

Is the Friesian *polled* mutation affecting performance traits in Holstein Friesian cattle?

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Summary

Breeding for polledness has just recently become of interest, since the routinely performed dehorning of calves has raised serious animal welfare concerns. The autosomal dominant inheritance of the *polled* locus and the availability of a direct gene test have a great potential for a rapid spread of the trait. Nevertheless, it is not clear whether the *polled* locus influences other traits than horn development as well.

In our study, we examined a possible influence of the *polled* locus on performance traits in Holstein Friesian cattle. For that, we compiled a data set of 1851 Holstein Friesian cows descending from 19 heterozygous polled sires. The genetic horn status of the cows was analyzed and the performances of horned and polled daughters were compared using independent two sample t-tests and multi-factor ANOVAs. As phenotypes, the routinely estimated breeding values for milk performance, fertility and body conformation were abducted. To investigate a pleiotropic effect, the performances of all polled and horned daughters of the sires were compared. However, the results of the independent two-sample t-test and the ANOVA were inconclusive, contradicting a pleiotropic effect of the *polled* locus. To compare the performance within the different families a nested ANOVA for multiple factors was used, where the factor genotype was nested within the factor sire. Indeed, we found significant results for the traits yield deviation of milk, fat and protein yield, as well as for the total merit index and the relative estimated breeding values for milk production, protein yield, body, total conformation, fertility, paternal calving traits. Intriguingly, the effect of the *polled* locus between the different sire groups is partly vice versa. A possible explanation for that is that the *polled* locus could be linked to putative QTLs influencing those performance traits, but the linkage phases differ between different families. To investigate this more closely we contrive to consider the kinship between the sires in the data set.

The exclusion of a pleiotropic effect of the *polled* locus could reduce the skepticism towards including polledness in the practical breeding strategies. Therewith, breeding for polledness as a non-invasive alternative to dehorning could increase the animal welfare in the cattle industry on a long-term base.

Keywords: polled cattle, Holstein Friesian, milk performance

Introduction

All along, horns were a typical feature in the bovine species, although they entail a series of

disadvantages for the modern beef and dairy industry. Therefore, dehorning is a wide spread and common practice, but there are serious animal welfare concerns regarding dehorning as an invasive method (Medugorac *et al.*, 2017; Rothammer *et al.*, 2014; Wiedemar *et al.*, 2014). Breeding genetically hornless (polled) cattle provides a non-invasive alternative to dehorning (Allais-Bonnet *et al.*, 2013). In most of the European cattle breeds polledness is present in a very low frequency (Rothammer *et al.*, 2014). It is inherited in an autosomal dominant manner and the *polled* locus was identified on the bovine chromosome 1 (Medugorac *et al.*, 2012; White and Ibsen, 1936). A direct gene test for polledness is available. Hence, there is a great potential for a rapid spread of the trait by selective breeding. The *polled* locus is characterized by genetic heterogeneity with two identified mutations, one of Celtic and one of Friesian origin. Both mutations are perfectly associated with the polled phenotype (Allais-Bonnet *et al.*, 2013; Carlson *et al.*, 2016; Rothammer *et al.*, 2014; Wiedemar *et al.*, 2014). However, the functional causality is still not understood, since both mutations are not located within any known coding regions, regulatory elements or splice sites (Medugorac *et al.*, 2012). Intriguingly, there are conjectures that the *polled* locus influences other traits than horn development as well. A previous study found differences in performance of polled and horned Holstein Friesian bulls. Polled bulls were found to be in an inferior position for the relative estimated breeding values for milk production. In contrast, they were in a superior position to the horned bulls for the paternal component of fertility and for traits like type and muscularity. However, it is unclear if these differences are caused by the *polled* locus itself or if they are due to a lower genetic gain in the polled breeding lines (Segelke *et al.*, 2013). Nevertheless, the assumption of a pleiotropic effect of the *polled* locus, especially on performance traits, has kept the cattle breeding industry skeptical towards focusing on breeding for polledness in Holstein Friesian cattle.

Aim of this study was to investigate whether a difference in performance traits of Holstein Friesian cattle are associated with the *polled* mutations. Two hypotheses would explain a correlation between the *polled* locus and performance traits. Firstly, the *polled* locus could have a pleiotropic effect. Secondly, the *polled* locus could be in linkage with quantitative trait loci (QTLs) affecting the performance traits.

Material and Methods

To investigate these hypotheses, genetic material of 1851 daughters descending from 19 heterozygous polled bulls was collected. Since the frequency of the *polled* allele in the Holstein Friesian population is very low (>0.01) (Segelke *et al.*, 2013), we assumed half of the daughters of each sire to carry one *polled* allele (originating from the sire) and the other half to be genetically horned. The genetic horn status of all cows was analyzed and Pearson's Chi Squared test was performed to ascertain that the observed proportion of horned and polled daughters per sire is equal at 0.5. An overview of the data set used for the analysis is given in Table 1.

Table 1. Overview of the complete data set used for the analysis.

No. of Animals	Horn status		Sires	Farms	Breed		Year of birth
	polled	horned			(Black) Holstein	Red Holstein	
1851	920	931	19	359	1057	794	2008-2015

As phenotypes, yield deviations (YD) of the traits milk, fat and protein yield, fat and protein percentage and somatic cell score of the first three lactations were regarded.

Furthermore, we analyzed the relative estimated breeding values for milk yield, protein yield, fat yield, protein percentage, fat percentage, somatic cell score, dairy type, body, feet & legs, udder and temperament, respectively. Additionally, the total merit index (RZG) as well as the relative estimated breeding values for milk production (RZM), somatic cell score (RZS), total conformation (RZE), functional herd life (RZN), fertility (RZR), paternal calving traits (dRZK) and milking speed (RZD) were used. These phenotypes are collected routinely for estimation of breeding values. A list of all phenotypes in the analysis can be seen in Table 3 (supplementary results).

To investigate the first hypothesis, that aside from horn status the *polled* locus also influences performance, we performed both an independent two-sample t-tests and a multi-factor analysis of variances with the following model for the multi-factor analysis of variances:

(1)

Where:

y_{ijklm} : observation on the n^{th} animal of i^{th} genotype, j^{th} breed, k^{th} sire, l^{th} year of birth and the m^{th} farm

μ : mean

s_k : fixed effect of the k^{th} sire ($k= 1, \dots, 19$)

g_i : fixed effect of the i^{th} genotype ($i=$ horned and polled)

b_j : fixed effect of the j^{th} breed ($j=$ Black Holstein and Red Holstein)

a_l : fixed effect of year of birth of the l^{th} animal (2008-2015)

f_m : fixed effect of the m^{th} farm

e_{ijklm} : random error associated with the measurement on the n^{th} animal of the i^{th} genotype, j^{th} breed, k^{th} sire, l^{th} year of birth and the m^{th} farm

To investigate the hypothesis that the *polled* locus is linked to QTLs influencing performance traits, independent two-sample t-tests were conducted for each sire group. Subsequently, we performed a nested analysis of variances using the following model where the factor genotype was nested within the factor sire.

Results

Only heterozygous polled animals carrying the Friesian mutation were considered in the analysis. Using Pearson's Chi-squared test, all animals deviating from the expected proportion were excluded. Data were checked for outliers and observations outside of plus-minus four standard deviations were excluded from the analysis. The summary statistics for all traits are shown in Table 3 (supplementary results).

To investigate whether the *polled* locus has a pleiotropic effect, independent two sample t-tests for all traits were performed comparing the performance of all heterozygous polled and all horned animals in the data set. For RZR a significant difference and for RZG a suggestive difference between polled and horned animals was found with the polled group being inferior to the horned group. The results are shown in Table 4 (supplementary results). To prevent a bias due to other factors that could influence the performance, an ANOVA for multiple factors was carried out and significant differences for the traits YD fat content of lactation no. 1 ($p=0.03$) and RZD ($p=0.02$) were evident (results not shown).

For the examination of the second hypothesis, that the differences in performance between polled and horned animals are due to a linkage to a putative QTL, independent two-sample t-tests for the daughter groups of each sire for all traits were performed and significant differences were found. The results are shown in Table 5 (supplementary results). The results of the nested ANOVA for multiple factors are presented in Table 2. The significance level of the effect of the horn status within the daughter groups of each sire is displayed.

Table 2. Significant results of the nested ANOVA for multiple factors for the traits yield deviations (YD) milk, protein and fat yield and somatic cell score of the respective lactations. The relative estimated breeding value (rEBV) for protein kg, body and udder. Total merit index (RZG) and the relative estimated breeding value for milk production (RZM), total conformation (RZE), fertility (RZR), paternal calving traits (dRZK).

Sire	YD milk, kg; lact.2	YD fat, kg; lact.1	YD protein, kg; lact.1	YD protein, kg; lact.2	YD scs; lact.1	YD scs; lact.2	rEBV protein kg	rEBV body	rEBV udder	RZM	RZE	RZR	RZG	dRZK
298078	** ▲			▲	* ▼	* ▼								
298101							*** ▼	* ▼		* ▼		. ▼	* ▼	** ▼
298164					* ▲				** ▲		* ▲			* ▲
476865			* ▲						* ▲		* ▲			
506286	. ▼													* ▲
599458						. ▼		. ▼				* ▲		
679585	* ▲				* ▲							*** ▼		
832435	* ▲						* ▲			* ▲			. ▲	
916401				. ▼										
917382	* ▲	. ▲												
917391	. ▼					* ▲								
917470			. ▼		* ▲		. ▼	* ▼		. ▼		* ▲	. ▼	* ▲
917510	. ▼	. ▼			* ▼			** ▼	*** ▲		** ▲	. ▼		
923151			** ▼											
927004							*** ▲			** ▲	. ▼		** ▲	

Significance levels: . (p-value < 0.1); * (p-value < 0.05); ** (p-value < 0.01); *** (p-value < 0.001). Up arrow (▲) indicating the polled group being superior to the horned group; down arrow (▼) indicating the polled group being inferior to the horned group.

Discussion

For the underlying hypothesis of the *polled* locus having a pleiotropic effect, we found only RZR to be significant and a suggestive significance for RZG according to an independent two-sample t-test. Intriguingly, those two traits were not significant in the subsequently performed ANOVA. Instead, two other traits (YD fat content of lactation 1 and RZD) appeared to be significantly affected by the *polled* locus. Due to the ambiguity of those results, it seems to be unlikely, that the *polled* locus has a pleiotropic effect. Nevertheless, there are undeniable differences between the polled and horned animals in the data set. To regard our second hypothesis, that the *polled* locus is linked to putative QTLs affecting performance traits, independent two-sample t-tests for the daughter groups of each sire were performed. Indeed, significant differences between the polled and the horned daughters of the regarding sire were found. To avoid a possible bias due to other factors influencing the performance traits, a nested ANOVA was carried out where the genotype was nested within the factor sire. Several significant differences were found for most of the traits already stated in the t-test and for additional traits. Intriguingly, the effect of the *polled* locus between the different sire groups is partly vice versa. That is, the *polled* locus has in some daughter groups a positive effect on a performance trait, whereas in other daughter groups it has a negative effect on the same trait. Even more interesting, in the remaining daughter groups the *polled* locus has no influence on this trait. One possible explanation is that the *polled* locus could be linked to putative QTLs influencing those performance traits but the linkage phases differ between different families. For closer inspection, we contrive to consider the kinship between the sires in the data set.

If we can ascertain that the correlation between polledness and performance traits is due to linkage and not caused by a pleiotropic effect of the *polled* locus, the skepticism towards

including polledness in the practical breeding strategies could be reduced. Therewith, breeding for polledness as a non-invasive alternative to dehorning could increase the animal welfare in the cattle industry on a long-term base.

Acknowledgements

The authors are grateful for the financial support from the H. Wilhelm Schaumann Foundation.

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Supplementary Results

Table 3. Summary statistics of the data set for the traits yield deviation (YD) milk, protein and fat yield, protein and fat percentage and somatic cell score of the first three lactations. The relative estimated breeding value (rEBV) for milk, protein and fat yield, protein and fat percentage, dairy type, body, feet & legs, udder and temperament. The total merit index (RZG) and the relative estimated breeding value for milk production (RZM), somatic cell score (RZS), total conformation (RZE), functional herd life (RZN), fertility (RZR), paternal and maternal calving traits (dRZK & mRZK) and milking speed (RZD).

Trait	Lactation No.	n	mean	sd	min	max
test days	1	1846	9.1	1.2	5.0	12.0
test days	2	1121	8.4	2.3	3.0	16.0
test days	3	575	8.7	2.0	3.0	12.0
YD milk, kg	1	1839	769.61	515.28	-1412.59	2784.63
YD milk, kg	2	939	963.14	493.02	-1012.27	2984.37
YD milk, kg	3	500	1076.75	469.71	-1252.13	2393.12

YD fat, kg	1	1834	20.62	20.00	-60.82	104.16
YD fat, kg	2	937	26.88	21.97	-65.07	126.73
YD fat, kg	3	500	26.12	21.89	-74.79	129.40
YD protein, kg	1	1839	25.43	15.50	-31.89	89.37
YD protein, kg	2	936	30.96	16.01	-42.79	84.84
YD protein, kg	3	499	31.95	15.68	-34.18	68.41
YD fat, %	1	1837	-0.114	0.235	-1.053	0.828
YD fat, %	2	938	-0.118	0.224	-0.846	0.797
YD fat, %	3	501	-0.172	0.210	-1.027	0.507
YD protein, %	1	1833	-0.003	0.104	-0.352	0.415
YD protein, %	2	936	-0.015	0.100	-0.456	0.388
YD protein, %	3	500	-0.040	0.086	-0.361	0.316
YD somatic cell score	1	1823	-0.024	0.534	-2.793	3.012
YD somatic cell score	2	931	-0.054	0.624	-3.733	3.877
YD somatic cell score	3	500	-0.103	0.496	-2.644	2.208
rEBV milk kg	-	1851	235.33	556.68	-1379.00	1933.00
rEBV fat kg	-	1849	5.13	19.20	-68.00	67.00
rEBV protein kg	-	1851	7.46	15.85	-45.00	65.00
rEBV protein %	-	1851	-0.004	0.136	-0.470	0.520
rEBV fat %	-	1850	-0.047	0.286	-1.120	0.960
rEBV dairy type	-	1851	106.4	6.8	81.0	130.0
rEBV body	-	1850	99.9	8.9	68.0	124.0
rEBV feet & legs	-	1849	100.5	6.3	77.0	123.0
rEBV udder	-	1851	108.2	7.8	80.0	136.0
rEBV temperament	-	1851	100.4	4.4	75.0	121.0
RZM	-	1851	104.9	10.8	66.0	142.0
RZS	-	1850	101.1	7.9	71.0	126.0
RZE	-	1851	106.1	7.6	78.0	133.0
RZN	-	1851	102.6	6.7	78.0	125.0
RZR	-	1850	99.8	6.8	78.0	123.0
RZG	-	1851	106.0	10.2	70.0	139.0
dRZK	-	1850	100.5	3.9	86.0	114.0
RZD	-	1851	99.9	5.7	68.0	126.0

Table 4. Significant results of the independent two-sample t-test comparing the performance of polled and horned animals for the relative estimated breeding value for fertility (RZR) and the total merit index (RZG).

Trait	Df	Statistic (t)	p-value	mean horned	mean in polled	
RZR	1848	2.2373	0.0254	100.14	99.43	*
RZG	1849	1.7452	0.0811	106.37	105.54	.

Table 5. Significant results of the independent two-sample t-test for the daughter groups of each sire. Yield deviations (YD)

HB_sire	Trait	Lactation		Statistic (t)	p-value	mean in	mean in
		No.	Df			horned	polled
679585	YD fat, kg	1	37	-2.240	0.031	17.51	32.324
917382	YD fat, kg	1	182	-2.189	0.030	27.16	32.275
298078	YD fat, kg	2	15	-2.314	0.035	12.62	46.306
298078	YD fat, %	1	79	-2.031	0.046	-0.273	-0.214
298101	YD fat, %	1	163	-2.197	0.029	0.074	0.140
298101	YD milk, kg	1	164	2.203	0.029	460.21	311.128
917391	YD milk, kg	1	115	2.047	0.043	614.95	429.513
298078	YD milk, kg	2	18	-2.297	0.034	881.00	1704.966
917391	YD milk, kg	2	63	2.323	0.023	664.55	415.198
298101	YD protein, kg	1	164	2.158	0.032	25.03	20.473
923151	YD protein, kg	2	93	2.971	0.004	31.38	24.003
927004	YD protein, kg	3	26	-2.114	0.044	20.63	30.378
917391	YD protein, %	1	116	-2.002	0.048	-0.006	0.023
832283	YD protein, %	2	42	-2.442	0.019	0.036	0.096