SCC for Holsteins was 322,000 cells/mL. The highest SCC mean was observed between 130 to 290 days of lactation. Trends of decreasing for SCC mean along the years. Cows from the region 5 (Rio Grande do Sul) showed higher SCC mean. A increasing on SCC mean along the lactations is showed. The risk of culling was slightly higher for functional longevity than true longevity. The impact of SCS on functional and true longevity was high in cows from 1<sup>st</sup> to 4<sup>th</sup> lactation with high SCS, which the risk of culling varying from 0.90 (true longevity: 2<sup>nd</sup> lactation and class 2) to 1.2 (functional longevity: 4<sup>th</sup> lactation and class 5). Interestingly, for 5<sup>th</sup> lactation, cows with lower SCS have higher risk of culling (1.4) compared to cows with higher SCS (1.2). Inclusion of effect of SCS class by stage of lactation within lactation in the models do not proved to be beneficial.

**Conclusion:** The SCS can be used for indirect selection for improved longevity in Brazilian Holstein cows under tropical conditions.

**Keywords:** Dairy cattle; Funcional longevity; Somatic cell count; Risk of culling, Survival analysis

## INTRODUCTION

Longevity is a highly desirable trait that considerably affects overall profitability in the dairy production. In the decision of culling, the farmer will take into account production, health, fertility, mastitis and other auxiliary traits such as milking speed, milking temperament, and calving ease. Culling because of poor production is called voluntary culling, and culling other than for production is called involuntary culling [1,2]. Reduction of the rate of involuntary culling allows a higher voluntary replacement rate, which can increase the economic profit. Distinct definitions for longevity are used as: True longevity, which mainly depends on productivity, and functional longevity defined as the ability to delay involuntary culling [1] (Ducrocq et al., 1988).

Mastitis is among the most costly and complex disease in dairy industry. The economic effect include losses of milk production or milk sale, increased culling rates, higher Somatic Counts (SCC) in milk [3] and decreased milk quality. For decrease the levels of mastitis, the farmers can control the management and production systems or even use a more effective alternative, the selection for mastitis resistance.

Direct selection against clinical mastitis is difficult, because in most countries as Brazil, clinical mastitis events are not widely recorded and because it's low heritability, around to 0.02 [4,5,6]. As SCC are routinely recorded in most milk recording system, as Brazil do, SCC efficiently account for subclinical and chronic infections, the heritability of SCC is greater than clinical mastitis (0.11) [3,6]. In addition, the genetic correlation between both traits is positive and moderate to high (higher than 0.60) [7,5], suggesting that some genes can reduce both SCC and clinical mastitis. In this ways, the selection to decrease SCC can reduce the clinical and subclinical mastitis, which the SCC are been used as an indicator of mastitis diseases.

The longevity or survival rate of dairy cows can be influenced by SCC through the death or culling of clinically affected animals, as well as the culling of subclinical animals with high SCC [8]. Several studies reports that udder health traits, as mastitis and SCC, conduce a major reason of culling in dairy cattle [2,9]. Samoré et al [10] and Caraviello et al [5], using survival analysis, reported that higher concentrations of SCC were associated with higher rates of culling. Antagonistic genetic correlations

between SCC and longevity, ranging from 0.16 to 0.36, have been reported for dairy cows [11,12], indicating that elevated SCC is associated with reduced longevity.

In statistical analyses, the values of SCC of an individual cow changes over time, been called as time-dependent variable, allowing to consider the particular environmental conditions of a cow influencing her probability of being culled each day during her entire productive life and not only at some specific moments. Survival analysis methodology effectively uses information from time-dependent covariates and is able to hand censored information, so data from animals that are still alive at the time of analysis can be used [13].

Study of the association between SCC and longevity would benefit farmers, which help them take decisions from the indirect selection results improving the herd profit. In Brazil, no study has reported the association between SCC and longevity using a survival analysis approach in Holstein cattle. The main objective of the study was to assess the association of SCS on true and functional longevity in the Brazilian Holstein cattle using a piecewise Weibull proportional hazards model.

## **MATERIAL AND METHODS**

### **Data**

Length of productive life and somatic cell score (SCC) records were obtained from the Brazilian Association of Holstein Cattle Breeders (ABCBRH) and its affiliated state agencies. Two longevity traits were defined as in Ducrocq et al [1]: true longevity which is measured as the number of days from first calving to culling, and functional longevity which is approximated by correcting true longevity for within-herd-year level of production.

The database included 83,669 cows with both SCC and production records and 47,661 cows with only production data. Records from milk production of Holstein cows with at least a first calving occurring between 1989 and 2013 were included in the analysis. Cows younger than 20 or older than 40 months at first calving and cows without information on date of first calving were excluded from the data, as well as daughters of sires with fewer than five daughters.

The analysis was focused on culling early in life which is the most damaging for the herd profitability. For this purpose, when a cow had more than five lactations, her productive life record was

assumed to be censored on the day of her sixth calving. Cows that were still alive on December 31, 2013 and with less than six calvings were also considered as censored at this date. To avoid biases due to potential poor recording of calving dates, lactation lengths were bounded at 800 days. To account for a possible preferential treatment in their new herd, cows sold to another herd were considered as censored on the day of their last milk record in their first herd. When the exact culling date was known, it was used. However, it was often missing and then, the last known recording date was used as culling date. Overall, 33% of the cows had a censored record. From the test-day SCC were obtained SCS for each by lactation averaged (1<sup>st</sup> to 5<sup>th</sup> lactation). Somatic cell scores were grouped into 5 categories (1- 10 to 75 SCS x 1000/mL; 2 - 76 to 150 SCS x 1000/mL; 3 - 151 to 275 SCS x 1000/mL; 4 - 276 to 550 SCS x 1000/mL e 5 - 551 to 2750 SCS x 1000/mL).

The pedigree was traced back only for the sires of the cows. The pedigree file included 6.804 animals. Seven unknown sire or maternal grand sire groups were created based on year of birth and country of origin. Four groups of country of origin were created: the first group included Canada and the United States; the second group included Brazil, Argentina and Uruguay; the third group represented Europe (France, Spain, Italy, Belgium, New Zealand, Germany and the Netherlands); and the fourth group corresponded to sires with unknown origin.

## **Model**

Three analyses were performed to study the relationship between length of productive life and SCS using proportional hazard models where the hazard  $\lambda(t)$  of a cow at time  $t$  (i.e., the risk of culling given she is alive just prior to  $t$ ) was modelled using a piecewise Weibull hazard function. In the first analysis, an approximation of functional longevity was studied, in which all environmental effects found significant in the analysis of Kern et al [14] and described below were analyzed with SCS average by lactation. In the second analysis, the effects related to level of production were omitted to study the impact of each type trait on true longevity. In the third analysis, the functional longevity and all effect included in the first analysis were considered, with except of change of the effect of SCS average by lactation by the effect of 3 classes of SCS average by test-day control by lactation (1= 1, 2, 3; 2= 4, 5, 6; and 3= 7, 8, 9 and 10 test-day control). The analysis of the effect of SCS average according the test-day

control was performed to know behavior of the survival cows according to somatic cell count in different stages of lactation, since studies have showed variations according to stage of lactation.

The full model used for the three analysis above describe can be write as:

$$\lambda(t) = \lambda_{0,ls}(t) \exp\{\sum_m f_m(t) + hys_k(t) + s_i + 0.5 mgs_j\} \quad [1]$$

where  $\lambda(t)$  is the baseline hazard function of the cow at time  $t$ , defined as a piecewise Weibull hazard function of the form  $\lambda_{0,ls}(t) = \lambda\rho(\lambda\tau)^{\rho-1}$  with scale parameter  $\lambda$  and shape parameter  $\rho$  differing for each combination of the  $l$ th lactation (1 to 5) and  $s$ th stage of lactation  $s$  (1 to 4) resulting in 20 different Weibull functions, one per interval.  $\tau$  represents the time (in days) between the most recent calving and current time. Stages of lactation were defined according to the number of days from the most recent calving, with changes within each lactation at time 0, 270, 380, and day when dried-off.

In the three analysis describe above, the model included the following fixed environmental effects ( $\sum_m f_m(t)$  is the sum of fixed environmental effects): Age at first calving, was assumed to influence the whole productive life: it was time independent and was estimated for each one-month class of age at first calving between 20 and 42 months. All the other fixed effects were time-dependent. These included the interaction effects of region (region 1- Minas Gerais, 2- Other states of Brazil, 3- Paraná, 4- São Paulo, and 5- Rio Grande do Sul) by year of calving (from 1989 to 2013); variation in herd size class (with only one class for herds size with less than 5 cows, three classes of variation in herd size (decrease by more than 10%, stable size, increase by more than 10% for herds size with 5 to 19 cows) and five classes of variation in herd size (decrease by more than 15%, decrease by 5 to 15%, stable size, increase by 5 to 15%, increase by more than 15% separately for herds size with 20 to 49 cows and herds size with 50 cows or more)); SCS average by lactation (1 to 5 lactation) and SCS average by test-day control by lactation. The SCS by lactations and SCS by test-day control were considered separately, one at a time.

For the second analysis, the following fixed effects time-dependent related to level of production were omitted when studying the impact of SCS on true longevity: milk production class by year of calving within herd milk production class (from 1- worst (1- 20%), 2- (21 - 40%), 3- (41 - 60%), 4- (61 - 80%), 5- best (81 - 100%) for milk production, and 6- unknown); within herd milk production class by lactation number (1- first lactation, 2- later lactations); within herd fat content (from 1- worst (1- 20%) 2- (21 - 40%), 3- (41 - 60%), 4- (61 - 80%), 5- best (81 - 100%) and 6- unknown); within herd protein

content (from 1- worst (1- 20%) 2- (21 - 40%), 3- (41 – 60%), 4- (61 – 80%), 5- best (81 – 100%) and 6- unknown).

The following random effects were also considered in the three analysis:

$h_{yk}(t)$  is the  $q$ th random herd-year effect assumed to follow a log-gamma distribution (with shape and scale parameters both equal to  $\gamma$  in order to force its mean to be 1).

$s_i + 0.5 mgs_j$  represents the additive genetic contribution from the sire  $i$  and of the maternal grand sire  $j$  of the cow.

Estimates of all fixed effects were expressed as relative culling risks, defined as the ratio between the risk of culling under a particular environmental factor and a specific reference class. For example, the risk ratio associated with a score of  $p=5$  for SCS 1<sup>st</sup> lactation SCS average was defined as  $\exp\{t_p - t_3\}$ , score 3 being chosen as the reference class. All analyses were performed using the Survival Kit version 6 software [13].

## RESULTS

The overall mean number of somatic cell count (SCC) for Holsteins was 322,000 cells/mL, with a standard deviation of 377,000.

Figure 1 shows the effect of DIM on SCC for the first parity, the number of SCC and the SCC standard deviation. In general, the SCC showed a continuous increasing along the lactation until approximately 250 days. In 130 days after calving, an increase in SCC values was observed and a stable curve after 290 days until the end of lactation. Higher number of SCC records were shown around 210 to 215 of lactation. Large variations were found for standard deviation values along lactation, ranging from 106,700 cells/mL (30 days of lactation) to 360,800 cells/mL (240 days of lactation). The higher standard deviation value shows the presence of high number of animals with extreme SCC values, indicating the attendance of animals with different genetic and phenotypic potential and herd management response. The smallest standard deviation variation of somatic cell count was observed at the beginning of lactation (15 to 130 DIM) and can be explained by the reduced number of SCC observations in this period.

Figure 2 provides the opportunity to observe annually trends for SCC in Brazilian Holstein cows. Trends of increasing for SCC mean until 1995, showing the higher SCC mean during the study years.

After this year, were observed a linear decreasing in the SCC mean values until 2011, showing a slight peak of SCC value in this year.

The effect of region on SCC mean is showed in Figure 3. For region 2 and 3, the SCC mean were lowest compared the others regions studied. Cows from the region 4 (São Paulo) followed by region 1 (Minas Gerais), and region 5 (Rio Grande do Sul) have higher SCC mean, with values varying from 370,000 cells/mL (SP) to 365,000 cells/mL (RS). The Figure 4 demonstrate the distribution of SCC mean according the lactation numbers. A clear increasing on SCC mean along the lactations is showed, obtaining an average of SCC values of fifth lactation of 545,000 cells/mL.

For all analysis the censored records were around 32% of the total number of records considered. The average uncensored and censored time varying from 331 and 357 and 331 days, respectively. The similarity between this values is justify by inclusion of all data even the cows without somatic cell count information's.

### **Relative Risk of Culling**

Somatic cell scores had a highly statistically significant ( $P < 0.001$ ) association with functional and true longevity in Holsteins. This was tested using likelihood ratio tests comparing the full model (with classes of SCS) with a reduced model (without classes of SCS). The results are expressed as relative culling risk, defined as the ratio of the estimated risk of being culled under the somatic cell score relative to the average risk (or reference risk set to 1 for a score of 3). Values greater than 1 indicate a higher culling risk associated with somatic cell score. Relative culling of risk lower than 1 indicates a low culling risk.

Figure 5 shows the relationship between SCS with functional and true longevity according the five lactations. The association between functional and true longevity with SCS showed a similar trend, with a slight elevation of the values to functional longevity. As the SCS increased, the relative risk of culling also increased to 1<sup>st</sup> to 4<sup>th</sup> lactation for functional and true longevity. Opposite result can be observed to 5<sup>th</sup> lactation, which higher risk of culling was demonstrated for the class of SCS 1 and 2. Relative risk of culling to cows from the first lactation were a little higher when compare cows from second lactations, considering functional and true longevity. As for the other lactations can be observed the risk of culling increase according increase the lactation numbers. Cows from first lactation with a SCS



of 5 had a 1.17 for functional, and 1.21 for true longevity, times higher risk of being culled than cows with SCS at the reference level (class 3). However, cows from the 5<sup>th</sup> lactations were 1.40 (functional longevity) and 1.28 (true longevity) times more probably to be culling compared to class 3.

Figure 6 represents the survivor curves of the whole population in the different class of SCS according lactation numbers. Cows from fifth lactation (SCS5) lived shorter in the first days after calving than cows in from others lactations. This indicate that cows from fifth are stronger affected by SCS in the early productive life and less at the end of the productive life. For the fourth SCS lactation a less survivor functions was observed after 365 days until 710 days of productive life. Similar curve shape was founded for the others SCS lactations.

The table 1 is showing the relative risk of culling according different SCS within lactation-by-lactation stages. For the first and second lactation, the risk of culling were similar for the three lactation stages and higher risk were indicate for SCS 5 (1.11 to 1.17). The risk of culling showed higher values for the second and third stage for the case of third lactation, also for the class 5 (1.18 to 1.20). In fourth and fifth lactations, the class of SCS 1 showed higher risk of culling than class of SCS 5, similar were appoint in the Figure 5. However, in class of SCS 5 (1.20) on fourth lactation the risk values was similar to class 1 (1.22).

## **DISCUSSION**

The overall mean SCC for Holsteins in others countries were lower that found in this study (322,000 cells/mL). Norman et al. [16] and Caraviello et al [8], using US Holstein cows, reported a mean SCC 307,100 and 200,000 to 250,000 cells/mL. In the Figure 1, observing the curve behavior of somatic cell count, the higher SCC values were found in the interval of 180 to 250 days of lactation, which differed substantially of values observed in other studies with herds of Holstein and Jersey cows. Caraviello et al [8] identified the higher SCC values between 75 to 220 days after calving and Sewalem et al [2] in the beginning of lactation (10 to 30 days). The behavior of the somatic cell count during the first lactation is closely related to genetic factors and herd management, such as the milking time and also the cleaning of milking machine

Differences in SCC according the years of test day as showed in Figure 2, suggest that environmental conditions as temperature and rains may affect to SCC values. This situation suggests that udder disease prevention and control programs must be used by dairy producers to mitigate the effect of the environment on SCC, decreasing the udder diseases.

The difference in SCC mean values observed according the different region (Figure 3) are most likely caused by the environmental variations in climate, and in management practices adopted to control the health problems of mammary gland between the different studied regions. Kern et al [14] studied the functional longevity with same Holstein database, reported that regions showed a high impact in relative risk of culling according the different regions. The SCC values were lowest for region 2. This result is probably because this region includes several states with lower expression in milk production, thus expecting their cows to have low milk production and SCC.

The distribution of average SCC, as expected, increased with the lactation number (Figure 4). The increase of SCC according the lactation order is related a higher incidence of mastitis and higher lost in milk production [17,18]. The increase of SCC in the milk of cows with higher number of lactations could be partially explained by the increase of epithelial cells in the milk in cows more old and higher rate of infection of the mammary gland. High SCC milk is undesirable for milk processors because it reduces the shelf life of dairy products and diminishes the quality and quantity of milk protein, thereby reducing cheese yields. According to Barbano et al [19], even modest values of SCC (e.g., < 100,000 cells/mL) have been shown to reduce cheese yield.

The longevity or survival rate of dairy cows can be influenced by SCC through the death or culling of clinically affected animals, as well as the culling of subclinical animals with high SCC. In this ways, the results obtained in the study, with exception of 5<sup>th</sup> lactation, showed us that cows with lower SCS have higher survival than cows with higher SCS, being this achieves similar to both longevity traits (functional and true). Several studies pointed same effect between SCS and longevity as showed in our study using survival analysis [2,10,20] and using a linear model, which the genetics correlations between SCS and longevity, ranging from 0.16 to 0.36 [6,11,12], which means that higher SCS values can result in higher culling rate, and lower longevity, consequently. These results suggest that using SCS for indirect selection for improved udder health, e.g. decreasing the SCS, can also be expected to have a positive impact on longevity.

The individual cow SCS values can be used as a tool for involuntary culling decisions on herd dairies, such as clinical and subclinical mastitis incidence, and so the results can be compared with studies analyzing the rate of culling directly associated to mastitis incidence [10], due to the higher genetic correlations between SCS and mastitis (0.78) [21]. Caraviello et al [5], reported that when culling was due to mastitis (inclusion of mastitis information in the survival model), the relative risk of culling were more than 10 to 25 times greater compared to risk using only SCS. In our study we do not included the effect of mastitis in the model, due to still this trait do not measured in the Brazilian Holstein cows. Many countries such as Brazil do not have a national recording system for udder health, as mastitis for example, because they are often difficult and expensive to measure, being the SCS values the way to study the association between the mastitis and longevity.

Interestingly, cows in 5<sup>th</sup> with lower SCS (30,000 to 100,000 cells/mL) tended to have a slightly higher risk of culling than average and higher SCS cows. Shukken et al [22] have expressed concern that genetic selection to decrease SCS could lead to cows with reduced ability to recruit leukocytes and adequately respond to an intramammary infection. Our results appear to indicate that cows with extremely low SCC may suffer from a reduced capacity to resist mastitis, particularly in herds with poor environmental conditions or poor udder health management practices, being to exposed to mastitis pathogens. Caraviello et al [8] reported similar situation for Holstein and Jersey cattle from US.

The risk of culling according to stage by within lactation considering the 1<sup>st</sup> and 2<sup>nd</sup> lactation were higher for cows with high SCS (Class 5). However, no expressive differences were observed for the lactation stages of these first two lactations. From the 3<sup>rd</sup> lactation, in the extreme classes (classes 1 and 5) the risk of culling was higher than in the intermediate classes (classes 2, 3 and 4). Regarding lactation stages for lactations 3, 4 and 5, there was no linear trend for the risk of culling. However, it was observed that for lactations 3 and 5 the risk is higher for 3<sup>rd</sup> stage. For lactation 4, cows in 2<sup>nd</sup> stage presented lower survival than 1<sup>st</sup> and 3<sup>rd</sup> stages.

Different from our results, Grohn et al [23], reported that the rate of culling of New York cows' considering the mastitis effect, varying according to distinct stage of lactation, which higher culling were observed in the 3<sup>rd</sup> (61 to 120 d) and 5<sup>th</sup> (151 to 240 d) stage. Theses authors also, showed that mastitis was the biggest effect to culling compared others diseases, such as metritis, retained placenta and milk fever.

## **CONCLUSION**

The survival analysis is a way to understand the relationship between SCS and longevity of Brazilian Holstein cows. The impact of level SCS on functional and true longevity was high in cows with high SCS, except on 5<sup>th</sup> lactation where the cows with lower SCS also have higher risk of culling. The inclusion of effect of SCS class by stage of lactation within lactation in the models unproved to be beneficial. The results suggest a positive impact on longevity, when cows are selected to decreasing SCS values, and indirectly it is expected cows with higher udder health. In addition, future studies should consider using mastitis events, rather than lactation SCS (as in this study), because such data could give a more precise evaluation of the current udder health status of individual cows.

## **CONFLICT OF INTEREST**

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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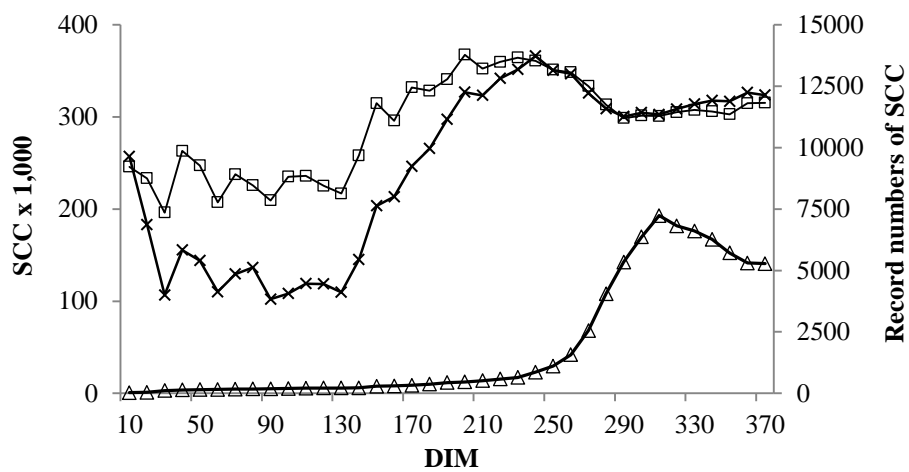
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**Table 1.** Relative risk of culling for classes of SCS, according the stage of lactation.

		Class SCS				
		1	2	3	4	5
1 lactation	1st stage	0.96	0.98	1.00	1.05	1.11
	2nd stage	0.96	0.98	1.00	1.03	1.14
	3rd stage	1.00	0.98	1.00	1.07	1.13
2 lactation	1st stage	0.92	0.90	1.00	1.02	1.14
	2nd stage	0.95	0.97	1.00	1.06	1.17
	3rd stage	0.97	0.93	1.00	1.05	1.14
3 lactation	1st stage	1.02	0.98	1.00	1.00	1.15
	2nd stage	1.06	1.00	1.00	1.07	1.18
	3rd stage	1.11	1.02	1.00	1.06	1.20
4 lactation	1st stage	1.13	1.00	1.00	1.05	1.12
	2nd stage	1.22	1.01	1.00	1.07	1.20
	3rd stage	1.19	1.02	1.00	1.01	1.13
5 lactation	1st stage	1.27	1.25	1.00	1.00	1.13
	2nd stage	1.25	1.10	1.00	1.03	1.11
	3rd stage	1.45	1.05	1.00	1.07	1.10

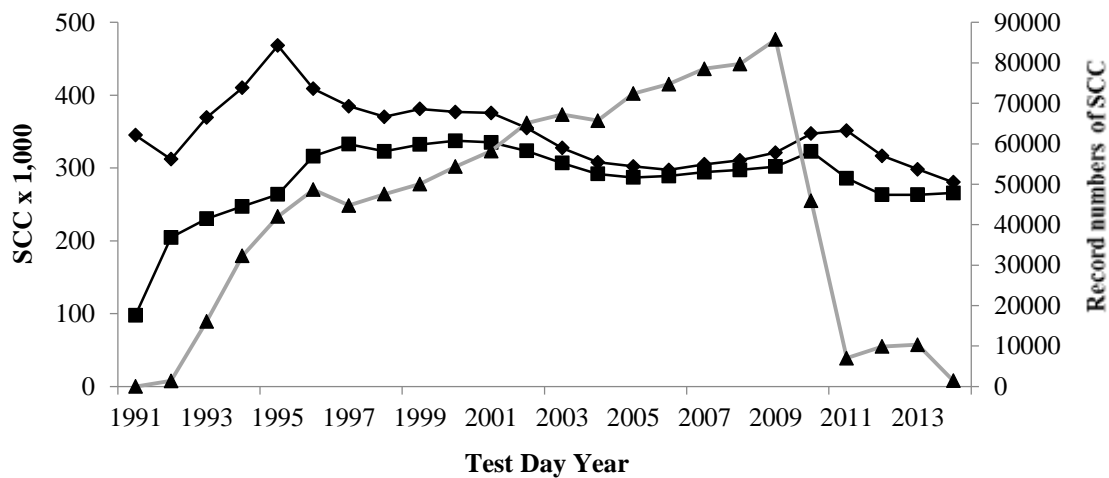
Reference: class 3 was set to 1. Class SCS= 1- 10 to 75 SCS x 1000/mL; 2 - 76 to 150 SCS x 1000/mL; 3 - 151 to 275 SCS x 1000/mL; 4 - 276 to 550 SCS x 1000/mL e 5 - 551 to 2750 SCS x 1000/mL. Lactation stage (1<sup>st</sup> stage -1, 2, 3 test day-control ; 2<sup>nd</sup> stage – 4, 5, 6 test day-control; and 3<sup>rd</sup> stage – 7, 8, 9 and 10 test day-control).



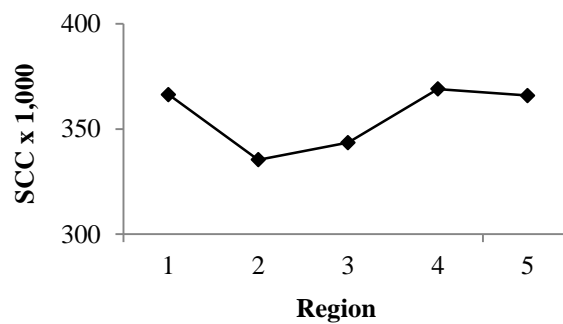
**Figure 1.** Effect of stage of lactation on SCC (cells/mL) for first parity in Brazilian Holstein cows.

Standard deviation (×), SCC in the first parity (□) and number of records (Δ).



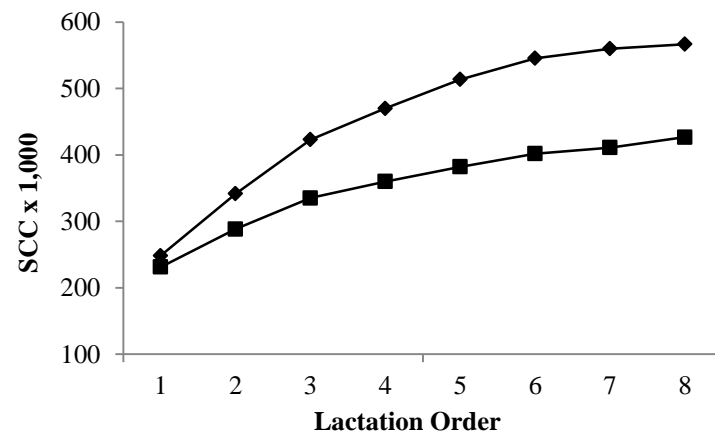


**Figure 2.** Effect of test-day year on SCC (cells/mL) for all lactations in Brazilian Holstein cows. Standard deviation (■), SCC mean in all lactations (♦), number of records (▲).



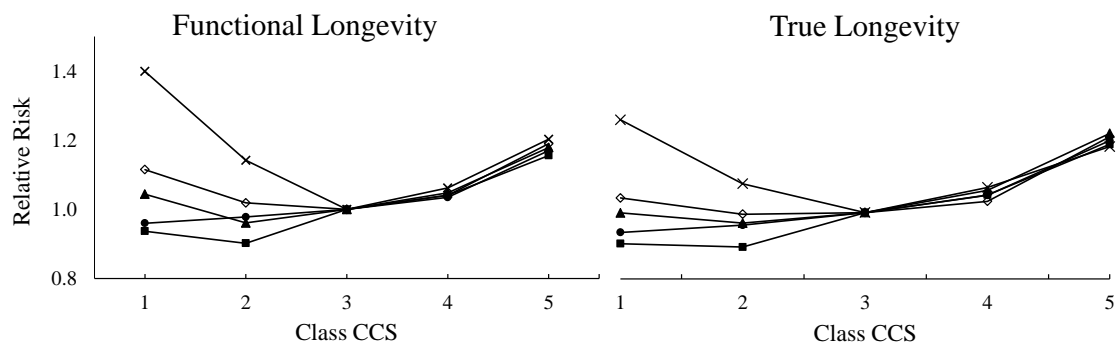
**Figure 3.** Effect of region on SCC mean (cells/mL) considering all lactations in Brazilian Holstein cows.

Region 1- Minas Gerais, 2- Other states of Brazil, 3- Paraná, 4- São Paulo, and 5- Rio Grande do Sul.

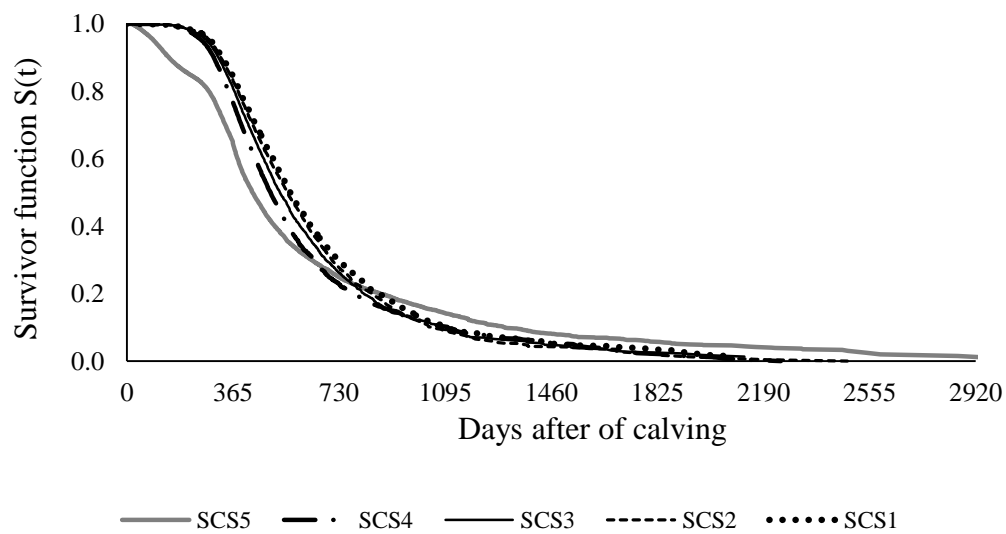


**Figure 4.** Distribution of average SCC values (cells/mL) in each lactation in Brazilian Holstein cows.

Standard deviation (■) and SCC mean (♦).



**Figure 5.** Relative risk of culling by classes for SCS and lactation order, according functional and true longevity (reference: class 3 was set to 1). Class SCS= 1 - 30 to 100 SCS x 1000/mL; 2 -101 to 190 SCS x 1000/mL; 3 -191 to 330 SCS x 1000/mL; 4 - 331 to 600 SCS x 1000/mL e 5 - 601 to 1770 SCS x 1000/mL. Lactation: 1 (●), 2 (■), 3 (▲), 4 (◇) e 5 (×).



**Figure 6.** Survivor curves for 5 class of SCS according the lactation numbers (SCS1- class of SCS in first lactation, SCS2- class of SCS in second lactation, SCS3- class of SCS in third lactation, SCS4- class of SCS in fourth lactation, and 4- class of SCS in fifth lactation).

## **CAPÍTULO V**

## CONSIDERAÇÕES FINAIS

A análise de sobrevivência poderá ser utilizada para futura avaliação genética da duração da vida produtiva das vacas Holandesa no Brasil. A metodologia permite modelar para a presença de covariáveis tempo dependentes e para a distribuição não normal dos dados de sobrevivência, permitindo a análise das informações de vacas censuradas. O modelo de Weibull estratificado conduziu à predição acurada (confiabilidade) dos valores genéticos, além de modelar adequadamente para as mudanças cíclicas do risco ao longo das sucessivas lactações, em que o risco é menor no início e maior no final das mesmas.

Os valores de herdabilidade foram baixos, porém estão em concordância com os valores reportados na literatura. Observou-se tendência genética positiva para a longevidade funcional, possibilitando inferir que alguma atenção à duração da vida produtiva das vacas Holandesas no Brasil tem sido praticada.

A produção de leite foi o fator que mais afetou as decisões de descarte das vacas. Vacas de alta produção são mais longevas que vacas de baixa produção de leite. Outro fator que fortemente influencia a duração da vida produtiva das vacas foi o efeito de região, ou seja, as vacas pertencentes à região do Paraná e São Paulo apresentaram maior chance de permanência no rebanho.

A seleção para a longevidade funcional baseada nas características de tipo mostrou-se uma estratégia pouco viável, o que diferiu expressivamente dos resultados relatados na literatura. Isto ocorreu possivelmente devido ao fato de que, em muitos rebanhos, a classificação para tipo não é realizada em todas as vacas, e sim em apenas algumas poucas, de acordo com o interesse do criador. Devido a isso, recomenda-se que todas e/ou o maior número possível de vacas sejam classificadas para tipo no rebanho.

Contudo, escore final, angulosidade, nivelamento da linha superior, textura do úbere e ligamento suspensório apresentaram as maiores estimativas de associação com a duração da vida produtiva. O uso do escore de células somáticas como preditor da longevidade funcional pode resultar em impacto positivo na duração da vida produtiva das vacas, além de diminuir a incidência de problemas com a saúde da glândula mamária.

De maneira geral, recomenda-se que informações da saúde da vaca, como a saúde da glândula mamária, através dos casos de mastite, os problemas reprodutivos, como aborto, e as causas de descarte e/ou morte das vacas passem a serem coletadas de forma rotineira nos rebanhos, possibilitando maior qualidade e clareza dos assuntos relacionados à saúde das vacas.

Considerando a importância da longevidade na atividade leiteira, espera-se que os resultados obtidos neste estudo possam contribuir e ser utilizados para uma futura avaliação da longevidade e para a definição de um índice de seleção a ser utilizado num futuro processo de seleção de animais da raça holandesa no Brasil.

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## APÊNDICES

## Apêndice 1. Instruções para publicação na revista Livestock Science

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The journal encourages authors to create an AudioSlides presentation with their published article. AudioSlides are brief, webinar-style presentations that are shown next to the online article on ScienceDirect. This gives authors the opportunity to summarize their research in their own words and to help readers understand what the paper is about. [More information and examples are available](#). Authors of this journal will automatically receive an invitation e-mail to create an AudioSlides presentation after acceptance of their paper.

### **Virtual Microscope**

The journal encourages authors to supplement in-article microscopic images with corresponding high resolution versions for use with the Virtual Microscope viewer. The Virtual Microscope is a web based viewer that enables users to view microscopic images at the highest level of detail and provides features such as zoom and pan. This feature for the first time gives authors the opportunity to share true high resolution microscopic images with their readers. [More information and examples](#). Authors of this journal will receive an invitation e-mail to create microscope images for use with the Virtual Microscope when their manuscript is first reviewed. If you opt to use the feature, please contact [virtualmicroscope@elsevier.com](mailto:virtualmicroscope@elsevier.com) for instructions on how to prepare and upload the required high resolution images.

## **AFTER ACCEPTANCE**

### **Online proof correction**

Corresponding authors will receive an e-mail with a link to our online proofing system, allowing annotation and correction of proofs online. The environment is similar to MS Word: in addition to editing text, you can also comment on figures/tables and answer questions from the Copy Editor. Web-based proofing provides a faster and less error-prone process by allowing you to directly type your corrections, eliminating the potential introduction of errors.

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## Apêndice 2. Instruções para publicação na revista Asian-Australasian Journal of Animal Sciences

*Asian-Australasian Journal of Animal Sciences* (AJAS) is the official journal of the Asian-Australasian Association of Animal Production Societies (AAAP). Anyone who would like to submit a manuscript is advised to carefully read the aims and scope section of this journal. Manuscripts submitted to AJAS should be prepared according to the following instructions. For issues not addressed in these instructions, the author is referred to the International Committee of Medical Journal Editors (ICMJE) "Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals" (<http://www.icmje.org>).

### AIMS AND SCOPE

AJAS aims to publish original and cutting-edge research results and reviews on animal-related aspects of the life sciences. Emphasis will be placed on studies involving farm animals such as cattle, buffaloes, sheep, goats, pigs, horses, and poultry. Studies for the improvement of human health using animal models may also be publishable.

AJAS will encompass all areas of animal production and fundamental aspects of animal sciences: breeding and genetics, reproduction and physiology, nutrition, meat and milk science, biotechnology, behavior, welfare, health, and livestock farming systems.

AJAS is subdivided into 10 sections.

- Animal Breeding and Genetics: quantitative and molecular genetics, genomics, genetic evaluation, evolution of domestic animals, and bioinformatics.
- Animal Reproduction and Physiology: physiology of reproduction, development, growth, lactation, and exercise; and gamete biology
- Ruminant Nutrition and Forage Utilization: rumen microbiology and function, ruminant nutrition, physiology and metabolism, and forage utilization.
- Swine Nutrition and Feed Technology: swine nutrition and physiology; evaluation of feeds, feed additives, and feed processing technology
- Poultry and Laboratory Animal Nutrition: nutrition and physiology of poultry and other non-ruminant animals.
- Animal Products: milk and meat science, muscle biology, product composition, food safety, food security, and functional foods
- Animal Biotechnology: molecular nutrition, transgenic animals, identification and manipulation of genes.
- Animal Health: immune modulation, infection and immunity, stress responses, vaccines and therapeutics, and animal models.
- Animal Behavior and Welfare: social and sexual behavior, adaptation, and animal welfare.



- Environment and Management: livestock waste management, livestock and environment, and livestock farming systems.

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## RESEARCH AND PUBLICATION ETHICS

The journal adheres to the ethical guidelines for research and publication described in the Guidelines on Good Publication (<http://publicationethics.org/resources/guidelines>) and the ICMJE Guidelines (<http://www.icmje.org>).

### 1. Authorship

Authorship credit should be based on (1) substantial contributions to conception and design, acquisition of data, and/or analysis and interpretation of data; (2) drafting the article or revising it critically for important intellectual content; (3) final approval of the version to be published; and (4) agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Every author should meet all of these four conditions. After the initial submission of a manuscript, any changes whatsoever in authorship (adding author(s), deleting author(s), or re-arranging the order of authors) must be explained by a letter to the editor from the authors concerned. This letter must be signed by all authors of the paper. Copyright assignment must also be completed by every author.

- Corresponding author and first author: AJAS allows a multiple corresponding authorship (maximum two) for one article. Only one author should correspond with the editorial office for one article. AJAS accepts notice of equal contribution for the first author when the study was clearly performed by co-first authors (maximum two).
- Correction of authorship after publication: AJAS does not correct authorship after final acceptance unless a mistake has been made by the editorial staff. Authorship may be changed before final acceptance when the authorship correction is requested by all of the authors involved with the manuscript.

### 2. Originality, Plagiarism, and Duplicate Publication

Submitted manuscripts must not have been previously published and not be under consideration for publication elsewhere. No part of the accepted manuscript should be duplicated in any other scientific journal without the permission of the editorial board of AJAS. Submitted manuscripts are screened for possible plagiarism or duplicate publication by CrossCheck upon receipt by the journal. If plagiarism or duplicate publication related to the papers of this journal is detected, the manuscripts may be rejected, the authors will be

announced in the journal, and their institutions will be informed. There will also be penalties for the authors.

A letter of permission is required for any and all material that has been published previously. It is the responsibility of the author to request permission from the publisher for any material that is being reproduced. This requirement applies to text, figures, and tables.

### **3. Secondary Publication**

It is possible to republish manuscripts if they satisfy the conditions of secondary publication of the ICMJE Recommendations ([http://www.icmje.org/urm\\_main.html](http://www.icmje.org/urm_main.html)).

### **4. Conflict of Interest Statement**

The corresponding author must inform the editor of any potential conflicts of interest that could influence the authors' interpretation of the data. Examples of potential conflicts of interest are financial support from or connections to companies, political pressure from interest groups, and academically related issues. In particular, all sources of funding applicable to the study should be explicitly stated.

### **5. Care and Use of Animals**

All animal experiments should be reviewed by the Institutional Animal Care and Use Committee (IACUC) for the care and use of animals. The manuscript must include a statement of IACUC compliance that should appear as the first item in the Methods section. If necessary, the editor or reviewers may request copies of these documents to resolve questions about IACUC approval and study conduct. AJAS retains the right to reject any manuscript on the basis of unethical conduct or misconduct of animal studies.

### **6. Process for Managing Research and Publication Misconduct**

When the journal faces suspected cases of research and publication misconduct such as redundant (duplicate) publication, plagiarism, fraudulent or fabricated data, changes in authorship, an undisclosed conflict of interest, ethical problems with a submitted manuscript, complaints against editors, and so on, the resolution process will follow the flowchart provided by the Committee on Publication Ethics (<http://publicationethics.org/resources/flowcharts>). The discussion and decision on the suspected cases will be carried out by ethics committee of AJAS.

### **7. Editorial Responsibilities**

The editorial board will continuously work to monitor and safeguard publication ethics: guidelines for retracting articles; maintenance of the integrity of the academic record; preclusion of business needs from compromising intellectual and ethical standards; publishing corrections, clarifications, retractions, and apologies when needed; and excluding plagiarism and fraudulent data. The editors maintain the following responsibilities: responsibility and authority to reject and accept articles; avoiding any conflict of interest with

respect to articles they reject or accept; promoting publication of corrections or retractions when errors are found; and preservation of the anonymity of reviewers.

## SUBMISSION AND PEER REVIEW PROCESS

### 1. Submission

All manuscripts should be submitted via the AJAS e-submission system (<http://submit.ajas.info/>). If there are difficulties, authors should contact the editorial office (<http://submit.ajas.info/community/contact/>).

### 2. Peer Review/Revision Process

The suitability of papers for publication in AJAS is judged by the members of the editorial board. The editor-in-chief has full responsibility for the papers submitted, which are evaluated in the order received. At the initial stage, the editor-in-chief may ask the associate editors to evaluate submitted papers for suitability for further review. Each paper that is deemed suitable will be evaluated by at least two members of the editorial board or other scientifically qualified reviewers. The editor-in-chief handles all correspondence with the author and makes the final decision as to whether the paper is recommended for acceptance or rejection, or needs to be returned to the author for revision. A reviewer may not be from the same institution as the author. Reviewers should examine the paper and return it with their report to the editor-in-chief as soon as possible, usually within 3 weeks. The identity and the report of the reviewers are made known to the editor-in-chief, but only the anonymous report is routinely sent to the author. The anonymity of the reviewers is preserved unless it is desired otherwise by all parties involved.

The reviewer recommends acceptance, acceptance after revision, resubmission after revision, or rejection. If both reviewers recommend acceptance or rejection, the decision stands. When their opinions differ, then the editor-in-chief may ask a third reviewer or associate editor to decide on the acceptance or rejection of that paper. The editor-in-chief may have to decide whether to accept or reject a manuscript for which review reports are overdue if the review process has not been completed within 2 months.

Papers needing revision will be returned to the corresponding author, and the author must return the revised manuscript to the editor-in-chief within 4 weeks; otherwise, the author will be notified that the paper has been withdrawn. The editor-in-chief may send the revised manuscript to associate editors to examine whether the manuscript has been revised as suggested by the reviewers.

If a paper is not suitable for publication, the corresponding author will be notified with a statement of reasons for rejection. The author may appeal if s/he believes an erroneous or unfair judgement has been made. A letter to the editor-in-chief presenting reasons why the decision should be reconsidered will be given due consideration. Most papers that eventually are published are first returned for revision. Common reasons for requesting revision are failure to follow style and form, lack of clarity or brevity, questions of fact or theory, poor organization of tabular material, and poor English.

## MANUSCRIPT PREPARATION

### 1. General Requirements

The manuscript must be double-spaced in Times New Roman font (size 10). All pages should be numbered consecutively in the top right hand corner, beginning with the title page.

The lines on all pages, including those pages for references and figure legends, must be numbered consecutively in the left margin, beginning with number one at the top of the title page. A 2.5 cm margin on both sides of the page is desirable.

Weights and measures must be expressed in the SI unit (metric) system and temperatures in the Celsius (centigrade) scale.

Tables, double-spaced, should be as few and as simple as is feasible. Each table should be on a separate sheet.

The legends for figures should be typed on a separate sheet. Photographs should be carefully prepared so that a clear image can be printed.

Manuscripts will be edited in the order received, and accepted papers will be published in the order submitted if at all possible.

Authors whose native language is not English are strongly encouraged to have their manuscripts proofread prior to submission.

Authors must declare any financial support or relationships that may pose a conflict of interest. Manuscript preparation is different according to the publication type, including Original Articles, Reviews, Technical Notes, Editorials, Book Reviews, and Correspondence. Other types may also be negotiated with the editorial board of AJAS.

### 3. Original Articles

Original Articles are reports of basic investigations. Although there is no limitation on the length of the manuscripts, the editorial board may abridge excessive illustrations and large tables. The manuscript for an Original Article should be organized in the following sequence: title page, abstract, keywords, main text (introduction, materials and methods, results, and discussion), implications (optional), acknowledgments (optional), references, tables, and figure legends. The figures may be submitted as separate files.

#### 1) Title page

The following items should be included on the title page: (a) the title of the manuscript, (b) author list, (c) each author's affiliation and e-mail, (d) the name, e-mail, and telephone number of the corresponding author, (e) when applicable, the source of any research funding and a list of where and when the study has been presented in part elsewhere, and (f) a running title of fewer than 45 characters.

The title of the manuscript should be typed in bold-faced print using both upper and lower case letters and set in the center of the page. Although the title should be as brief as possible, it is recommended to include the animal species involved in the research when applicable. Abbreviations are not permitted in the title.

Full names of all authors should be provided with the family name in italics. Indications of professorial rank or other professional titles should not be used. Naming an author on a paper implies that the person named is aware of the research reported and agrees with and accepts responsibility for any results or conclusions reported.

The address of the institution where the research was conducted should include the name of the institution, city, zip code, and country. If the affiliation is different from the first author, the authors should be marked "1," "2," "3," and so forth in Arabic numerals, which should appear in superscript at the top right-hand corner of the author's name and at the beginning of each affiliation.

## 2) Abstract

A structured abstract is required for original articles and an unstructured one for reviews papers.

The abstract, consisting of no more than 300 words, appears on a separate page following the title page. The abstract should summarize pertinent results in a brief but understandable form. A structured abstract should contain Objective (purpose/background), Methods, Results, and Conclusion sections. An unstructured abstract should be one paragraph without sections. References should never be cited in the abstract. Abbreviations that appear in the abstract that are not included in the standard abbreviation listing (Appendix 2) must be defined before they are first used.

## 3) Keywords

At the end of the abstract, up to six keywords that best describe the nature of the research should be listed. The term "Keywords" should appear in bold followed by a colon. The first letter of each keyword is capitalized and keywords are separated by semicolon. Keywords should include the animal species, variables tested, and the major response criteria. Keywords must be selected from the CAB Thesaurus (available from <http://www.cabi.org/cabthesaurus/>).

## 4) Headings

The article's major headings (Introduction, Materials and Methods, Results, Discussion [or Results and Discussion], and References) appear in roman bold-faced type.

First subheadings appear at the left margin on a separate line in bold-faced print, are not followed by punctuation, and only the first word is capitalized. First subheadings are used when subsections consist of several paragraphs.

Second subheadings appear at the beginning of the first line of a paragraph. They are italicized and do not require labeling (a, b, c, etc.).

### **Materials and Methods**

**Animals, experimental design, and diet**  
*Animals*

### 5) Introduction

The introduction starts on a new page following the abstract. The introduction briefly justifies the research and specifies the hypotheses to be tested. Extensive discussion of relevant literature should be included in the discussion of results, not in the introduction. To minimize length and avoid redundancy, generally no more than three references should be cited to support a specific concept.

### 6) Materials and Method

All animal experiments should be reviewed by IACUC for the care and use of animals. If specimens from human subjects were used in research, the authors must certify that the approval of the research from an appropriate IRB was obtained. The manuscript must include a statement of IACUC or IRB compliance or exemption in this section.

A clear description or original reference is required for all biological, analytical, and statistical procedures used in the experiment. All modifications of procedures must be explained. Diets, animals (breed, sex, age, body weight, and weighing conditions [i.e., with or without restriction of feed and/or water]), surgical techniques, measurements, and statistical models should be described clearly and fully. Brand names and company names and locations for all substances and equipment referred to in the text should be included in parentheses within the text, not in footnotes.

Statistics: Biology should be emphasized, but the use of incorrect or inadequate statistical methods to analyze and interpret biological data is not acceptable. Consultation with a statistician is recommended. Statistical methods commonly used in the area of animal sciences need not be described in detail, but adequate references should be provided. The statistical model, classes, blocks, and experimental unit must be designated. Any restrictions used in estimating parameters should be defined. Reference to a statistical package without reporting the sources of variation (classes) and other salient features of the analysis, such as covariance or orthogonal contrasts, is not sufficient. A statement of the results of statistical analysis should justify the interpretations and conclusions.

### 7) Results

Results should be presented in tabular form when feasible. The text should explain or elaborate on the tabular data, but numbers should not be repeated extensively within the text. Sufficient data, all with some index of variation attached, should be presented to allow the readers to interpret the results of the experiment. The discussion may be combined with the results in one section if desired.

### 8) Discussion

The discussion, whether in a separate section or combined with the results, should interpret the results clearly and concisely in terms of biological mechanisms and should integrate with the research findings of other studies to provide the readers with a broad base for understanding whether the hypotheses tested were accepted or rejected.

### 9) Implications (optional)

This section, consisting of no more than 100 words in one paragraph, follows the discussion and should explain in lay terms, without abbreviations, acronyms, or citations, what the findings of this research imply for animal production and/or biology. Though some speculation is permitted, this section should also caution the reader against overextrapolation of results. For manuscripts with direct applications, this section will consist of an interpretive summary.

### 10) References

In the text, references should be cited with Arabic numerals in brackets, numbered in the order cited. In the references section, the references should be numbered and listed in order of appearance in the text. The number of references is limited to 30 for Original Articles. All authors of a cited work should be listed if there are six or fewer authors. The first three authors should be listed followed by “et al.” if there are more than six authors. If a reference has a digital object identifier (DOI), it should be supplied. Non-published findings and personal communications should not be included in the list of references. Journals titles shall be abbreviated according to the conventional ISO abbreviations used by PubMed (<http://www.ncbi.nlm.nih.gov/nlmcatalog/journals>). A short list of journal title abbreviations is provided in Appendix 1. Sample references are given below. Other types of references not described below should follow *The NLM Style Guide for Authors, Editors, and Publishers* (<http://www.nlm.nih.gov/citingmedicine>).

### Sample References

#### (Journal Articles)

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2. Tizioto PC, Coutinho LL, Mourão GB, et al. Variation in myogenic differentiation 1 mRNA abundance is associated with beef tenderness in Nelore cattle. *Anim Genet* 2016 Mar 30 [Epub]. <http://dx.doi.org/10.1111/age.12434>
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4. Mahan DC, Weaver EM, Russell LE. Improved postweaning pig performance by adding NaCl or HCl to diets containing animal plasma [abstract]. *J Anim Sci* 1996;74(Suppl 1):58.

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9. Raosoft. Sample size calculator [Internet]. Raosoft Inc.; c2004 [cited 2016 Apr 1]. Available from: <http://www.raosoft.com/samplesize.html>
10. Metagenomics: sequences from the environment [Internet]. Bethesda, MD: National Center for Biomedical Information; 2006 [cited 2016 Feb 20]. Available from: <http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=metagenomics.TOC>

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11. Ha JK. Studies on beneficial and adverse effects of dietary buffers for lambs [dissertation]. Brookings, SD: South Dakota State University; 1981.
12. Yoon CH. Effects of lysine and sodium levels on growth performance, acid-base balance and lysine-arginine antagonism in broiler chicks [master's thesis]. Seoul, KR: Seoul National University; 1991.

(Conference Papers)

13. Moss KJ, Greening L. The effect of age and gender on the time taken for horses to learn an operant task. In: Proceedings of the British Society of Animal Science 2009; 2009 Mar 30-Apr 1; Southport, UK. Penicuik, UK: British Society of Animal Science; 2009. p. 1.
14. Patrias K. Computer-compatible writing and editing. Interacting with the digital environment: modern scientific publishing. 46th Annual Meeting of the Council of Science Editors; 2003 May 3-6; Pittsburgh, PA.

(Research Reports)

15. Page E, Harney JM. Health hazard evaluation report. Cincinnati, OH: National Institute for Occupational Safety and Health; 2001. Report No.: HETA2000-0139-2824.

11) Tables

Tables are used to present numerical data in a self-explanatory manner. They should be intelligible without consulting the text and should not duplicate data already given in the text or in illustrations. Any abbreviation used in a table must be defined in that table. Tables should be double-spaced with each table on a separate sheet. Tables should appear immediately after the references. The tables should be paginated in series with the text.



All tables should be cited in the text. Arabic numerals are used to number tables. The table number (i.e., Table 4.) is typed in bold face followed by a period. The title of the table continues on the same line with only the first letter capitalized. A period should not appear at the end of the title. Column headings should have the first letter of each word capitalized while the names of variables are to be typed with only the first letter capitalized (i.e., Average daily gain).

For numerals less than 1, a zero should be inserted to the left of the decimal point, and if possible, columns should be center-aligned. If there are no data for a particular entry, a hyphen should be inserted. If an explanation is necessary, an abbreviation can be used in the body of the table (e.g., ND) and it should be explained clearly in the footnotes.

References to footnotes in a table are to be specified by superscript numbers, independently for each table. Superscript letters are used to designate statistical significance. Use a lower case p to indicate probability values (i.e.,  $p < 0.05$ ).

Presentation of pooled standard errors, the general basis for statistical comparisons of means, is recommended when variance is homogeneous. These should be presented in a separate column or row. Standard errors can be attached to each mean by  $\pm$  signs when variance or SE is heterogeneous (e.g., unbalanced experiments or unequal numbers of observations in treatment means). The pooled standard error is the preferred estimate of experimental error because presenting individual standard errors tends to clutter up the table. For diet composition, major ingredient inclusion levels should be presented as a percentage of the total rather than in grams or kilograms of food.

## 12) Figures

Figures should be placed at the end of the manuscript with each figure on a separate page. Figure legends should be typed (double spaced) on a separate page. Figures should fit in one column (8 cm wide), or full-page width (17 cm wide). A minimum type size of 8 points (Times New Roman) is recommended so as to be readable in the final publication size. For tables containing multiple lines, solid, long-dash, short-dash, and dotted lines should be used, while gray or shaded lines should be avoided. Lines with different symbols for the data points may also be used to distinguish curves. Unnecessary backgrounds and grid lines should be removed from graphs. Each axis should have a description and a unit.

For bar charts, different fill patterns may be used if needed (black, white, gray, and stripes). Curves and data points should be identified using the following symbols ( $\bullet$ ,  $\circ$ ,  $\blacksquare$ ,  $\square$ ,  $\blacklozenge$ ,  $\diamond$ ,  $\blacktriangle$ ,  $\triangle$ ,  $+$ , and  $\times$ ). Symbols should be defined in the figure legend or in a key on the figure. The preferred file type for figures is JPEG, TIFF, or PPT. If figures are to be reproduced in grayscale (black and white), they should be submitted as such. If figures are to appear in color in the print journal, the files must be submitted in CMYK color (not RGB). The minimum resolution is 300 dpi for color and grayscale figures, and 600 dpi for line art.

Photomicrographs must have their unmagnified size designated either in the caption or with a scale bar on the figure.

A legend should be prepared to provide sufficient information and all abbreviations, and the symbols used in the figure should be defined in the legend.

## FINAL PREPARATION FOR PUBLICATION

### 1. Manuscript Corrections

Before publication, the manuscript editor may correct the manuscript such that it meets the standard publication format. The author(s) must respond within 2 days when the manuscript editor contacts the author for revisions. If the response is delayed, the manuscript's publication may be postponed.

### 2. Galley Proof

The author(s) will receive the final version of the manuscript as a PDF file. Upon receipt, within 2 days, the editorial office (or printing office) must be notified of any errors found in the file. No major changes including changes to the author list will be allowed at this stage. Any errors found after this time are the responsibility of the author(s) and will have to be corrected as an erratum.

## SUBMISSION FEE AND ARTICLE-PROCESSING CHARGES

There is no submission fee. For information on article-processing charge for papers accepted for publication, see "Subscription, page charge policy" (<http://www.ajas.info/authors/subscription.php>).

## Contact Us

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NOTICE: These instructions to authors will be applied beginning with the January 2017 issue.

## VITA

Elisandra Lurdes Kern nasceu em 13 de dezembro de 1987 no município de Alecrim, no estado do Rio Grande do Sul. É filha de Inacio Kern e Alma Kern. No segundo semestre de 2006, ingressou no curso de Zootecnia da Universidade Federal de Santa Maria, na unidade do Centro de Educação Norte do Rio Grande do Sul – CESNORS, localizado no município de Palmeira das Missões, e no dia 14 de julho de 2010, sob orientação da Prof<sup>a</sup>. Dr<sup>a</sup>. Fernanda Cristina Breda Mello, apresentou o Trabalho de Conclusão de Curso em Zootecnia. Em novembro deste mesmo ano, realizou o Estágio Supervisionado em Zootecnia na Universidade Federal do Rio Grande do Sul (UFRGS), sob orientação da Prof<sup>a</sup>. Dr. Fernanda Cristina Breda Mello e supervisão do Prof. Dr. Jaime Araújo Cobuci, recebendo, no dia 08 de janeiro de 2011, o título de Bacharel em Zootecnia. Iniciou, em abril de 2011, o curso de mestrado no Programa de Pós-Graduação em Zootecnia da Faculdade de Agronomia da UFRGS, na área de Melhoramento Genético Animal, sob orientação do Dr. Jaime Araujo Cobuci, obtendo em 2013, o título de Mestre em Produção Animal. Em abril de 2013, ingressou no curso de Doutorado em Zootecnia também pela UFRGS, desenvolvendo o trabalho de tese sobre a avaliação genética da longevidade de vacas da raça Holandesa, usando um modelo de riscos proporcionais Weibull estratificado. Realizou parte de sua tese no Institut National de la Recherche Agronomique (INRA), Jouy en Josas, Paris, França, no período de setembro de 2014 a setembro de 2015. Submeteu-se a banca de defesa de tese em março de 2016 pela UFRGS em Porto Alegre, RS.