



Estimation of genetic and environmental relationships between milk yield and different measures of mastitis and hyperkeratosis in Holstein cows

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ABSTRACT. In this research, records of milk yield, mastitis occurrence and teats hyperkeratosis of 453 Holstein cows from a large herd with calving dates from December 2012 to December 2014 was used to estimate genetic and environmental correlations between milk yield, mastitis and hyperkeratosis. The highest and the lowest number of mastitis occurrence were in winter (January to March) and spring (April to June), respectively. Also, the highest and the lowest averages of days in milk at the time of mastitis occurrence were observed in winter and spring, respectively (162.53 *vs.* 29.67 days). The total number of mastitis occurrence was 151 times and in average a sick cow was infected 1.649 times. Obtained results showed low heritabilities for measures of mastitis (ranging from 0.048 to 0.134). Genetic correlations between measures of mastitis and milk yield were negative (from -0.502 to -0.183). Genetic correlation between hyperkeratosis and mastitis was positive which showed that selection for udder type traits would decrease mastitis occurrence. The results of this study can provide a background about the relationships between aforementioned traits and serve as a starting point for further study on these traits.

Keywords: dairy cow, genetic relationship, iteration, udder type.

Relações genéticas e ambientais entre produção de leite e tipos diferentes de mastite e hiperqueratose em vacas holandesas

RESUMO. Os registros de produção leiteira, ocorrência de mastite e hiperqueratose dos úberes de 453 vacas holandesas de um grande rebanho, com datas de parição entre dezembro 2012 e dezembro de 2014, foram usados para estimar as correlações genéticas e ambientais entre produção de leite, mastite e hiperqueratose. O número mais alto e o mais baixo da ocorrência de mastite aconteceram respectivamente no inverno (janeiro – março) e na primavera (abril - junho). As médias mais altas e baixas em dias/leite no período da ocorrência da mastite foram registradas respectivamente no inverno e na primavera (162,53 *vs.* 29,67 dias). O número total de ocorrência de mastite foi 151 vezes e, em média, uma vaca doente foi infectada 1649 vezes. Os resultados revelaram baixa herdade para as medidas de mastite (entre 0,048 e 0,134). Correlações genéticas entre mastite e produção de leite foram negativas (entre -0,502 e -0,183). A correlação genética entre hiperqueratose e mastite foi positiva e mostrou que a seleção de características de úbere poderia diminuir a ocorrência de mastite. Os resultados podem proporcionar uma base para o relacionamento entre as características mencionadas acima, como também servem de ponto de partida para mais estudos sobre essas características.

Palavras-chave: vacas leiteiras, relacionamento genético, iteração, tipo de úbere.

Introduction

The main goal of breeding programs is to increase milk yield of dairy cattle. Intensive selection along with improvement in housing, feeding, health measurements and management and use of artificial insemination lead to considerable production per animal.

Average estimated breeding value for milk yield of Holstein cows of Iran increased by 700 kg from

1971 to 2005 (Abdollahpour, Moradi Shahrabak, Nejati-Javaremi, Vaez Torshizi, & Mrode, 2013). It was stated that genetic selection for milk yield cause unfavorable side effects including increased probability of metabolic, reproductive and other health disorders such as mastitis (Rupp & Boichard, 1999; Lucy, 2001; Wolf, Wolfová, & Štípková, 2010; Martin, Wensch-Dorendorf, Schafberg, & Swalve, 2013).

Because of animal suffering, high incidence rate and considerable economic loss, mastitis has received special attention within the last years. Mastitis is defined as inflammation of the mammary gland and occurs as a result of the introduction and multiplication of pathogenic microorganisms in the udder (Harmon, 1994). Bacteria are the main cause of disease and the route of infection is usually through the teat canal. Mastitis is a multifactorial and complex disease because it has numerous causative pathogens, a wide variety of physiological responses, and a multifactorial background in which several genes and many environmental factors are involved. Risk factors associated with mastitis are often related to management practices, including milking technique and equipment, housing, cleanliness of the environment, hygienic quality of feed and water, preventive health measures and stress. Effect of different factors on mastitis has been investigated. Non-management factors such as season, parity, lactation stage, breed, udder conformation, milk production, milking speed and reproductive disorders are also known to be associated with mastitis (Schukken, Grommers, Van de Geer, Erb, & Brand, 1990; Barkema et al., 1998; Hagnestam, Emanuelson, & Berglund, 2007). The incidence of clinical mastitis increases with increasing parity and is highest in early lactation, especially in first-parity cows (Barkema et al., 1998; Hagnestam et al., 2007). In a previous study by Ghavi Hossein-Zadeh and Ardalan (2011a), retained placenta, milk fever, pluriparity, winter calving, early stages of lactation and number of somatic cells in previous lactation introduced as significant risk factors for mastitis. In addition to aforementioned factors, genetic structure and immune system of the cow play an important role in defense mechanisms in the cow against mastitis (Ohtsuka et al., 2010).

The teat canal is a primary barrier against invasion of mastitis pathogens into the udder. Between milkings, the smooth muscles surrounding the teat duct should be contracted and the teat canal tightly closed to impede bacterial passage from the teat orifice into the interior of the gland (Nickerson, 1994). A teat-end in good condition is an important resistance factor to bacterial colonization of the mammary gland (Michel, Seffner, & Schulz, 1974). Changes in teat tissue by milking, teat canal integrity, and teat tissue pliability may favor penetration of bacteria into the udder (O'Shea, 1987). Severe teat-end lesions are associated with a higher prevalence of subclinical mastitis and bacterial colonization (Sieber & Farnsworth, 1981; Bhutto, Murray, & Woldehiwet, 2010; Paduch, Mohrb, & Krömker, 2012). The huge variation in the frequency of callosity between herds using

similar milking systems suggests that a major genetic influence to susceptibility should not be overlooked (Shearn & Hillerton, 1996). Hyperkeratosis means excessive keratin growth and is a normal physiological response to the forces applied to the teat skin during milking. The onset and severity of hyperkeratosis is influenced by the effects of climate, seasonal and environmental conditions, milking management, herd milk production level and genetics of individual cows (Ohnstad et al., 2003).

To our knowledge, there is no study that investigated genetic relationship between hyperkeratosis with mastitis and milk yield. Therefore, the aim of this study was to estimate heritability as well as genetic and environmental correlations between 305-day milk yield (Milk305), milk yield in the first test-day (Firstmilk), mastitis occurrence (Mast), number of mastitis occurrence (NumMast), days sick (DS), hyperkeratosis (Hup), number of hyperkeratotic teats (NumHyp) and sum of the degree of hyperkeratotic teats (DegHyp) in Holstein cows.

Material and methods

Records of milk yield, mastitis occurrence and hyperkeratosis of 453 Holstein cows (with 1812 teats) from a large herd with calving dates from December 2012 to December 2014 in Qazvin province of Iran were used in this study. It was a new established herd and all cows were in their first or second lactations. These cows were the daughter of 80 sires, with the mean of 5.66 and range of 1 to 33 daughters per sire. One teat-end observation was available for each individual teat. Three criteria were defined for mastitis as follows: Mastitis occurrence (Mast; binary; '0' as no mastitis occurrence and '1' with at least one case of mastitis during lactation), number of mastitis occurrence (NumMast), and days sick (DS) as sum of days that a cow was treated for mastitis. Also, three criteria were defined for hyperkeratosis: Hyperkeratosis (Hyp; binary; '0' represented four healthy teats with no ring and '1' denoted at least one teat is in group two), number of hyperkeratotic teats (NumHyp) (with 5 classes; from '0' as none hyperkeratotic teat to '4' as four hyperkeratotic teats), and sum of degree of hyperkeratotic teats (DegHyp). Teat-end hyperkeratosis was scored based on a four class scale (Mein et al. 2000): in group one the teat-end is smooth with a small, even orifice; group two had raised ring with no roughness or only mild roughness and no keratin fronds; group three with raised roughened ring with isolated fronds of

old keratin extending 1-3 mm from the orifice and in group four a raised ring with rough fronds of old keratin extending >4 mm from the orifice.

Cows were housed in covered barns with concrete floors and free stalls (sawdust and sand were used as bedding) and fed manually throughout the year with alfalfa hay, corn silage and wheat straw as forages and a mixture of barley grain, corn grain, soybean meal, whole cottonseed, cottonseed meal, canola meal, wheat bran, corn gluten and beet pulp and vitamin and mineral premix as concentrate. The following model was used for analyzing the studied traits:

$$y_{ijkl} = \mu + YS_i + L_j + A_k + e_{ijkl}$$

where y_{ijkl} = dependent variable (Milk305, Firstmilk, Mast, NumMast, DS, Hyp, NumHyp, DegHyp) observed for animal k ; μ = mean of the trait; YS_i = fixed effect of year-season of calving; L_j = fixed effect of lactation number; A_k = random additive genetic effect of the k th cow; e_{ijkl} = random residual effect of observation k .

At first, univariate analyses were performed to obtain heritabilities. Then, two-trait threshold animal model analyses were performed to estimate genetic and environmental correlations. As diseases has threshold nature, in order to estimate heritabilities, genetic and environmental correlations, TM program (Legarra, Varona, & Lopez de Maturana, 2011) was used that is capable of analyzing threshold traits along with continues traits using Gibbs sampling approach, simultaneously. Considering recommendations of TM manual, threshold nature of studied traits and few numbers of data, number of iterations was set to 500,000 rounds and one-fifth of them were discarded as burn-in period. Also, a thin interval of 1000 rounds was used.

Results and discussion

Mean (standard deviation) of Firstmilk and Milk305 were 37.78 kg (9.66) and 10943 kg (1644.43), respectively. The NumMast and average of days in milk at the time of mastitis occurrence separated by season of occurrence (Table 1).

Table 1. Number of mastitis occurrence and average days in milk at the time of mastitis occurrence separated by season of occurrence.

Season	Number of mastitis incidence	Average days in milk
Spring	15	29.67
Summer	37	69.32
Fall	43	95.98
Winter	56	162.53

The most and the least NumMast were observed in winter and spring, respectively. Investigating risk factors for mastitis, Ghavi Hossein-Zadeh and Ardalán (2011a) reported that calving in winter is associated with highest likelihood of mastitis. Also, the highest and the lowest averages of days in milk at the time of mastitis occurrence were observed in winter and spring, respectively (162.53 *vs.* 29.67 days). Probably, cause of mastitis in winter and fall (wet seasons) is mostly due to infecting pathogens whereas in spring and summer (dry seasons; that most of mastitis cases were occurred at early stages of lactation) is mainly due to high tension of production at peak yield. In general, 20.09% of cows were infected with mastitis. Sum of the NumMast was 151 times and in average a sick cow was infected 1.649 times. Also, mean of DS per mastitis case was 3.55 days and overall mean of days in milk at the time of mastitis was 107.5 days. In a study conducted by Ghavi Hossein-Zadeh and Ardalán (2011a), lactational incidence of mastitis in 20 large herds was reported to be 18.9%. Reports of other researchers for mastitis incidence ranged from 1.7 to 54.6% (Emanuelson, Oltenuacu, & Gröhn, 1993; Kelton, Lissemore, & Martin, 1998; Mrode, Pritchard, Coffey, & Wall, 2012; Martin et al., 2013). Frequency of mastitis incidence was in the range of previous studies.

Posterior means of variance components and heritabilities from univariate analyses are shown in Table 2. Heritability of different measures of mastitis was relatively low (from 0.048 to 0.134). Ghavi Hossein-Zadeh and Ardalán (2011b) estimated heritability of mastitis in the first three lactations which ranged from 0.06 to 0.09. Also, heritability of mastitis for Holstein cows of Germany was 0.09 (Martin et al., 2013). In another research (Mrode et al., 2012), heritability of mastitis in the first, second and third lactations was estimated to be 0.04, 0.06 and 0.08, respectively, for Holstein cows of UK. These authors also estimated heritability of NumMast in the range of 0.05 to 0.10, and genetic and phenotypic correlations between Mast and NumMast were 0.52 and 1.00, respectively.

Heritability of different measures of hyperkeratosis was from 0.363 to 0.594. Estimates of parameters for hyperkeratosis are rare in the literature. Lojda, Stavikova and Polacek (1982) estimated heritability of teat-end shape using regression of daughter on dam 0.414. Also, using animal model, Chrystal, Seykora and Hansen (1999) estimated heritability of teat-end shape in

the first, second and ≥ 3 lactation cows that ranged from 0.44 to 0.56. In a recent study (Kramer, Erbe, Bapst, Bieber, & Simianer, 2013) that considered each quarter as a distinct organ, range of heritability for Hyp was from 0.12 (for rear left teat) to 0.26 (front right) for Brown Swiss cows in Switzerland. Discrepancies between estimates from different studies can be due to the subjective nature of visually scored teat-ends, different scales used for teat-end scoring (Chrystal et al., 2001), differences between statistical analysis and breeds. It seems condition of all four teats together has higher heritability relative to consider it as binary.

Estimates of genetic and environmental correlations among traits are shown in Table 3. In contrary to the results of previous study (Rupp & Boichard, 1999; Martin et al., 2013), genetic correlation between Milk305 and measures of mastitis was negative (from -0.183 to -0.502). Rupp and Boichard (1999) estimated a genetic correlation of 0.45 between milk yield and Mast. Vallimont, Dechow, Sattler and Clay (2009) and Wolf et al. (2010) reported genetic correlation between milk yield and NumMast case as 0.25 and 0.34, respectively. However, Irano et al. (2014) reported a negative genetic correlation between cumulative 305-day milk yield and Mast in Holstein cows in tropical condition of Brazil. Genetic correlation between Firstmilk and measures of mastitis was relatively similar to those of Milk305 and measures of mastitis. Although,

standard deviations were high in this study, environmental correlations between Firstmilk and measures of hyperkeratosis showed that each factors that increase Firstmilk would increase hyperkeratosis and subsequently increase likelihood of mastitis in the remaining parts of lactations (due to positive environmental correlation between hyperkeratosis and mastitis).

Genetic correlations between different measures of hyperkeratosis and mastitis were from -0.106 to 0.442. There is no published research that reports this genetic correlation. Also, environmental correlations ranged from -0.005 to 0.397. Formerly, it was believed that presence of smooth or rough rings on teat-ends is not associated with significant increase in the risk of mastitis (Sieber & Farnsworth, 1981), but other researches changed this approach (Lewis, Cockcroft, Bramley, & Jackson 2000; Neijenhuis, Barkema, Hogeveen, & Noordhuizen, 2000). It was stated that teats having moderate rings or moderate hyperkeratosis compared to teats with no ring or rough ring have lower risk of both clinical mastitis and somatic cell count ≥ 200000 cells mL⁻¹ (Neijenhuis et al., 2000; Breen, Bradley, & Green, 2009). Neijenhuis, Barkema, Hogeveen, and Noordhuizen (2001) reported that cows with mastitis had higher teat-end scores than their healthy counterparts, especially between the second and fifth months of lactation. Similarly, Lewis et al. (2000) reported an increased risk of sub-clinical mastitis for poorer teat-end scores.

Table 2. Posterior means of variance components from univariate analysis of 305-day milk yield (Milk305), milk yield in first test-day (Firstmilk), mastitis (Mast), number of mastitis occurrence (NumMast), days sick (DS), hyperkeratosis (Hyp), number of hyperkeratotic teats (NumHyp) and sum of degree of hyperkeratotic teats (DegHyp).

Traits	σ_a^2	σ_e^2	σ_p^2	$h^2 \pm SD$	Median	HPD
Milk305	6.93×10^5	1.867×10^6	2.56×10^6	0.246 ± 0.168	0.216	0.0003-0.5641
Firstmilk	12.848	65.260	78.108	0.163 ± 0.145	0.126	0.0003-0.5000
Mast	0.052	1.000	1.052	0.048 ± 0.032	0.039	0.0000-0.1378
NumMast	0.073	1.002	1.075	0.069 ± 0.044	0.058	0.0190-0.1568
DS	0.128	0.823	0.951	0.134 ± 0.062	0.123	0.0840-0.2218
Hyp	0.614	1.000	1.614	0.363 ± 0.107	0.419	0.2148-0.5850
NumHyp	191.630	135.070	326.700	0.594 ± 0.224	0.618	0.1880-0.9181
DegHyp	5.007	3.982	8.989	0.551 ± 0.173	0.540	0.2052-0.9130

σ_a^2 : additive genetic variance; σ_e^2 : environmental variance; σ_p^2 : phenotypic variance; h^2 : heritability; S.E.: standard error; HPD: highest posterior density regions intervals for heritability.

Table 3. Estimates of genetic (above diagonal) and environmental correlations (below diagonal) for 305-day milk yield (Milk305), milk yield in first test-day (Firstmilk), mastitis (Mast), number of mastitis occurrence (NumMast), days sick (DS), hyperkeratosis (Hyp), number of hyperkeratotic teats (NumHyp) and sum of degree of hyperkeratotic teats (DegHyp).

Trait	Milk305	Firstmilk	Mast	NumMast	DS	Hyp	NumHyp	DegHyp
Milk305		0.732 \pm 0.340	-0.502 \pm 0.465	-0.288 \pm 0.527	-0.183 \pm 0.513	0.150 \pm 0.505	0.165 \pm 0.476	0.253 \pm 0.470
Firstmilk	-0.027 \pm 0.289		-0.383 \pm 0.570	-0.250 \pm 0.640	-0.330 \pm 0.610	-0.090 \pm 0.640	-0.247 \pm 0.568	-0.268 \pm 0.591
Mast	0.034 \pm 0.212	-0.025 \pm 0.190		0.882 \pm 0.096	0.959 \pm 0.044	0.442 \pm 0.304	-0.106 \pm 0.540	-0.068 \pm 0.562
NumMast	0.236 \pm 0.380	0.072 \pm 0.393	0.954 \pm 0.014		0.935 \pm 0.092	0.110 \pm 0.570	0.134 \pm 0.486	0.050 \pm 0.440
DS	0.223 \pm 0.411	0.083 \pm 0.370	0.995 \pm 0.004	0.955 \pm 0.007		0.069 \pm 0.517	0.068 \pm 0.454	-0.033 \pm 0.434
Hyp	-0.043 \pm 0.266	0.030 \pm 0.238	0.000 \pm 0.000	0.159 \pm 0.303	0.192 \pm 0.340		0.998 \pm 0.002	0.994 \pm 0.010
NumHyp	-0.123 \pm 0.406	0.118 \pm 0.389	0.197 \pm 0.336	-0.005 \pm 0.563	0.102 \pm 0.543	0.995 \pm 0.004		0.993 \pm 0.008
DegHyp	-0.114 \pm 0.399	0.320 \pm 0.330	0.296 \pm 0.290	0.281 \pm 0.478	0.397 \pm 0.436	0.998 \pm 0.001	0.926 \pm 0.108	

However, Chrystal et al. (2001) and Bhutto et al. (2010) declared no significant association between udder shape and teat-end lesions with quarter somatic cell count. Nonetheless, these authors pointed out that there is some association between mammary infection and udder shape and teat-end lesions. On the other hand, Paduch et al. (2012) expressed that highly calloused teat-ends had an increased microbial load in teat canal in comparison to less-calloused teat-ends. Investigating association between teat dimensions and somatic cell count, Zwertvaegher et al. (2013) declared that selection based on teat characteristics would improve milk quality and udder health. Although, there was some discrepancy between studies, but most of researches approve that some association exist between hyperkeratosis and teat-end callosity with mastitis and milk quality.

Conclusion

Result of the present study shows that with recording for udder traits it can be possible to evaluate udder health conditions elaborately and also it can be used for management and breeding purposes. Correlation between mastitis and hyperkeratosis indicate that a common genetic component exist for them; therefore, selection to improve teat-end shape would decrease mastitis incidence and improve udder health. Considering high heritability of hyperkeratosis it can be included in selection index, but further studies with higher number of data is needed to obtain steadier parameters with smaller standard deviations.

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