# Estimation of heritability for immunoglobulin concentrations in colostrum and serum in Holstein cows and their calves

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**Abstract:** In the present study, colostrum quality in Holstein cows and their genetic parameters for passive transfer traits in calves were estimated. In order to get an estimation, colostrum Brix (ColBrix), colostrum immunoglobulin G (ColIgG) of Holstein cows, and serum total protein (STP) and serum immunoglobulin G (SIgG) levels of the calves of selected cows raised in a private livestock company in Turkey were assessed. Variance components and genetic parameters were estimated by MTDFREML using a mixed model. The heritability for ColBrix, ColIgG, STP and SIgG was estimated to be 0.19  $\pm$  0.07, 0.19  $\pm$  0.07, 0.36  $\pm$  0.10 and 0.34  $\pm$  0.10, respectively. According to the results of bivariate analysis, genetic correlations between the traits varied between -0.98 and +0.98. There was a sufficient genetic variance for SIgG in the calves in the population and thus a significant genetic improvement may be achieved by selection in order to increase the passive transfer. It will be worthwhile to continue the efforts for genetic improvement studies which have the potential to reduce calf deaths in the future.

Keywords: dairy cattle; passive transfer; calf survival; genetic parameter; MTDFREML

Breeding goals in dairy cattle have focused on increasing milk production for many years. Today, many traits are evaluated in selection programs in dairy cattle breeding. These traits are usually milk production and quality traits, udder health and reproductive performance traits. In recent years, functional traits have also been included in the selection programs. Among these functional traits are efficient life and other health traits such as paratuberculosis resistance (Johne's disease), metritis, ketosis, mastitis resistance, feed efficiency and methane emissions, milking traits such as milking speed and temperament, and immune response (Egger-Danner et al. 2015).

While genetic parameters for colostrum quality in dairy cows (Fleming et al. 2016) and in pigs (Balzani et al. 2016) were estimated in previous

studies, heritability for serum total protein (STP) and some immunoglobulins (Ig) were estimated in some studies (Jensen and Christensen 1975; Jensen et al. 1982; Mallard et al. 1983; Muggli et al. 1984; Mazengara et al. 1985; Gilbert et al. 1988a; Burton et al. 1989; Detilleux et al. 1994). On the other hand, while in some studies genetic parameters for natural antibodies associated with mastitis (Thompson-Crispi et al. 2013) and antibody development against paratuberculosis (Pritchard et al. 2017) were estimated in the Holsteins, some other studies focused on the development of resistance to the bovine respiratory disease through selection (Snowder et al. 2006; Snowder 2009; Cockrum et al. 2016).

The immune system is vital in terms of controlling contagious and other diseases. It is sug-

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gested that in dairy cattle improvement, immune response traits should be included in the selection indexes in terms of decreasing the incidence and effect of diseases (Thompson-Crispi et al. 2013). For this reason, genetic selection for an immune response may be a promising and appropriate approach to increasing the natural disease resistance in dairy cattle (Thompson-Crispi et al. 2012).

In recent years, in addition to STP and Ig, antibody-mediated immune response (AMIR) via antibodies with subsequently acquired immunity traits, cell-mediated immune response (CMIR), and the possibility of using other immune response traits in the selection of dairy cattle have been investigated (Thompson-Crispi et al. 2012; Heriazon et al. 2013; Thompson-Crispi et al. 2014; Denholm et al. 2017). The studies done in Canada and in the USA in the past 10 years demonstrated that the Holstein cows with superior or higher immune responses had lower occurrence of mastitis, produced higher quality milk and colostrum and responded better to vaccinations (Wagter et al. 2000). A technology developed to increase the immune response traits, AMIR and CMIR by genomic selection is now routinely used in Canada (Thompson-Crispi et al. 2014).

Calf health and vitality have a great influence on lifelong production and performance. It is also an important cause of poor welfare. In those facilities where calf deaths are particularly high, the risk of other infections of the digestion and respiratory systems is increased in calves in the early stages of the life. Colostrum is important in removing meconium by the effect of a laxative, producing energy for the protection of body temperature, and future efficiency and productivity in calves. However, the most important benefit of colostrum for calves is that it provides passive transfer of immunity against bacterial diseases that cause deaths. Newborn calves have no immunoglobulins as the placenta is impermeable to Ig. However, thanks to these substances in the colostrum, calves can be protected from diseases until their (active) immune systems develop. Total IgG,  $IgG_1$  and  $IgG_2$  are the major components of the humoral immune system, which is part of the subsequently acquired immune response that protects cattle against bacterial and viral attacks (Cockrum et al. 2016). It was also reported that bovine colostrum improved the survival of low and normal birth weight piglets during their first 10 days of life (Viehmann et al. 2015).

The purpose of this study was to estimate variance components and heritability for the colostrum quality traits and STP and SIgG in Holstein cattle. Thus, it was aimed to determine whether these traits can be improved by selection.

#### MATERIAL AND METHODS

#### Material

The study was carried out between January 2017 and April 2018 in the herd that is a member of Cattle Breeders' Association of Bursa, (40°29'31" N; 29° 59'56" E) in Bursa Turkey. The climate of Bursa has a hot-summer Mediterranean climate, with hot, dry summers and mild to cool, wet winters. In total 1 120 colostrum and serum samples were used in the study. The number of cows in first, second, third and fourth parity was 436, 305, 172 and 207, respectively. On the farm there are semiopen free stalls and precautions were taken with fans against heat stress during the research study. The cows roamed freely in the roaming area inside and outside the cowshed and similar roughage and compact feed were given in total mixed ration all the year round.

# Taking serum and colostrum samples and their analyses

Colostrum samples were taken at the first milking after calving and stored at -20 °C until the analysis. Blood samples of the calves were taken from the jugular vein by the veterinarians of the facility 36 h after birth and centrifuged, and the serums obtained were stored at -20 °C. Analyses of colostrum and serum samples were performed using a digital refractometer (Palm Abbe Digital Refractometer PA203; Misco, Cleveland, OH, USA). After the colostrum results were read as ColBrix in the refractometer, STP results as g/dl, SIgG results as mg/ml, ColBrix values were converted to CollgG (mg/ml) values using the conversion table as recommended by the manufacturer. Records of the sires with less than five calves were not evaluated. The colostrum consumed by the calves was divided into three groups as low (below 70 mg/ml), medium (70–115 mg/ml) and high (above 115 mg/ml) IgG content and a fixed effect was established for serum traits.

## Statistical analyses

For colostrum traits, the calving year (2017, 2018), calving season [spring (March, April, May), summer (June, July, August), fall (September, October, November), winter (December, January, February)], calf's gender (male, female), and number of lactations (1, 2, 3, 4+) were taken into account as fixed effects. Colostrum quality groups (low, medium, high) were included in addition to the model used for serum traits. Colostrum and serum samples were primarily analyzed in the GLM procedure by analysis of variance (SPSS v16.0; SPSS Inc., Chicago, IL, USA).

(Co)variance components were estimated by using REML and MTDFREML under a mixed model (Boldman et al. 1995). The animal's genetic effect was included as a random factor in the model. Initially univariate analysis was performed, subsequently it was followed by bivariate analysis. As a result of bivariate analysis, genetic and Pearson correlations between all traits were estimated. If the value of the -2 log likelihood variance in the simplex function was less than  $10^{-9}$ , then the convergence was considered to be accomplished. The model used was given by the matrix notation below.

$$y = \mathbf{X}b + \mathbf{Z}a + e \tag{1}$$

where:

y — the observed value of the vector for each trait;

X, Z - the design matrices of these effects;

 the vector of fixed effects (four effects were used for ColBrix and ColIgG and five effects for STP and SigG);

a and e – the direct additive genetic effects (individual)
and residual effect, respectively.

The assumptions used in the analyses are as follows:

$$var(a) = \mathbf{A}\sigma_a^2, var(e) = \mathbf{I}\sigma_e^2$$
 (2)

where:

A – the numerator relationship matrix with 2 770 animals:

 $\sigma_a^2$  and  $\sigma_e^2$  – direct additive genetic variance and residual variance, respectively;

I – the identity matrix whose order was equal to the number of observations in *y*.

Heritability ( $h^2$ ) was calculated using these variance components. Subsequently, in a series of bivariate analyses based on the same model, genetic and Pearson correlations among all traits were derived from co-variance component estimates using the following equation:

$$r = \frac{cov(y_1y_2)}{\sqrt{var_{y_1} \times var_{y_2}}} \tag{3}$$

#### **RESULTS**

A general evaluation regarding the effects of the statistical model used in the research on colostrum and serum traits was done. Based on this, it was found that the effects of calving season and lactation number on ColBrix and IgG, and of the season and colostrum quality group on STP and SIgG were significant (Table 1). The descriptive statistics for ColBrix, ColIgG, STP and SIgG are presented in Table 2. The general means and standard error for the traits were found as  $27.3 \pm 0.12\%$ ,  $93.5 \pm 0.70$  mg/ml,  $6.7 \pm 0.03$  g/dl, and  $14.4 \pm 0.18$  mg/ml, respectively.

# Genetic parameter estimation

Variance components and heritability estimates obtained from the model for all traits are presented in Table 3. The heritability for ColBrix and ColIgG were estimated to be  $0.19 \pm 0.07$ . Heritability for serum traits was almost twice that of colostrum. It was estimated that the heritability for STP and SIgG was 0.36 and 0.34, respectively.

Table 1. Effect of fixed factors on colostrum and serum traits

Factor	ColBrix	ColIgG	STP	SIgG
Calving year	ns	ns	ns	ns
Calving season	P < 0.05	P < 0.05	P < 0.05	P < 0.05
Parity	P < 0.05	P < 0.05	ns	ns
Calf gender	ns	ns	ns	ns
Colostrum quality groups	-	_	<i>P</i> < 0.05	P < 0.05

ColBrix = colostrum Brix; ColIgG = colostrum immunoglobulin G; ns = non-significant; P < 0.05 = the difference between the means of at least two levels of a factor was significant; SIgG = serum immunoglobulin G; STP = serum total protein

Table 2. Descriptive statistics of colostrum and serum traits

Variable	п	Mean	SEM	SD	Minimum	Maximum
ColBrix	1 120	27.3	0.12	4.16	13.9	43
ColIgG	1 120	93.5	0.70	23.36	19.7	165.9
STP	1 120	6.7	0.03	1.10	3	11
SIgG	1 120	14.4	0.18	5.89	2.2	38.1

ColBrix = colostrum Brix (%); ColIgG = colostrum immunoglobulin G (mg/ml); n = number of observations; SIgG = serum immunoglobulin G (mg/ml); STP = serum total protein (g/dl)

The genetic and Pearson correlations between colostrum and serum characteristics are presented in Table 4. Estimated genetic correlations were very strong. Pearson correlation coefficients were weaker, except for the correlations between traits within both groups.

# **DISCUSSION**

The results obtained from this study have demonstrated that a satisfactory level of genetic progress can be achieved for passive transfer by selection. A moderate estimate of heritability of the serum traits is an indication of this particular result. Nevertheless, it is possible to say that genetic progress to be gained in the colostrum traits may be slower. Besides, highly genetically related traits can be used in indirect selection.

While the findings obtained in this study were lower than the heritability reported in the literature (0.41–0.56) for colostrum and serum  $IgG_1$  in cattle (Gilbert et al. 1988b), they were similar to the value reported in the literature for colostrum in sheep, but higher (0.18) than the value reported for serum (Gilbert et al. 1988a). It has been suggested that the genetic selection for  $IgG_1$  in colos-

Table 3. Variance components and heritability for colostrum and serum traits

Trait	$\sigma_a^2$	$\sigma_e^2$	$\sigma_p^2$	$h^2 \pm SE$
ColBrix	3.20	13.83	17.04	$0.19 \pm 0.07$
ColIgG	103.7	444.4	548.1	$0.19 \pm 0.07$
STP	0.41	0.72	1.13	$0.36 \pm 0.10$
SIgG	11.60	22.16	33.77	$0.34 \pm 0.10$

 $\sigma_a^2$  = direct additive genetic variance;  $\sigma_e^2$  = environmental variance;  $\sigma_p^2$  =phenotypic variance; ColBrix = colostrum Brix (%); ColIgG = colostrum immunoglobulin G;  $h^2$  = heritability; ns = non-significant; SIgG = serum immunoglobulin G; STP = serum total protein

trum and lamb-calf serum would be effective and that an indirect increase in calves' viability might be possible (Gilbert et al. 1988a, b). However, Muggli et al. (1984) found lower heritability for  $IgG_1$  in calf serum (0.03 and 0.13), but higher maternal heritability (0.23 and 0.07) in two herds.

In a study carried out by Burton et al. (1989), heritability estimates ranged from 0 to 0.20 for IgG, 0 to 0.45 for IgA, and 0 to 0.26 for IgM, depending on the sampling period, and were 0.07, 0.08, and 0 for IgG, IgA, and IgM, averaged over all sampling periods. As a result of the study, it was reported that serum Ig levels could be used to make calves more resistant to diseases (Burton et al. 1989). On the other hand, the heritabilities for STP and  $IgG_2$  in Danish Red Cattle were 0.09 and 0.12, respectively, and the genetic correlation (0.49) between them was weaker than the value in this study (Jensen and Christensen 1975). Jensen et al. (1982) found heritability in Holstein cows for serum TP, albumin, IgG<sub>1</sub>, IgG<sub>2</sub>, IgA, IgM to be 0.10-0.20.

The heritabilities for  $IgG_1$ ,  $IgG_2$ , IgM and IgA in Holstein cows were identified as 0.10, 0.10, 0.09, and 0.14, respectively (Mazengera et al. 1985). In the meantime, while Mallard et al. (1983) identified the heritability in Holstein cattle of various ages for  $IgG_1$  and IgA as between 0.0 and 0.28, for  $IgG_2$  as 0.68 and for IgM as between 0.08 to 0.85, they

Table 4. Genetic (below diagonal) and Pearson correlations (above diagonal) between traits

	ColBrix	ColIgG	STP	SIgG
ColBrix	_	$1.00 \pm 0.00$	$0.24 \pm 0.03$	$0.26 \pm 0.03$
CollgG	$-0.98 \pm 0.59$	_	$0.24 \pm 0.03$	$0.26 \pm 0.03$
STP	$0.98 \pm 0.52$	$0.98 \pm 0.56$	_	$0.97 \pm 0.00$
SIgG	$0.98 \pm 0.60$	$0.98 \pm 0.60$	$0.98 \pm 0.08$	_

ColBrix = colostrum Brix (%); ColIgG = colostrum immunoglobulin G; SIgG = serum immunoglobulin G; STP = serum total protein

reported that selection might be possible to change the Ig content and reduce the diseases.

It was reported that a significant genetic variation occurred in the total number of neutrophils in the serum in cattle and for IgG<sub>1</sub>, IgG<sub>2</sub>, and IgM levels prior to calving (Detilleux et al. 1994). According to the researchers, this variation proved that it could be used in the selection of cattle that developed innate immunity without causing adverse effects on milk production. The genetic factors affecting resistance to the bovine respiratory disease (BRD) have been investigated recently (Snowder 2009). Estimates of heritability for resistance to BRD in the weaned calves ranged from low to moderate (0.00-0.26) (Snowder et al. 2006). Passively acquired maternal antibodies in the weaned calves play an important role in calf resistance against BRD. For this reason, the potential of the mother for maternal antibody production in high volume or density for BRD antigens might be a critical factor in reducing BRD in the weaned calves (Snowder 2009). Thompson-Crispi et al. (2013) estimated the heritability for IgG and IgM that are natural antibody isotypes as 0.32 and 0.18, respectively, and reported that IgM reduced the clinical mastitis risk. In another study, heritabilities for IgG, IgG<sub>1</sub>, and  $IgG_2$  were estimated to be 0.15, 0.11, and 0.24 (Cockrum et al. 2016). The researchers reported that since the cattle had immune system traits and BRD had a moderate heritability, health of the herds could be improved through genetic selection.

Denholm et al. (2017) found that genetic selection for immune-related traits may be possible. They suggested that additional cellular markers that reflect the immune function better or the traits that include immune tests should be investigated in future studies. This study also emphasized that in addition to the blood leukocyte subgroups considered to increase the health and well-being of dairy cows, the serological immunity traits that could be measured both in cow's milk and blood could be important.

Estimates of heritability for AMIR the  $21^{st}$  day was found higher than the  $14^{th}$  day for both  $IgG_1$  and  $IgG_2$  (Thompson-Crispi et al. 2012). The average heritability estimates via series of bivariate analysis for  $IgG_1$  and  $IgG_2$  were found 0.29, 0.34 on the  $14^{th}$  day and 0.16, 0.41 on the  $21^{st}$  day, respectively (Thompson-Crispi et al. 2012).

In another study, heritability for AMIR on the 14<sup>th</sup> and 21<sup>st</sup> day was estimated to be 0.44 and

0.31, respectively, and these traits were reported to be quantitative (Heriazon et al. 2013). Moderate estimation of the heritability of these traits suggests that genetic improvement may be possible to reduce the impact of the disease to the lowest level and enhance immunity in improving the overall health status. The occurrence of diseases in CMIR and AMIR cows was examined and high immune response cows were shown to have a decreased incidence of diseases such as mastitis, metritis, ketosis or retained placenta (De La Paz 2008). De La Paz (2008) also found that the categorization for AMIR or CMIR was not a significant predictor for the incidence of displaced abomasum. Similarly, Pinedo et al. (2009) investigated the association between paratuberculosis infection and the general humoral and cellular immune status of adult cows and found that cows with higher levels of CMIR were less likely to demonstrate the infection.

It was reported that the improvement for high AMIR in cattle could lead to better quality colostrum production and reduce the passive transfer failure in the calves (Fleming et al. 2016). The passive transfer failure in dairy female calves in the USA was determined to be 19% and annual calf mortality (7.8% in suckling period, 1.8% weaning) was 9.6% (NAHMS 2010). It was reported that the most common causes of mortality were digestive system diseases (e.g. diarrhoea) before weaning and BRD after weaning (NAHMS 2010). In the calves with higher IgG concentrations, fewer respiratory diseases were seen (Furman-Fratczak et al. 2011). In addition to the other bioactive components in colostrum, while high IgG concentrations improve the health of calves, they also reduce the risk of disease and mortality.

The estimates of genetic correlations are very strong in the present study. The standard error of genetic correlations in the different traits evaluated in this study was very high, which may be related to the limited number of data. It was very important to obtain more accurate predictions for these traits that can be used in indirect selection. According to these results, it can be said that these traits cannot be used successfully in indirect selection in this herd. This relationship is even more important, especially between ColBrix and SIgG. That is to say, taking serum samples from calves and determining the IgG values may not always be possible in farm conditions. Determining them in the laboratory is also a costly and time consuming method. On the

other hand, in establishments that determine the quality of colostrum with optical or digital refractometer, the colostrum Brix value may be a selection criterion for calf SIgG. However, it should be taken into account that the heritability for ColBrix is about half of what is predicted for SIgG.

Accurate genetic selection for a trait and effective improvement methods for successful genetic progress should be used. For genetic improvement program to be successful in a population depends on the heritability and selection intensity of the relevant trait. In order to be able to develop effective selection programs in new traits, it should be possible to predict breeding values with high accuracy and large databases should be developed for this purpose. Since the work required to record the data for new traits requires extra effort from the manufacturer, it is necessary to provide immediate benefits for the manufacturers.

#### **CONCLUSION**

There is a sufficient genetic variance in the population to improve by selection the SIgG, which is a passive transfer marker in the calves. Therefore, significant genetic progress can be achieved by selection to increase the passive transfer. It will be worthwhile to continue the efforts for genetic improvement studies that have the potential to reduce calf mortality in the future.

### **Conflict of interest**

The authors declare no conflict of interest.

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