

Genetic studies of carotenoid concentration in the plasma and milk of New Zealand dairy cattle

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Abstract Blood and milk samples were collected in 1992/93 from 2-year-old heifers in the Livestock Improvement Corporation's Sire Proving Scheme, for analysis of carotenoid concentration (CC). The trial comprised heifers in 127 spring-calving North Island dairy herds, where blood samples were taken from a total of 2744 heifers (Holstein-Friesians (F), Jerseys (J), and their crosses) in early/mid lactation (spring), and from a sub-group of the same animals in autumn. These heifers were the daughters of 157 young F and J sires, and 15 older sires. Whole-lactation yields of milk, fat, and protein, and fat% and protein% were also obtained. Sire-model restricted maximum likelihood (REML) analyses were carried out to estimate genetic and phenotypic parameters. Purebred F and J breed means for plasma CC in spring were 14.1 and 17.9 $\mu\text{g ml}^{-1}$ ($P < 0.001$), respectively, and for milk CC 5.24 and 7.50 $\mu\text{g g}^{-1}$ fat ($P < 0.001$), and corresponding heritabilities for the two traits were 0.46 ± 0.08 and

0.11 ± 0.10 . The repeatability of plasma CC across seasons was 0.64 ± 0.02 . The genetic correlation between spring plasma CC and milk CC g^{-1} fat was 0.66 ± 0.22 , whereas genetic correlation estimates for spring plasma and milk CC g^{-1} fat with other traits were: with milk volume -0.01 ± 0.16 and 0.41 ± 0.35 , with fat yield 0.06 ± 0.22 and 0.29 ± 0.54 , and with fat% 0.03 ± 0.13 and -0.26 ± 0.29 . It was concluded that plasma CC was a repeatable and heritable trait, whereas milk CC g^{-1} fat was lowly inherited; plasma CC was essentially uncorrelated with the standard milk production traits; if bulls were selected for lower plasma CC, there would be reduced CC in both the plasma and milk of their daughters.

Keywords cattle; carotenoid concentration; heritability; genetic correlation; milk; plasma

INTRODUCTION

Yellow coloration of fat in the meat and dairy products from pasture-fed cattle in New Zealand reduces acceptability of these products to many consumers. The colour is mainly due to the presence of carotenoids (β -carotene and lutein) deposited in the adipose tissue and in milkfat. In beef cattle, Morgan & Everitt (1968, 1969) found correlations between beef fat colour and the carotene concentration in either blood plasma (0.67) or beef fat (0.92). Plasma carotenoid concentration (CC) is correlated with milkfat colour and with milkfat CC (McGillivray 1960a). These relationships suggest that the CC in adipose tissue and in milkfat may both be under the same control. If this control is at least partly influenced by genetic factors, then genetic selection could change CC in adipose tissue and in milkfat, and the degree of yellowness in beef or dairy cattle could be altered if there was ever a requirement for this change to meet a different market specification. To achieve such an objective, a selection criterion would need to be chosen, and the options available for this include analysing CC

in plasma, milkfat, or fat biopsy samples. The plasma sampling option has the advantage of being feasible in both sexes, and possibly at a young age.

Genetic parameters are required before such a scenario could be evaluated further. Evidence for the fact that CC is genetically determined comes from two types of data. Firstly, breed differences are known for CC in plasma (Morgan & Everitt 1969) and in milkfat (McGillivray 1960a; Winkelman et al. 1999). Secondly, within breeds, Morgan & Everitt (1968) found a high concordance among monozygous twin pairs ($r = 0.85$) for subcutaneous fat colour, and for plasma CC, suggesting that CC is heritable, although the actual heritability can be uncertain if deduced from twin data. Heritable differences in CC were also reported by Winkelman et al. (1999) for milk. Further, there is evidence for useful phenotypic variation, from the wide range (2- to 3-fold) of plasma CC among animals of the same breed grazing together (Knight et al. 1994).

Plasma CC largely reflects the carotene content of the diet consumed over the previous 1–3 weeks. If a group of cattle has been grazing together for at least 3 weeks, then plasma CC reflects their ability to absorb β -carotene, whereas the subcutaneous fat CC reflects the historical absorption of β -carotene over the previous 10–12 weeks, or even over the life of the animal. Two previous projects (Knight et al. 1993, 1994) have shown that the ranking of cattle on plasma CC was not affected by stress, short-term (48 h) starvation, or short-term changes in the carotenoid content of the diet.

The current project estimates the heritability of plasma CC and milkfat CC, and the phenotypic and genetic correlations between these CCs and whole-lactation yields (of milk, fat, and protein), or milk composition (fat%, and protein%). Preliminary results of the between-breed comparison of plasma CC were presented by Newman et al. (1994).

MATERIALS AND METHODS

Trial design and sample collection from cows

The trial involved Holstein-Friesian (F) and Jersey (J) heifers and their crosses, sampled from the Livestock Improvement Corporation's (LIC) Sire Proving Scheme. The scheme consisted of 157 young sires (20 born in 1987 and 137 in 1988) and 15 older sires, whose semen was used in many herds to generate heifers in late winter/early spring 1990, for recording in their first lactation in 1992/93. A

total of 127 Sire Proving Scheme cow herds from three of the six LIC regions (Auckland, Taranaki, and Wellington-Hawke's Bay) provided animals whose blood was sampled for the trial.

Blood samples for plasma CC were obtained from 2744 2-year-old animals in early/mid lactation (late October to mid December 1992). A second blood sample was obtained from a sub-group of the same animals in late lactation (March 1993), to estimate the repeatability of plasma CC ($n = 757$ animals). These animals were from as many of the same herds as possible in the Taranaki and Wellington-Hawke's Bay regions. Blood samples were obtained from the tail of each animal, and drawn into 10-ml heparinised vacutainer tubes. The tubes were gently shaken, and placed immediately on ice in a dark container. At the end of the day, blood samples were centrifuged; each plasma sample was removed, placed in a plastic tube, frozen immediately, and stored at -20°C for subsequent analysis.

As part of the LIC's normal Sire Proving Scheme routine in each herd, herd-test milk samples were taken for each cow by pooling milk samples from an afternoon (Day 1) and the following morning's milkings. Surplus fresh milk from each pooled milk sample was retrieved on the evening of Day 2 at the central depot ($n = 819$ animals from which milk samples were collected, in 47 of the herds (29 F-sired and 18 J-sired herds) in the Taranaki region, and in the Manawatu province, part of the Wellington-Hawke's Bay region) between November 1992 and January 1993, for analysis of milk CC. At the end of the 1992/93 season, the relevant full-lactation records were obtained from the LIC for the herds in our study to provide data on full-lactation milk volume, fat yield, and protein yield; percentage fat and protein values were derived from these data (2321 animals in common with the heifers from which blood was collected).

Sample collection from bulls

In February 1993, the 1988-born bulls used to generate heifers in the study had blood collected for plasma CC (72 from Newstead, Hamilton, and 44 from Awahuri, Palmerston North). Overall, 73 were F and 43 were J bulls, comprising 85% of the original bulls in the Sire Proving Scheme from that age group.

Carotenoid analyses

Plasma CC was analysed by the method of Knight et al. (1994), in which 1 ml of plasma was denatured

with 1 ml of ethanol, and carotenoids were extracted with 3 ml petroleum spirits (Analar 40–60°C). The absorbance of the extract was measured in a spectrophotometer (Pye Unicam SP8/100) at a wavelength of 450 nm. Milk CC was analysed by a modification of the method of Indyk (1987) in which 2.5 ml of milk was mixed with 5 ml ethanol and 1 ml potassium hydroxide. The mixture was heated to 70°C in a waterbath for 10 min, and after cooling in a cold waterbath the carotenoids were extracted with 3 ml hexane/di-ethyl ether (9:1 v/v) and the absorbance of the extract was measured in the spectrophotometer at a wavelength of 450 nm. An extinction coefficient of 2590 (Indyk 1987) was used to calculate the μg carotene equivalents per ml of milk from the absorbance measured with the spectrophotometer. Since the methods used to extract both plasma and milkfat extracted all the carotenoids (i.e., β -carotene, lutein, and traces of other carotenoids), the data are presented as μg carotenoid ml^{-1} plasma and, for milk, as both μg carotenoid ml^{-1} milk and μg carotenoid g^{-1} milkfat. The percent milkfat in the full lactation from the LIC herd-test results was used to convert the μg carotenoid ml^{-1} milk to a “ g^{-1} milkfat” basis.

Data analysis

Inspection of the CC data showed that the distributions were skewed, and data were therefore transformed to natural logarithms (as was done for milk CC by Winkelman et al. 1999). Four CC traits were analysed, spring and autumn plasma CC, milk CC ml^{-1} milk and g^{-1} milkfat, and five full-lactation traits, milk volume, fat yield, protein yield, fat%, and protein%. All data (plasma and milk) were analysed by restricted maximum likelihood (REML) techniques (Johnson & Thompson 1995), using a sire model. There was only one daughter per dam, so the dam data were not included in our files. The relationship matrix for the sires was included, however, to take account of the common ancestry of some of the sires under evaluation. A single-trait repeatability model was also used for plasma CC in a separate analysis, to take account of the repeated records available from spring and autumn, on a sub-sample of animals.

The fixed effects included in all models accounted for herd (or herd \times sample-time, in the case of plasma CC), and breed of heifer (J, $^{3/4}$ J, $^{5/8}$ J, $^{1/2}$ J, $^{1/2}$ F, $^{5/8}$ F, $^{3/4}$ F, F), and a covariate for date of calving was included when significant ($^{1/2}$ J indicated Jersey-sired, and $^{1/2}$ F Friesian-sired cross-

bred animals). A quadratic term for the covariate was also tested (as discussed by Winkelman et al. 1999). In our case there was only a narrow range of dates for cows sampled (effectively indicating the range of days in milk, within a herd), and the quadratic term was only significant for spring plasma CC; accordingly, it was included in the model for this one trait. An alternative model was tested where the fixed breed effect was replaced by a breed covariate (% F genes) and an individual heterosis term (with heterosis values of 1 for $^{1/2}$ J, $^{1/2}$ F, 0.5 for $^{3/4}$ J, $^{3/4}$ F, and 0 for J and F); the small classes of $^{5/8}$ -bred animals were discarded for this analysis. Numbers of samples per breed group, as a percentage of the spring CC numbers were: 26% (J), 6.2% ($^{3/4}$ J), 1.2% ($^{5/8}$ J), 2.7% ($^{1/2}$ J), 9.0% ($^{1/2}$ F), 1.5% ($^{5/8}$ F), 10.6% ($^{3/4}$ F), and 43% (F).

For the bulls' plasma CC data, the effects of breed and location were removed by least squares analysis, and correlations were then calculated between each bull's adjusted plasma CC value and his breeding value for plasma CC obtained only from his daughters (effectively adjusted for breed, herd, and calving date).

RESULTS

The overall means, phenotypic standard deviations, and breed effects for each concentration trait (Table 1) show coefficients of variation for plasma CC and milk CC of 25–31%; considerably larger than for fat% and protein% at 9.8 and 7.0%, respectively. Milk volume and the other two yield traits were intermediate. The breed effects showed higher concentrations of milkfat and protein in the Jerseys, as expected, but also higher CCs in the plasma and milk in the Jerseys (purebred J and F heifers differed by factors of 1.27 for spring plasma CC, 1.33 for autumn plasma CC, 1.76 for CC ml^{-1} milk and 1.43 for CC g^{-1} fat). The F-cross and J-cross groups were generally intermediate between the F and J breeds, although trends with percentage-Friesian were clearer for the traits with lower coefficients of variation, namely the yields, and fat% and protein%. An example is given showing the effects of breed-cross (% F genes) on plasma CC in spring (Fig. 1). The linear regression was $-0.043 \mu\text{g} \text{ml}^{-1}$ per % increase in F genes, and the intercept (J mean) was $17.8 \mu\text{g} \text{ml}^{-1}$, with a correlation between CC and F% of -0.94 . On the transformed scale in which the CC analyses were done, the regression of \log_e spring plasma CC on

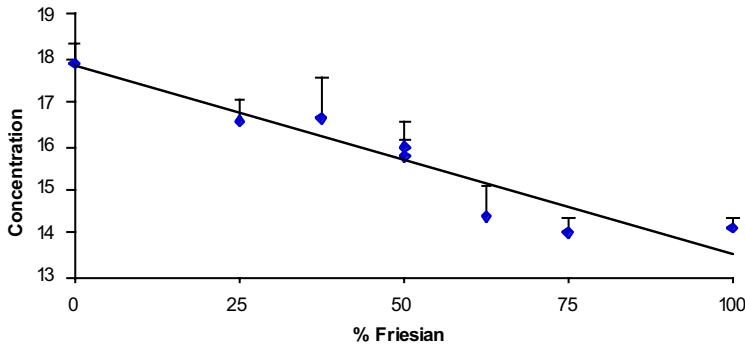


Fig. 1 Effect of breed or breed-cross (percentage of Friesian genes) on the plasma carotenoid concentration ($\mu\text{g ml}^{-1}$) of cows in spring (standard error bars are shown).

% F genes was $-0.0023 \pm 0.0003 \log_e$ units per % ($P < 0.001$), and the individual heterosis estimate was not significant.

The single-trait REML heritability estimates for all nine traits (Table 2) were based on pooled within-sire-breed estimates. Heritabilities were high for three of the six concentration traits (range 0.46–0.66; spring plasma CC, fat%, and protein%), but low to medium for the other three (0.11–0.27; autumn plasma CC, CC ml^{-1} milk, and CC g^{-1} fat) and also for the three volume and yield traits (0.08–0.28). Perhaps the only surprise was the low estimate for fat yield. The repeatability estimate for

plasma CC was 0.64 ± 0.02 , based on samples taken in spring and autumn.

From separate analyses within sire breeds (not shown), there was a trend for the heritability estimates of all CC traits to be higher in the J than the F breeds for spring CC, autumn CC, and milk CC ml^{-1} milk, but not milk CC g^{-1} fat (although standard errors were larger than for the pooled estimates in Table 2). Heritability estimates from the multi-trait analysis (pooled within sire breeds) gave similar values to the single-trait heritabilities shown in Table 2, except for CC g^{-1} fat (mean heritability = 0.15, where the various estimates had

Table 1 Means, standard deviations (SD), coefficients of variation (CV), and breed effects (carotenoid traits being back-transformed from \log_e means) for spring and autumn plasma carotenoid concentrations (CC), milk CC (ml^{-1} milk, and g^{-1} fat), and for whole-lactation milk volume, fat and protein yields, and composition data.

	Plasma CC		Milk CC		Milk volume (l)	Fat yield (kg)	Protein yield (kg)	Fat %	Protein %
	Spring ($\mu\text{g ml}^{-1}$)	Autumn ($\mu\text{g ml}^{-1}$)	($\mu\text{g ml}^{-1}$)	($\mu\text{g g}^{-1}$ fat)					
<i>n</i>	2744	757	819	720	2321	2321	2321	2321	2321
Mean	16.05	12.5	0.35	6.59	2763	138	101.9	5.09	3.73
Phenotypic SD	4.83	3.85	0.09	1.66	396.0	18.0	13.0	0.50	0.26
CV %	30.1	30.8	25.7	25.2	14.3	13.0	12.8	9.8	7.0
Log_e (trait)									
Mean	2.71	2.45	-1.14	1.82					
Phenotypic SD	0.30	0.31	0.27	0.26					
Least squares means for breed									
J	17.89	14.21	0.44	7.50	2324	131.52	92.81	5.70	4.03
³ / ₄ J	16.58	13.67	0.42	7.22	2447	133.73	94.77	5.50	3.91
⁵ / ₈ J	16.61	12.38	0.46	8.09	2550	137.34	100.00	5.40	3.93
¹ / ₂ J	15.97	10.43	0.32	5.97	2659	138.36	99.84	5.24	3.78
¹ / ₂ F	15.78	11.35	0.31	6.10	2847	141.70	104.65	5.02	3.69
⁵ / ₈ F	14.42	11.64	0.36	7.27	2965	145.87	107.01	4.95	3.62
³ / ₄ F	14.03	10.24	0.26	5.47	2935	141.59	105.26	4.86	3.60
F	14.14	10.70	0.25	5.24	2977	138.06	105.35	4.68	3.56
s.e.d. (J versus F)	0.47	0.56	0.02	0.31	48	2.17	1.57	0.06	0.03

to be derived from sub-sets of the traits, with a range for CC g⁻¹ fat from 0.10 to 0.26). It proved not possible to run a 9 × 9 analysis because of the high correlations between some pairs of traits, such as milk volume × protein yield with a phenotypic correlation of 0.87.

Also given in Table 2 are the phenotypic and genetic correlations among the CC traits and milk volume, fat yield, protein yield, and percentages. For plasma CC (spring and autumn), the phenotypic correlations with milk volume or fat and protein yields ranged from 0.06 to 0.12 (all significant), while the genetic ones ranged from -0.01 to 0.08 (none significant). For milk CC (CC ml⁻¹ milk and CC g⁻¹ fat), the phenotypic correlations with milk volume or fat and protein yields were -0.15 to 0.10 (two significant), and the genetic correlations were -0.22 to 0.61 (one significant). All the phenotypic correlations of CC with fat% and protein% were close to zero except for CC ml⁻¹ milk (0.27-0.32), and these two were the only genetic correlations which were significant, i.e., CC ml⁻¹ milk with fat% (0.36 ± 0.19, *P* < 0.06), and CC ml⁻¹ milk with protein% (0.57 ± 0.19, *P* < 0.003).

Correlations for the bulls' plasma CC (*n* = 116) with their daughters' spring plasma CC were estimated from raw data on bulls (*r* = 0.37; *P* < 0.001) or from bull plasma CC adjusted for location and breed (*r* = 0.42; *P* < 0.001). In effect, these were similar to genetic correlations, because the daughters' data for spring plasma CC were combined and represented by the bulls' REML breeding values. Correlations estimated in the same way between the bulls' plasma CC and their daughters' milk CC g⁻¹ fat were: from bulls' raw data (*r* = 0.24; *P* < 0.05), or from adjusted bull plasma CC (*r* = 0.10; *P* > 0.05).

DISCUSSION

Mean plasma CC values of 16.1 µg ml⁻¹ in spring and 12.5 µg ml⁻¹ in autumn in heifers in this study were similar to concentrations found by McGillivray (1957, 1960b) for pasture-fed cows in spring and autumn. Higher plasma CC in J than F heifers was consistent with earlier reports of differences between these breeds (McGillivray 1960a; Morgan et al. 1969). These differences in plasma CC between breeds were reflected in the CC in milkfat; least squares means for concentrations in J (7.5 µg g⁻¹ fat) and F (5.2 µg g⁻¹ fat) heifers were similar to those found in J and F heifers by Winkelman et al. (1999) at similar stages of

Table 2 Single-trait heritabilities (on diagonal), genetic correlations (below diagonal), and phenotypic correlations (above diagonal), for plasma carotenoid concentrations (CC), milk CC ml⁻¹ milk and g⁻¹ fat, and whole-lactation milk volume, fat and protein yields, and composition data. (All values ± s.e.)

Trait	Plasma CC spring	Plasma CC autumn	Milk CC (µg ml ⁻¹)	Milk CC (µg g ⁻¹ fat)	Milk volume	Fat yield	Protein yield	Fat%	Protein%
Plasma CC spring	0.46 ± 0.08	0.69 ± 0.02	0.41 ± 0.03	0.49 ± 0.03	0.12 ± 0.02	0.08 ± 0.02	0.11 ± 0.02	-0.06 ± 0.02	-0.05 ± 0.02
Plasma CC autumn	0.98 ± 0.05	0.27 ± 0.12	0.40 ± 0.04	0.49 ± 0.04	0.07 ± 0.03	0.06 ± 0.03	0.07 ± 0.03	-0.05 ± 0.03	-0.03 ± 0.03
Milk CC (µg ml ⁻¹)	0.59 ± 0.16	0.66 ± 0.19	0.23 ± 0.11	0.94 ± 0.01	-0.15 ± 0.04	0.10 ± 0.04	0.01 ± 0.04	0.32 ± 0.03	0.27 ± 0.03
Milk CC (µg g ⁻¹ fat)	0.66 ± 0.22	0.70 ± 0.28	0.78 ± 0.11	0.11 ± 0.10	0.02 ± 0.04	-0.01 ± 0.04	0.05 ± 0.04	-0.05 ± 0.04	0.05 ± 0.04
Milk volume	-0.01 ± 0.16	0.03 ± 0.19	-0.22 ± 0.24	0.41 ± 0.35	0.28 ± 0.07	0.72 ± 0.01	0.87 ± 0.01	-0.45 ± 0.02	-0.44 ± 0.02
Fat yield	0.06 ± 0.22	0.02 ± 0.28	0.49 ± 0.32	0.29 ± 0.54	0.14 ± 0.26	0.08 ± 0.05	0.82 ± 0.01	0.26 ± 0.02	-0.01 ± 0.02
Protein yield	0.04 ± 0.16	0.08 ± 0.19	0.24 ± 0.23	0.61 ± 0.31	0.76 ± 0.07	0.51 ± 0.18	0.25 ± 0.06	-0.17 ± 0.02	0.04 ± 0.02
Fat%	0.03 ± 0.13	-0.01 ± 0.16	0.36 ± 0.19	-0.26 ± 0.29	-0.86 ± 0.07	0.32 ± 0.20	-0.46 ± 0.13	0.66 ± 0.08	0.63 ± 0.02
Protein%	0.05 ± 0.14	0.01 ± 0.17	0.57 ± 0.19	0.25 ± 0.36	-0.52 ± 0.12	0.48 ± 0.24	0.11 ± 0.16	0.72 ± 0.07	0.51 ± 0.08

lactation. Concentrations of CC in milkfat in this study were lower than those reported for samples of bulk milk taken from dairy factories in the Manawatu and Taranaki provinces over the October–December period (Keen & Wilson 1992) and for earlier reported concentrations (McGillivray 1957, 1960b). These differences may reflect cow-age effects, since heifers have lower plasma CC than mature cows (McGillivray 1960b). The high phenotypic correlation for plasma CC in blood samples collected in spring and autumn (0.69) agrees with earlier reports of the consistent ranking of heifers in a group on plasma CC even when there were large changes in the carotenoid intake of the group (Knight et al. 1994). The phenotypic correlation of 0.49 between spring plasma CC and milk CC g⁻¹ fat was lower than reported correlations between plasma CC and subcutaneous fat CC in beef steers ($r = 0.58$, Morgan et al. 1969; $r = 0.71$ – 0.74 , Knight & Death 1999) and yellowness of beef fat ($r = 0.67$, Morgan & Everitt 1969).

The repeatability estimate for plasma CC in this study (0.64) was identical to the value found by Winkelman et al. (1999) for test-day milk CC g⁻¹ fat (called fat colour (FC) by them). Phenotypic standard deviations for all the CC traits were similar (on a log basis) at 0.25–0.31, and the value for milk CC g⁻¹ fat in this study and for FC in the Winkelman et al. (1999) study coincided at about 0.25 log_e units. Heritability estimates for milk CC g⁻¹ fat were lower at 0.11 in the present study than reported by Winkelman et al. (1999) for FC at 0.36, although their separate breed estimates were 0.44 for Friesians and 0.15 for Jerseys. Other heritability estimates for CC traits in our data were higher, ranging from 0.23 to 0.46. The trend was for breed differences in the heritability of CC traits to be in the opposite direction in our data compared with Winkelman et al. (1999). Heritability estimates for lactation milk volume, fat yield, and protein yield were 0.28, 0.08, and 0.25 in the present data, compared with 0.25, 0.17, and 0.20, respectively, in the Winkelman et al. (1999) results. The estimates for fat yield and milk CC g⁻¹ fat seem to be consistently lower than for the other traits, although a third New Zealand data set with genetic parameters for milk volume, fat yield, and protein yield (Johnson et al. 2000) gave heritabilities of test-day production, averaged over three stages of lactation, of 0.27, 0.26, and 0.21, respectively. Heritabilities of fat% and protein% were higher than for the corresponding yield traits, as expected.

Although the heritabilities in Table 2 had standard errors of relatively small size (0.05–0.12), the genetic correlation estimates had much larger standard errors, which meant that predictions of correlated responses to any selection that might be applied to CC traits could be inaccurate. Most of the genetic correlations were not significantly different from zero. Winkelman et al. (1999) concluded that “genetic correlations between milk colour and milk and protein yields were negative, and the correlations with fat yield were close to zero”, but their conclusions based on FC instead had only one yield trait significant (fat yield), where the estimate was -0.25 ± 0.10 .

Our experiment provided some limited data to look, hypothetically, at the consequences of selecting for a change in milk CC using the bulls' plasma CC as a predictor. This predictor was significantly correlated with daughters' plasma CC (0.42), indicating that plasma CC from bulls and cows (although at different ages) is approximately the same trait. As expected, cross-correlations between bulls' plasma CC and daughters' milk CC were lower (0.10), but the positive phenotypic correlations (0.40–0.49) and genetic correlations (0.59–0.70) in cows between plasma CC and milk CC (Table 2) indicated again that much of the biochemical pathway to controlling plasma CC and milk CC must be in common. Therefore, selecting bulls for lower plasma CC is expected to reduce CC in the plasma and, thus, the milk of their daughters.

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